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Science Symposium
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Paradise Valley College
Phoenix College
South Mountain College
Foreword

The 9th Annual Science Symposium was held on May 15, 2003. Students enrolled in General Organic Chemistry II, CHM 236 from Paradise Valley College (PVC), Phoenix College (PC) and South Mountain College (SMC), participated in the event. I want to thank Dr. Michael Bishop of South Mountain College for his leadership and the participation of his students.

Each contributor was responsible for selecting and researching their topic, preparing a paper and orally presenting their project to their peers. This booklet contains each of those papers.

As an instructor and faculty advisor for this symposium, I want to thank and congratulate each participant for their effort, courage and dedication. By participating these individuals perpetuate this event annually. I am both proud and honored to present the work of these individuals.

I would like to dedicate this symposium to the men and women of our armed forces. These people protect our freedoms by risking their lives for every one of us. The events of 9/11 will never be forgotten. As educators and students we acknowledge the freedoms we have to meet, learn, discuss and debate any topics we so choose.

William I. "Hank" Mancini, PhD
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Motion Sickness

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April 25, 2003
Abstract

This report gives an overview of motion sickness. Topics covered include what causes motion sickness, what motion sickness is, measures taken in order to prevent motion sickness and treatment options once one has become sick. It also explains briefly why each of these treatment methods is and is not recommended as well as who is most affected by motion sickness.
I. Motion sickness

Motion sickness is not fully understood despite the fact that it affects so many people and can be debilitating to those affected by it. Motion sickness is caused by a signal differential between the semicircular canals of the ear and otolith (pictured below), sight and somatosensory receptors in the skin, joints and muscles. This phenomenon is called Visual Vestibular Conflict (VVC). An otolith is one of the small particles of calcium carbonate in the utricle of the inner ear. Pressure of the otoliths on the hair cells of the macula provide sensory inputs such as acceleration and gravity. The VVC occurs when the parts of the ear send signals to the brain that the body is moving while the eyes, skin and nerves tell the brain that the body is stationary. This contradiction is thought to be the reason why the body produces the symptoms of motion sickness.

![Diagram of semicircular canals and otoliths](image)

II. Symptoms

The most common symptoms of motion sickness are salivation and nausea. Other symptoms include anxiety, dizziness, and even vomiting. The sensation of nausea is due to complex neural communication between the central nervous system and the gastrointestinal (GI) tract similar to when there is bad food in the stomach that the body must rid itself of. When stimulation of the GI tract is intense and prolonged, the result is usually vomiting, arguably the worst of the known symptoms of motion sickness. It is not known why the central nervous system stimulates the GI tract. Obviously there is no stimulation coming from the stomach. If scientists knew why the GI tract was affected, they could treat the cause of motion sickness rather than just treating the symptoms, which is the only known, means of treatment today.

III. Who motion sickness affects

Motion sickness is most common in people ranging in age from four to twenty, and is rare in people ages two and below as well as in people fifty and above.

IV. Prevention techniques

The best way to deal with motion sickness is to prevent it. If someone knew that they would be in a situation from which motion sickness could result, such as an airplane ride or a long car ride they could follow some simple steps to aid in the prevention of motion sickness. Some of these measures with brief explanations are as follows:
1- Eat a small meal consisting primarily of carbohydrates about three hours before leaving. Being hungry will make the stomach uncomfortable and easier to upset, also carbohydrates are relatively easy for the body to break down.

2- Do not eat food with high fat or protein content less than 24 hours prior to departure. Foods high in fat and protein are difficult to digest and may remain in the stomach longer than foods comprised of simpler molecules.

3- Do not eat spicy foods less than 24 hours prior to departure. Spicy foods have a tendency to upset the stomach causing heartburn and diarrhea, which will make a potentially bad situation worse.

4- Do not eat foods high in salt less than 24 hours prior to departure. Salty foods can dehydrate the body causing nausea and dizziness.

5- Do not read while in motion. This increases the contradiction between the sensory organs. It is better to focus your sight on the horizon or at least out the window.

6- Avoid alcohol. Similarly to number four, alcohol can dehydrate the body. Also it increases the effect of dizziness. People who become anxious before long trips or flights should consider an alternate method for relaxation.

7- Direct airflow toward the face. The sensation of air flow moving across the skin sort of tricks the nerves just below the surface into believing that the body is actually in motion (such as walking) more so than remaining stationary. Similarly, tapping the feet will produce this same effect.

V. Treatments-Drug

If these measures fail to prevent the onset of motion sickness, the sufferer should consider treating the symptoms with medication. There are a number of remedies, some approved by the Federal Drug Administration (FDA) and some that are not. I will cover some treatments in each category. The table on the following page lists some of the most common drugs available today which are marketed for motion sickness and their results in one study of effective treatment of the symptoms. As mentioned above the treatment of vertigo is primarily symptomatic. There are three major goals when treating these symptoms, the first of which is to eliminate the hallucination of movement and motion. The second goal is to reduce the accompanying psychoaffective signs such as nausea, vomiting and anxiety. The final goal is to enhance the process of vestibular compensation which allows the brain to find new sensory equilibrium in spite of the conflict of information it recieves.

The FDA recognizes only four over-the-counter medications that are safe and effective for preventing and treating nausea, vomiting and dizziness associated with motion sickness. The generic or “common” names of the approved drugs followed by the brand names are cyclizine hydrochloride (Marezine), meclizine hydrochloride (Bonine), dimenhydrinate (Dramamine) and diphenhydramine hydrochloride (Benadryl). While diphenhydramine HCl is not marketed for motion sickness, the FDA has approved it to treat the symptoms. These four drugs are the only ones approved by the FDA for over-the-counter use, however many more drugs are marketed for the treatment of motion sickness as is evident in the following table.
Fig. 1. Drugs and combination of drugs ranked according to their effectiveness.
A popular over-the-counter medication is meclizine hydrochloride also called "gravel" in Canada and prescribed for almost everything from nausea to diarrhea. Meclizine HCl is a histamine-receptor blocker that presumably prevents motion sickness by blocking the muscarinic receptors in the Central Nervous System. These drugs are commonly referred to as antihistamines some common anti histamines are pictured in the figure below\(^3\) and on the following page.

The most commonly used over the counter medication is dimenhydrinate hydrochloride. Dimenhydrinate HCl is safe to use on animals. Many people use this drug to calm their pets on long car rides. This is the foremost drug in the minds of Americans when they think of motion sickness cures. Dimenhydrinate HCl reduces the optokinetic nystagmus, making the following ability of the eye less accurate it also interferes with the ability to fixate adequately on a visual task during motion. Dimenhydrinate HCl has been found effective in many interesting trials. An example of one of these was a trial at sea including 140 subjects in a double blind experiment. The percent of the control group to get motion sickness was found to be 57.69%, the placebo group was 43.47%. With the use of dimenhydrinate HCl, the cases of sickness were about cut in half at 22.23% and with the use of scopolamine the percent was just 16.66%. Scopolamine is the next drug that I will cover.

**Synthetic Cholinergic Blocking Agents: Aminoalcohol Ethers**

![Chemical structures of benzphetamine (Cogentin®) and chlorphenoxamine (Phenergan®) and diphenhydramine (Benadryl®) or pheneridine (Norflex®)](image-url)
The most common prescription medication used for the treatment of motion sickness symptoms is scopolamine (Trans-Derm Scop patches). Scopolamine is an anticholinergic, which is delivered via a cutaneous patch consisting of a drug reservoir that contains 1.5 mg of scopolamine, mineral oil, and polyisobutylene in between a polyester film and an adhesive layer. The patch is applied to an area of intact skin behind the ear and delivers a continuous dose of scopolamine to the systemic circulation for 3 days. Scopolamine prevents motion-induced nausea by inhibiting vestibular input to the central nervous system (CNS), resulting in inhibition of the vomiting reflex. It may also have a direct action on the vomiting center. Scopolamine has had promising results in lab tests, however these tests were performed on lab rats and mice, not on human beings. In a two-year trial with rats and mice dosages of scopolamine ranging from 100mg/kg to 1600 mg/kg, were ingested by the animals without any carcinogenic activity found. One adverse effect was that the rats and mice had lower mean body weights and lower weight gain across the board. Another side effect was that several of the rats died from esophageal obstruction resulting from food build up. This problem was considered
secondary to the inhibitory effects of scopolamine on salivary gland secretion and on esopon esophageal smooth muscle involved in swallowing. Basically it was due to the fact that scopolamine causes severe dry mouth in some cases and thusly can effect the muscles of the throat. One unnerving effect was that in female rats with doses at 1200mg/kg and higher the estrous cycle was significantly increased scientists conducting the experiment did not have an explanation as to why this would occur.

The chemical scopolamine has a short action time in the body, this is the reason it is dosed in time release patches rather than ingested orally. Interestingly, the process used to make scopolamine is a rather long and involved one. Below is the chemical synthesis of the molecule scopolamine. This process is beginning with 3-alpha-acetoxytrop-6-ene, the process involved to synthesis that molecule is even more involved and requires more precise reaction conditions.

**Figure 4. Synthesis of (+)-scopolamine from 3α-acetoxytrop-6-ene.**

```
3-alpha-acetoxytrop-6-ene

H₂C₆N

OAc

CF₃CO₂H or Formic acid 80% HOOH → H₃C₆N

O acetylsopine

H₂C₆N

OAc

Kunz Hydrolysis

1) HCl

2) nitrobenzene, 85°C

H₂C₆N

OAc

Purify by partition chromatography on cellulose with butanol-N-NHCl

OH

Acid Hydrolysis

H₂C₆N

OH

Purify by partition chromatography on cellulose with butanol-N-NHCl

(+)-scopolamine
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**VI. Treatments-Herbal**

Some people are partial to herbal remedies. Usually because they hear of negative effects of drugs like the tests above for scopolamine. However, extreme caution must be exercised when using herbal medications because they have not been tested by a government-regulated agency. Simply because there is no information that a drug is harmful does not mean that it is safe. A common misconception is that if something occurs is nature that it is not harmful to the human body. It is certain that these same people who will state that opinion would not eat wild mushrooms off of a forest floor because they know that the natural chemicals contained in some of these “natural” sources are fatal.

The main herbal remedy associated with motion sickness is Ginger. As of today there are no known tests sanctioned by the FDA that have shown Ginger to be
effective as a motion sickness suppressant. The mechanism of its action is unknown, but many people believe in its ability to repress nausea wholeheartedly.

A recent study performed in Taiwan was reported to show that when test subjects were submitted to circularvection and eye stimulation to induce motion sickness (consisting of spinning on a platform and with a drum over the head painted with black and white stripes inside) Ginger was indeed effective. The test was performed on 78 people. Two dose sizes were given 1000mg and 2000mg as well as placebo. The tests were run every week at the same time of day. The testers concluded that ginger both slightly increased the time that it took to become ill and decreased the intensity of the nausea when it did occur. The time it took to become ill increased from 9.34 minutes to 10.46 and the intensity when rated on a scale from one to ten went from 8.2 to 7.1 respectively.

For an even safer alternative to drugs experts suggest controlled breathing and music audiotape. In that study subjects were made to what they rated as mildly nauseous and then asked to implement these techniques. The testers concluded that the breathing and music did give significant protection against motion sickness although only about half as effective as drugs ie: scopolamine and meclizine, but they had absolutely no adverse side effects.

VII. Conclusion

Motion sickness is a contradiction of signals sent from various parts of the body to the brain. Even though motion sickness is not well understood, there are a number of agreed upon methods for preventing this affliction as well as treatment methods however, the patient has to decide what works best for them whether it’s preventative, pharmaceutical, herbal or natural. There is no cure that is the best choice for everyone. There is still very little hard evidence as to what actually causes motion sickness and until scientist find out there really can be no cure, only the treatment as symptoms emerge.
References


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Carisoprodol (Soma)

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Abstract

This report discusses carisoprodol (Soma), a nonscheduled skeletal muscle relaxant that is congener of meprobamate, a schedule IV controlled substance. A background and normal dosage of carisoprodol is discussed. The many ways to obtain carisoprodol are included. This paper also explains why carisoprodol is a potential prescription drug of abuse.
I. Introduction

Carisoprodol is a prescription skeletal muscle relaxant drug. It is a dicarbamate usually prescribed for muscle spasms.¹ Carisoprodol metabolizes into three primary metabolites called hydroxymeprobamate, hydroxycarisoprodol and meprobamate.²³ Carisoprodol is found to convert to meprobamate by N-dealkylation.⁴ Meprobamate is an antianxiety carbamate ester introduced in 1955.³ Meprobamate is a schedule IV prescription drug that is controlled.³ It is a controlled substance due to its side effects including physical dependence.³ Because of the metabolite meprobamate, carisoprodol does have abuse potential. Physicians need to be careful when prescribing carisoprodol especially for long term use.³ The FDA Drug Abuse Advisory Committee proposed in February 1997 that carisoprodol addiction studies be performed, recognizing its abuse potential.⁶

Carisoprodol was approved in 1959 by the Food and Drug Administration.⁶ The chemical name for carisoprodol is N-isopropyl-2-methyl-2-propyl-1, 3-propanediol dicarbamate.⁷⁸ In tablet form, carisoprodol also contains the inactive ingredients magnesium stearate, povidone, lactose monohydrate, microcrystalline cellulose, sodium starch glycolate and pregelatinized starch.⁷ In non-tablet form, carisoprodol is a crystalline, white powder that has a bitter taste and a characteristic smell.⁷ The molecular weight is 260.33 and the molecular formula is C₁₂H₂₄N₂O₄.⁷ Carisoprodol is very soluble in acetone, chloroform and alcohol, but is only a little soluble in water.⁷ The structural formula is shown in the following diagram (Figure 1⁷).

Figure 1 Structural Formula of Carisoprodol

\[
\text{CH}_2\text{CH}_2\text{CH}_3
\]
\[
\text{H}_2\text{NCOOCCH}_2\text{CCH}_2\text{OOCNHCH(CH}_3)_2
\]
\[
\text{CH}_3
\]

SOURCE: Package insert from generic carisoprodol distributed by Geneva Pharmaceuticals

The National Institute on Drug Abuse rated carisoprodol number 54 out of 234 abused drugs in the year 1987.⁵ Brand names of the drug carisoprodol include Sodol®, Rela®, and the commonly prescribed Soma®.⁹ A Soma® Compound is also made. The compound contains 200-mg carisoprodol with 325-mg aspirin. Aspirin is broken down by hydrolysis into acetate and salicylic acid.⁸ Normal adult dosage for carisoprodol 350
mg is one tablet three to four times a day with the final dose taken at bedtime. Carisoprodol begins taking effect after thirty minutes and lasts between four to six hours. The half-life is approximately eight hours.

Carisoprodol does not have a direct effect on skeletal muscle. The mechanism of carisoprodol is believed to be caused by sedation, but is still not definitive. Carisoprodol is believed to inhibit interneuronal transmissions in the spinal cord and descending reticular formation. Interneuronal activity blocking is the reason found for muscle relaxation in animals when carisoprodol is used. Some side effects that may occur from a normal adult dosage include weakness, drowsiness, vertigo, headache, irritability, dizziness, impairment of coordination and alertness, and clumsiness.

The prescription drug carisoprodol is relatively easy to obtain, partially because it is available through veterinary mail-order catalogs. Another reason carisoprodol is easy to obtain is because physicians are often unaware of the abuse potential, or that meprobamate, its primary metabolite, is a controlled substance. Abusers of carisoprodol may resort to seeing multiple doctors or forge prescriptions to obtain high quantities. Prior to the 1990’s, carisoprodol had not been recognized as a drug of possible abuse. Physicians are also not aware that longer term use increases the likelihood that the patient will abuse carisoprodol. However, some states are acknowledging that carisoprodol does have potential abuse. Starting January 1, 1998, Alabama made carisoprodol a schedule IV.

II. Background

Carisoprodol is a skeletal muscle relaxant that is not a controlled substance. The primary metabolite of carisoprodol is a schedule IV drug, known for its abuse potential. There are recorded studies of abuse of carisoprodol, yet the first state to make carisoprodol a schedule IV drug is Alabama in 1998. Carisoprodol has been readily available since 1959. It continues to be readily available in present time because of lack of knowledge from the physician for potential abuse, lack of knowledge from the physician that carisoprodol metabolizes into a controlled substance, veterinary mail-order suppliers not needing a prescription, and patients that have multiple doctors and/or forge prescriptions.

All information for this report was obtained from professional journals and periodicals, and medical professional reference guides, including a package insert. Professional articles were obtained from Arizona State University’s Noble Science, Hayden Main, and Law Libraries. The University’s databases were accessed on campus, in the Noble Science Library. A broad search of many databases was conducted by simply typing in “carisoprodol.” Many articles were not available and had no call number to locate. Call numbers that were located required pulling the hard copy, bound in a book, off the shelf and a photocopy being made. Other articles were found using Paradise Valley Community College’s database access during a class time provided by the instructor.

III. Body
Meprobamate is a controlled substance and is the primary metabolite of carisoprodol. This would not be an issue if more physicians were aware of the potential abuse of carisoprodol like its congener meprobamate. Documented cases of suspected drug abusers who were analyzed for centrally acting drugs, when stopped for driving under the influence were reviewed by Logan et al, from the Washington State Toxicology Laboratory, Bureau of Forensic Laboratory Services in Seattle, Washington. Blood samples were analyzed at the Washington State Toxicology Laboratory by means of gas chromatography and EMIT or immunochemical analysis by enzyme immunoassay. According to Logan et al, a UV analysis is not adequate for testing because carisoprodol lacks chromophores, or multiple bonds. Drivers who were stopped that only had carisoprodol and meprobamate in their systems had problems with balance, slurred speech, swaying, nodding off, unsteadiness, and appeared dazed. Carisoprodol is relatively easy to obtain for several reasons.

Prescriptions for carisoprodol are easier to obtain than narcotics from doctors, often because the doctor is unaware of the abuse potential compared to the narcotics that they are well aware of. Carisoprodol is also easier to purchase because the drug can be obtained without a prescription through a veterinary mail-order. Carisoprodol is also less expensive than most narcotics and is sometimes used as a replacement for “harder” drugs or a supplement. According to Nancy Elder, M.D. from University of Missouri-Columbia School of Medicine, carisoprodol is often abused alone or in combination with alcohol or narcotics. It is common for physicians to be unaware of the abuse potential of carisoprodol.

Most physicians are aware of the abuse of many prescription drugs including narcotics. Unfortunately, skeletal muscle relaxants are not highly recognized as a potential drug of abuse. According to Roy Reeves, Chief of Psychiatry, G.V. (Sonny) Montgomery VA Medical Center, and Professor of Psychiatry and Neurology at University of Mississippi School of Medicine, et al., patients using the medication for a longer period or ones that have history of abusing other substances are more likely to have a substance abuse problem with carisoprodol. According to Littrell, Pharm D of Chandler Medical Center and College of Pharmacy and University of Kentucky, et al, chronic use of carisoprodol can lead to dependence of its metabolite, meprobamate, which can have severe withdrawal symptoms including coma and seizures. Over usage should be taken seriously, as coma and respiratory depression could occur.

Roy Reeves, et al, conducted a survey to question how many physicians who prescribe carisoprodol are aware of its abuse potential and that its metabolite is already a controlled substance. The survey was conducted by listing several controlled substances and a few that are not, including carisoprodol, and asking which drugs were either controlled substances or their metabolites are. Results from the survey showed that nearly all physicians surveyed or 95% were aware that meprobamate, the metabolite of carisoprodol, is a controlled substance. More results were that only 59% of physicians surveyed were aware that carisoprodol has abuse potential and a shocking 18% knew that carisoprodol metabolizes into a controlled substance. Caution needs to be practiced by physicians when prescribing carisoprodol.

Roy Reeves, et al., Nancy Elder, et al, S Sikdar, from Drug De-addiction and Treatment Centre, Department of Psychiatry, Postgraduate Institute of Medical Education & Research in Chandigarh, India, and Littrell, et al, all agree that physicians need to use
caution when prescribing carisoprodol to patients because of its potential for substance abuse.2,3,11,13,14 Davis and Alexander M.D., Jefferson County Coroner/ Medical Examiner’s Office in Birmingham, Alabama agree, “It is clear that some individuals do abuse carisoprodol.”4 Davis and Alexander make reference to a study performed by Olsen that tested the elimination of carisoprodol from the human body.4 His study shows that there are some people who metabolize carisoprodol slower than others. This causes a slower production of meprobamate in these individuals, leading to a higher concentration of carisoprodol in their bloodstream.10

IV. Conclusion

There is documented proof that carisoprodol metabolizes into a controlled substance called meprobamate. According to recent surveys, many physicians are unaware of this occurrence or that carisoprodol can be potentially abused, particularly because of how the medication relieves muscle pain by sedation. The FDA has recognized the need to review the substance for possible abuse. Several states have recognized this problem and have taken the initiative to make the substance a controlled substance at their individual state levels, to be able to regulate the dispensing of the substance. Carisoprodol should be made a controlled substance. Reasons to change carisoprodol into a controlled substance include its abuse potential, and its primary metabolite is already a controlled substance.

Carisoprodol is easy to obtain generally speaking, and compared to other abused substances. This is attributed to carisoprodol costing less, and being available through a mail-order veterinary catalog without a prescription from a medical doctor. Prescription forgery and patients who visit multiple doctors are more ways the drug can be obtained. Often, physicians are more willing to prescribe carisoprodol for several reasons. One reason is that carisoprodol is not controlled like narcotics. Another is that many physicians who readily prescribe carisoprodol to patients are unaware that carisoprodol has abuse potential. An even smaller percentage of physicians are aware that carisoprodol metabolizes into a controlled substance that is known to be a drug of dependence. There is enough evidence to support that the drug has abuse potential.
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Beta Catenin

Brooke Marshall

Mouse cerebral cortex expressing Beta Catenin
Abstract

Beta Catenin is a protein that induces brain folding. Brain folding refers to the crests and valleys that are on a human's cerebral cortex. This folding creates a surface area that is over a thousand times larger than the same mass without the folding. The possibilities of Beta Catenin are endless.
The cerebral cortex is the outermost one-eighth inch layer of the brain that is associated with higher order thoughts (1). This area of the brain is responsible for human’s abilities to read, speak, learn, remember and create along with the ability to problem solve. The intellectual capacity of the higher mammals is due to the increases in the cerebral cortex size through evolution (2). From primates to humans the cerebral cortex has not gotten any thicker, but the surface area has increased exponentially due to folding, wrinkling and creasing (1). The increased size of the cerebral cortex comes from a disproportionate expansion of the surface area. This layer is solely made up of neurons that are layered on top of each other. The skull size has also remained nearly unchanged since the primates so the cerebral cortex was forced to fold into crests and grooves, respectively called gyri and sulci (2). Martin Raff from University College London said “increased production of Beta Catenin was involved in increasing brain size during evolution” (1). Beta Catenin could be the link that gave humans the intellectual evolutionary leap.

The gyri and sulci of the cerebral cortex gives the human brain one thousand times the surface area compared to the same sized brain without the sulci and gyri. For example; the human cerebral cortex is only twice as thick as a mouse’s, but has over twelve hundred times the surface area because of the wrinkling (1). A protein called Beta Catenin has been found to directly affect the brains cerebral cortex size (1). Anjen Chenn and Christopher Walsh of Bringham and Women’s Hospital along with Beth Israel Deaconess Medical Center in Boston found that this protein’s interaction is credited with increasing brain size (1).

Beta Catenin is a protein in the Armadillo repeat family. This protein is alpha-alpha super helix with a molecular weight of 3,467 amu. The molecular formula of Beta Catenin is 6(C6H11N1O2Se). There are also thirty-two residues of Beta Catenin that are known. This is a multiply bonded system with an average bond length of 1.33Å (3). Beta Catenin is too large to draw out, but the basic appearance is in the picture below.
Once the different hypotheses of why the brain gets larger were examined, Beta Catennin was found to be the key. Beta Catennin affects the cerebral cortex by influencing the cell numbers in the brain and the cell fates (2). Specifically, Beta Catennin is involved in signaling and promoting cell division. When Beta Catennin is active the cells divide continuously. Usually when Beta Catennin has completed it’s function in promoting cell divisions the protein will get deactivated, break down and cause the cells to stop dividing (4). In normal circumstances, Beta Catennin breaks down quickly once enzymes attach phosphate groups to the amino end of the protein (5).

The experiment that led to the discovery of how Beta Catennin affects brain size was performed with transgenic mice that carried a human engineered form of Beta Catennin. Researchers connected the gene carrying Beta Catennin to a promoter that is activated in developing cells in the nervous system. This allowed the engineered Beta Catennin to be expressed in the mice. The resulting embryos had dramatically larger brains along with key changes in the cerebral cortex area. The cerebral cortex had normal thickness but had massive increases in the surface area (1). The horizontal growth was so extreme that it resembled the gyri and sulci of the higher mammals. Christopher Walsh, one of the original researchers, stated, “It looked as if these wrinkles don’t require any special genetic tricks. It seems to be a passive response to having a brain that’s bigger than your head” (5).

The horizontal expansion of the cerebral cortex was the result of increased numbers of proliferating precursor cells (2). The embryos also had an abnormally large amount of neural precursor cells (1). The enlargement in the numbers of precursor cells could be the result of three different things: increased mitotic rates, decreased cell deaths or fate choices (differentiate or proliferate). The mice study also proved that mitosis did not happen at a faster rate because of Beta Catennin. Cell deaths or apoptosis (programmed cell deaths) did not slow either. Beta Catennin did however play a roll in the progenitor division that expanded the progenitor pool exponentially. There was a two-fold increase in proportion of precursor cells that re-entered the cell cycle. This
proves that Beta Catenin influences cells to reenter the cell cycle rather than to differentiate (2).

Neural precursor cells can differentiate into several different types of brain cells. Beta Catenin increases neural precursor cells by making cells that would normally differentiate to keep dividing instead. This in turn makes a larger cortical sheet that folds and creases due to its larger size. It is not yet completely determined on how Beta Catenin affects neural precursor cells but there are two possibilities. The first is that Beta Catenin may interact with proteins called Wnts. Wnts influence the multiplication of neural precursor cells. The second idea, which Walsh and Chenn believe is that Beta Catenin works with adherens junctions where Beta Catenin has been found in larger numbers than normal (1). Beta Catenin is a component of adherens junctions that interact with the proteins of the TCF/LEF (T cell factor/lymphoid enhancer binding factor) family. TCF/LEF is a transducer of Wnt signals. Wnt is a family of secreting, signaling molecules that regulate cell growth and fate (2). TCF/LEF are expressed in overlapping patterns during developing mammalian brains (2). Adherens junctions are located on the cell surface and they play an important role in determining when daughter cells stop dividing and begin to differentiate. Basically, Beta Catenin functions in the decision of precursor cells to proliferate or differentiate during mammalian neuronal development. This suggests that Beta Catenin can regulate cerebral cortex size by controlling the generation of neural precursor cells (1). Beta Catenin was also found to not disrupt the normal developmental sequence of neuronal differentiation once they left the cell cycle (2).

The mice that were used in the first set of experiments had brains that were two to three times the size of an average mouse brain. Walsh stated, “The first mice in our experiments grew heads so large they could not survive after birth. We’ve done more recent tests in which the over activity of the gene was toned down” (6). Since these mice were not able to survive past birth, researchers were forced to look towards another strain of Beta Catenin that would not affect the brain so dramatically. Recently a strain of Beta Catenin was developed that increased the size of the mouse brain up to 40%. This new strain allowed the mice to survive past birth. Oddly enough these mice also exhibited more aggression than normal (5). Could it be our species “intelligence” that enables humans to be the ultimate predator at the top of the food chain?

Beta Catenin has also been found to help a developing embryo determine it’s front from it’s back (1). When an egg is fertilized, Beta Catenin will accumulate on the opposite side of where the sperm penetrated the egg. This accumulation happens before the first cell cycle is over. The protein will only be found in the cytoplasm on one side of the embryo. By the sixteen to thirty-two cell cycle, Beta Catenin will also be found inside of the nuclei of the cells on the same side. This heavily saturated area of the embryo will become the dorsal side of the developing embryo. The entire process of dorsal identification with Beta Catenin happens within one to two hours after fertilization. It is said that Beta Catenin has a dorsal-ventral polarity in early embryos (7). Beta Catenin allows the formation of neural tissue and the notochord in the dorsal
region (8). Beta Catenin will remain active until glycogen synthase kinase-3 phosphorylates the protein at the amino end (7).

Beta Catenin could help some mentally retarded people increase their intellectual capacity. This is also proved by the fact that mentally retarded people were found to have a much smoother brain than people of normal intelligence (4). If we could force the wrinkling of mentally retarded people’s brains, theoretically we could make them of normal intelligence. This protein could have the ability to end mental retardation due to underdeveloped cerebral cortexes. With the help of Beta Catenin we could also produce our own species of super humans. People of such extreme intelligence that they would surpass the geniuses of our day. These super humans would be able to utilize so much more of what every human today has already. For example; it has been said that the human eye can see in other wavelengths of light beside the small color spectrum we see right now. It is not our eyes that are underdeveloped, it is our brains that are unable to process and interpret the information. Imagine being able to see in ultraviolet, x-rays or infrared light wavelengths. Now imagine how different everything would look with these added capabilities. Our brains currently are unable to process this information, but if we speed up evolution through the use of Beta Catenin, this could be a reality. Walsh even stated that Beta Catenin “may be important for the major transition between rodents and primates: may be involved in changes between chimps and humans” (6).

Another possibility that could soon become a reality is the idea of other animals capable of a human level of intelligence. Humans and other animals could “speak the same language” and communicate in ways never even imagined today. If increases in human’s cerebral cortex size gave humans the evolutionary advantage, we could see the rest of the animal kingdom catch up. The big question to be asked is if we want to give up our position at the top of the food chain?

The possibilities of Beta Catenin seem endless. This protein could do things that were never imagined of before. Beta Catenin was isolated this past summer and in July of 2002, the first experiment dealing with brain folding was completed. No one imagined the effect that it would have on the mouse brain. There are many other studies that are currently underway, but the results are not in yet. This is an exciting field to be in and I can guarantee that in the next few years, amazing things will be discovered in relation to Beta Catenin’s interaction in the brain. If Beta Catenin can increase intelligence then…. Harvard here I come!!!!!
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Botulinum Toxin: Beyond Cosmesis

It’s Promise as a Therapeutic Alternative

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Abstract

Botulinum Toxin type-A has virtually revolutionized the minimally invasive approach to cosmetic enhancement of the upper face. Despite its rather "frivolous" reputation at the moment, where members of the public view Botulinum toxin as the "anti-wrinkling" drug, it will become clear that this toxin has far more uses than as a beauty enhancer. Botulinum toxin, administered in small amounts, has shown to have therapeutic advantages that are proving to be very promising for those suffering from any of a number of neurological disorders that lead to uncontrollable muscle spasm and contraction, possibly providing temporal relief with limited contraindications for millions of sufferers.

Introduction

More than 1.6 million Botox® procedures were done in 2001, nearly 46% more than the year before, making it the nation's most popular non-surgical cosmetic procedure, according to the American Society for Aesthetic Plastic Surgery -- that was before a television advertising campaign. And in 2002, Botox® injection continued to rank first among all cosmetic procedures, increasing a modest 4% from the previous year but more than 2400% since 1997. It has now been estimated that last years revenues for the botulinum toxin market was worth $335.6 million, and are projected to reach $1,430.2 million by 2009.

Botox® or Myobloc® (botulinum toxin type-A and type-B; respectively) injection procedures are utilized for much more than smoothing out a few wrinkles. Since 1989, botulinum toxin therapy, pioneered and incorporated as Oculinum, Inc. by Dr. Alan Scott in the late 1970s, received FDA approval to market Oculinum in the United States as an orphan drug to treat strabismus and blepharospasm associated with dystonia, including denig essential blepharospasm or VII nerve disorders in patients 12 years of age and older. Shortly thereafter, Oculinum was acquired by Allergan, Inc. in order to market and develop more applications for the neurotoxin and eventually renamed it as Botox®. But what IS Botox®? Or better yet what is botulinum toxin.

Botox® is one trade name for botulinum toxin type-A. (Figure 1) In this way, Botox® is related to botulism. Botulism is a form of food poisoning that occurs when someone eats something containing a neurotoxin produced by the bacterium Clostridium botulinum. There are eight different strains of Clostridium botulinum that produce toxins, labeled A through G(A,B,C1,C2,D,E,F,G),of which type A is best understood due to its strongest toxicity and only serotypes A and B have been approved for clinical use to date. The other serotypes of botulinum toxin have been effective in producing some therapeutic benefit; however, duration of action (botulinum toxin type-F) and lower potencies may make these less attractive alternatives than botulinum type-A.
The mechanism of action (Figure 2) of botulinum toxin is a weakening or paralysis (depending on dose) of muscles by preventing the release of acetylcholine from the nerve axons, a neurotransmitter that triggers muscle contraction. Essentially, the botulinum toxins block the signals that would normally tell your muscles to contract. Say, for example, it attacks the muscles in your chest — this could have a profound impact on your breathing. When people die from botulism, this is often the cause — the respiratory muscles are paralyzed so it is impossible to breathe. In fact, it is one of the most poisonous substances known to man, with just a few nanograms of the poison being capable of killing a man. This is why, with current events being what they are, botulinum toxin’s status as a possible neural agent for a WMD (Weapons of Mass Destruction) has come to the forefront of our common psyche.

**Figure 2: Mechanism of Action of Botulinum Toxin**
Release of acetylcholine at the neuromuscular junction is mediated by the assembly of a synaptic fusion complex that allows the membrane of the synaptic vesicle containing acetylcholine to fuse with the neuronal cell membrane. The light chain of botulinum toxin cleaves specific sites on the SNARE proteins, preventing complete assembly of the synaptic fusion complex and thereby blocking acetylcholine release.

**Historical Perspective**
Botulinum toxin in its purified form was developed, not for its potential as a therapeutic drug, but as a biological agent of destruction - i.e. germ warfare. After its molecular structure was determined, techniques were developed to produce the toxin in quantity. Of course, it would not be fair to say that all the research done was for nefarious reasons, but a lot of the research was undertaken under the auspices of the military, not only to produce the toxin (Figure 3), but...
also to develop interventions in the event of exposure to it. Today, a number of States named by the U.S. State Department as "state sponsors of terrorism" have developed or are developing botulinum toxin as a biological weapon. The Japanese cult Aum Shinrikyo tried but failed to use botulinum toxin as a biological weapon. If botulinum toxin poisoning is caught in the early stages, injection of an antitoxin made from horse serum can lessen the severity of disease by neutralizing the toxin that has not yet bound to nerve endings. But because of the risk of serious side effects such as anaphylaxis, a life-threatening allergic reaction, and serum sickness (an unpredictable allergic reaction to the horse serum, which can lead to anaphylaxis), the equine antitoxin cannot always be used, and it is never given to infants. Treatment of botulism is currently only preventive: An experimental vaccine is available, but no drug has been developed yet. Therapeutic treatment could be effective at any one of the three stages of toxicity - binding of toxin, internalization, or catalytic activity. But for a creation with such sinister and deadly underpinnings, it is amazing how much 'buzz' botulinum toxin's future is generating.

Current Indications

Botulinum toxin, administered in small amounts, has shown to have therapeutic advantages that are proving to be very promising. In patients suffering from any of a number of neurological disorders that lead to uncontrollable muscle spasm and contraction, the botulinum neurotoxin can provide relief for anywhere from 6 weeks up to many months. Over time, it appears that neurons "resprout" (Figure 4) from the paralyzed nerve ending terminals, providing new avenues of communication with the muscle. After the neurons resprout, the muscle returns to its previously abnormal state of constant spasm. Because of the neurons' ability to "resprout" and re-establish communication between the nerve and muscle, Botox® therapy is only a short-term fix. It needs to be re-administered regularly.

Botulinum toxin currently has FDA approval on a number of therapies and the industry is seeking to expand FDA approval even further. Besides its cosmetic appeal for its use on glabellar lines with Botox® COSMETIC, Botox® botulinum toxin type-A is FDA approved for the treatment of blepharospasm and strabismus, and botulinum toxin type-B has been approved for treating cervical dystonia. After repeated use of high doses, antibodies can develop in some individuals, making further treatment ineffective indefinitely. Because of Myobloc's® unique mechanism of action and antigenicity, Myobloc may be effective in patients with cervical dystonia who have developed antibodies to or who have not responded to Botox®.

Strabismus - is a misalignment of the eyes; the eyes appear to be looking in different directions. There are several different types of strabismus, which is usually treated surgically. Botulinum toxin can be an effective alternative to surgery for many patients, although studies comparing Botox® therapy and surgery have not been done.
Blepharospasm - is the involuntary forceful closure of the eyelids. Usually the first symptom is uncontrollable blinking. Eventually the eyelids remain completely closed all the time, and even though the patient’s vision may be normal they are functionally blind. When accompanied by oromandibular dystonia, the condition is known as Meiges syndrome. Injections of botulinum toxin into muscles in the face and periorcular region (around the eye) have become the treatment of choice for patients with blepharospasm.19, 20

Cervical Dystonia or Spasmodic torticollis - also known simply as torticollis, is an asymmetric muscular spasm in the neck that results in forceful turning of the head to one side. Additionally, the head may be pulled forward or backward. It is the most common of the focal dystonias (a dystonia is a state of abnormal muscle tone; blepharospasm is another dystonia and, indeed, is the second most common focal dystonia). Torticollis can accompany other movement disorders and can be very painful. Botulinum toxin injections are the primary and most effective form of treatment for cervical dystonia. Injections are made directly into the affected neck muscles. A crucial element to successful botulinum toxin injections is that the appropriate muscles are injected.21

Alternative Indications

Alternatively, botulinum toxin type-A has met with non-FDA approved formulary requirements for the following indications.22

Anal Fissure - Many people who are otherwise healthy have an anal fissure problem. This means that the skin around the anus cracks and leaves the site open to infection. The usual treatment is an intervention that reduces the action of the anal sphincter, as spasms have been associated with anal fissure. Local injections of Botox® are considered as an alternative to surgery with a 70-80% success rate.23

Cramping and Spasms - The use of some form of the botulism toxin is being investigated for a wide range of muscular or spastic disorders. Cramps in the neck, shoulders, back, limbs, hands or feet are among them. Writer's cramp is an example of a condition that causes interference with normal living and work that may benefit from this type of regimen.24, 25

Excessive Sweating - The medical term for excessive sweating is hyperhidrosis. Botulinum type-A injections into the palmar surface of the hands can block the chemical transmitter that causes sweating.26, 27

Hemifacial spasm - Hemifacial spasm is the sudden, simultaneous contraction of the muscles on one side of the face. The spasm can subside immediately or persist for several seconds and can occur several times a day. It can be painful and embarrassing. Botulinum toxin is an alternative therapy to oral medication and surgery.

Oromandibular dystonia - Oromandibular dystonia involves continuous, bilateral (both sides) spasms of the face, jaw, neck, tongue, larynx, and in severe cases, the respiratory system. Botulinum toxin injections are most effective when the correct muscles are injected. If the jaw muscles are injected, the facial muscles should not be affected, but injections into the face muscles may affect expressions. About 70 percent of people with oromandibular experience some reduction of spasm and improvement of chewing and
speech after injection of botulinum toxin into the masseter, temporalis, and lateral pterygoid muscles.  

**Spasmodic dysphonia** - Spasmodic dysphonia is the sudden interruption of speech due to a spasm of the laryngeal muscles (the vocal cords). Botulinum toxin therapy has proven effective at ameliorating the symptoms and restoring speech fluency, although one kind of spasmodic dysphonia (abductor spasmodic dysphonia) poses an airway obstruction risk if certain muscles are injected. Local injections of botulinum toxin into the vocal cord muscles have proven to be the most effective treatment for spasmodic dysphonia. The treatment weakens the vocal muscles so that spasms are greatly diminished and speech is greatly improved. The treatment can also reduce the breathiness and help decrease the effort required to speak.  

**Investigated Indications**

Botulinum toxin can also be used to treat patients who have inappropriate muscle contractions or even contractures due to stroke or cerebral palsy, especially when combined with rehabilitation. While Botulinum toxin is approved to treat a number of medical conditions, its use is being investigated for an amazing array of new medical applications.

**Cerebral Palsy** - Botox® helps in the treatment of cerebral palsy when it is injected into spastic or dystonic muscles. This reduces muscle stiffness, allowing physical therapists to stretch muscles and stimulate normal growth. The benefits that accrue include an improved range of motion and an acceleration of locomotion activities such as crawling or walking in children affected by cerebral palsy. Note that the FDA has not yet approved Botox® in the treatment of cerebral palsy.

**Migraine Headaches** - While the treatment of migraine headaches with the botulinum toxin is not 100 percent perfected, large studies are underway to determine its effectiveness and to determine what dosages might work best. More than half of patients who suffer from migraine headaches appeared to experience relief from their pain when receiving Botox® injections for other conditions. In clinical tests, only about fifteen to twenty percent of patients reported no effect. Some patients, though, experienced relief for up to six months when Botox® was injected into muscles of the forehead, side of the head, back of the head near the neck, eye area, or brow area.  

On the other side of the coin, a recent study supervised by Dr. Murad Alam found that one percent of the 320 patients receiving Botox® injections reported the occurrence of "life-altering headaches." Dr. Alam also recommended additional clinical trials to further define the situation.

**TMJ** - Botox® has been used to treat TMJ (temporomandibular joint disorder) since 1998. Although the FDA or insurance formularies have not yet approved its use for the treatment of TMJ, the procedure shows great promise and is far more desirable than surgery.
Contraindications

Despite any alarm triggered by the word "botulism," the botulinum toxin is a safe and effective agent when used appropriately by physicians who are professionally trained to use it. The doses required are tiny amounts, which are very carefully injected into specific muscles. Even very small amounts of the toxin can, however, have undesirable side effects.

Side effects are minimal and typically relate to the local injection of Botox® COSMETIC. Soreness or mild bruising, while uncommon, may occur around the injection site. Makeup may be worn after treatment, but care should be taken to avoid pressing or massaging the area for several hours. A temporary headache is not uncommon after injections in the forehead area, especially after the first treatment. In rare instances, patients may develop temporary weakness of the neighboring muscles, a temporary droopy brow or eyelid. All of these possible effects are likely to be mild and temporary, and in most cases, do not significantly limit routine activities.\footnote{32}

The main side effect is weakness in the group of muscles that is being treated. Drooping of the eyelids (ptosis) for example, can develop when blepharospasm is treated. Muscular weakness is often short-lived though. Some patients develop flu-like symptoms, but this is relatively rare. The obvious adverse effects are due to injection of too high a dose or of diffusion of the toxin to unintended sites. This may take the form of excessive weakness in the injected muscles or of weakness in muscles adjacent to injected muscles, e.g. dysphagia or dysphonia after injection of neck muscles. Other side effects may be attributed to a serum sickness-like reaction due to the protein content in the toxin preparation. This may result in malaise, itching, flu-like symptoms or even chest infections and headaches. Pain at the injection site may well be expected. Rashies rarely occur. As a rule, the administration of Botulinum toxin is a safe procedure when given by a trained physician. Informed consent is taken by the administering physician prior to the procedure to ensure that the patient understands the potential side effects.\footnote{33}

Unfortunately, the long-term effects (greater than 5 years) of receiving regular injections are not known at this time. Nor has its safety been determined for pregnant women, women who are breast feeding or children and geriatrics who receive chronic therapy. Obvious relative contraindications to the administration of botulinum toxin type-A include pregnancy, as the potential teratogenicity of the drug remains unknown. Thus, the drug should not be given to pregnant mothers until the cessation of breastfeeding. Dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy. Contraindications to botulinum toxin use include a history of hypersensitivity to the toxin, human albumin, or saline and neuromuscular disorders, such as Eaton-Lambert syndrome and myasthenia gravis. Several medications potentiate the effects of botulinum toxin, such as aminoglycosides, penicillamine, quinine, and calcium channel blockers. Toxin injection should not be performed for these patients.
Conclusion

This same substance that is produced in spoiled food as an agent of death also carries significant therapeutic potential. This neurotoxin, properly administered by a trained physician, can offer hope to patients whose quality of life is diminished by such diseases, which is characterized by inappropriate contraction of muscles causing the adoption of oftentimes bizarre and disabling postures. While botulinum toxin type-A may not be without some risks it is proving to be the non-intrusive answer to millions suffering from neurological disorders.
Bibliography


Luminol – The Blood Detector

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Abstract

Blood has many interesting characteristics. This report will discuss its uses on crime scenes. Also, the earliest cases where blood tied a crime to its criminal and the use of the chemical, luminol, to discover hidden blood are both topics described in the paper. The chemical reaction of luminol and studies done that test luminol's usefulness are all addressed along with future prospects for the use of this chemical.
**Introduction**

Crime scene investigation has become a popular topic in current culture. From the Discovery Channel to CBS, everybody has a television show that revolves around the fascinating world of forensic science. Forensic science itself is a multi-faceted topic that ranges from fiber samples to hair samples and, most important to this paper, blood samples. There is an extensive history of blood and its relation to crime with so many cases that an entire encyclopedia could be written about them. Blood is also tied into chemistry, as most forensic topics are, by the chemicals, such as luminol, that can be used to discover its existence and its characteristics. Luminol itself has both good and bad qualities, all which will be described in this paper.

**Background**

Blood is a captivating topic for many people – Awe-inspiring for some, grotesque and frightening for others. Basically, everybody has blood (approximately ten pints to a body or about nine percent of the average body’s total weight) \(^1\). In blood there are different ingredients: plasma, platelets, and most important to the topic of luminol, hemoglobin. Hemoglobin is a protein in the blood that carries oxygen around the body \(^2\). Hemoglobin possesses peroxidase-like activity, which means there are “...enzymes that accelerate the oxidation of several... organic compounds by peroxides...” \(^3\). One such organic compound is luminol. The significance of this characteristic will be discussed later in the paper.

**Figure 1** Iron in Hemoglobin

![Haem group image](https://example.com/hemoglobin.png)

*Haem group*

The part of the haemoglobin molecule containing iron (the central green atom)

*Source: www.example.com*

People have known for a long time that blood, especially spilled blood, usually indicates that a crime has been committed. One case in 1721, had a man named William Shaw hanged because his daughter was found bleeding profusely. When asked if her
death was her father’s fault, she replied yes. Later, a suicide note was found stating she
was going to kill herself because of disagreements with her father. In truth, he did not
actually physically kill her. (1). In 1869, blood patterns were used to find Pierre Voirbo
guilty of murdering a man and trying to clean it up. Blood, however, seeps into things
such as cracks in tile and fibers in rugs and because of this Voirbo was caught (1).

Qualitative tests for blood began showing up in 1900. The English began using a
chemical called benzidine that would turn blue if blood was present. Because benzidine
was later identified as a definite carcinogen, the benzidine test was replaced by the
Kastle-Meyer test, which used phenolphthalein instead. The indicator color was now
pink but the accuracy of the test was still the same (1). In 1902, a German doctor named
Paul Uhlenhuth discovered a test that would distinguish between animal blood and
human blood. It was called the Bordet test (1). The Bordet test was very useful because
in many cases, bloodstains were excused because they were explained away as animal
bloodstains and there was no way to prove otherwise. Not so in the case of a French
murderer in 1902, who said the blood stains on his clothes came from skinning a rabbit
but the Bordet test proved the blood was human and he was sent to the guillotine (1). In
1939, the luminol test was first used to screen for blood and became one of the most
commonly used chemicals on crime scenes to detect blood stains that someone had meant
to keep hidden (5).

The Luminol Reaction
Luminol, also known as 5-Amino-2,3-dihydro-1,4-phthalazinedione in the
chemical world, has a molecular formula of C₅H₇N₄O₂ and a molecular weight of
177.162g/mol (6). Luminol’s percent composition is C 54.24%; H 3.98%; N 23.72%; O
18.06%, with a yellow crystalline physical state and a melting point of 329-332⁰C (6).
The actual reaction of luminol involves hydrogen peroxide, luminol itself, and the iron in
hemoglobin, which will in turn produce a blue glow if blood is in fact present (2).

Figure 2 Luminol At Work

In this picture, it is seen how something that looks clean in the light can show a very
different, and gruesome picture of truth once luminol and darkness are added. In this
same way, crime scene investigators locate hidden blood and can then identify where,
how, and possibly who committed the crime.
Figure 3 Incriminating Footprint

As seen in figure 3, evidence such as a footprint can be left behind at a crime scene. With modern technology, a forensic expert may be able to determine the type of shoe and possibly tie a suspect to the crime if a match is made. One problem with luminol and prints is that it is dilute which means that some smearing may occur making a match harder to find (7). In place of luminol to find actual bloody prints, substances such as merbromin and ortho-tolidine are used because they rapidly evaporate making smearing less likely to occur (7).

Figure 4 The Luminol Reaction

\[
\begin{align*}
\text{Luminol} & \rightarrow \text{Luminol (A)} \\
\end{align*}
\]

\[
\begin{align*}
\text{3-Aminophthalate}^* (3-APA^*) \\
\text{Source: www.chm.uri.edu}
\end{align*}
\]

In this reaction, the reactants have more energy than the products. This causes the molecules to give off visible light “photons” to shed the extra energy (2). The hydrogen peroxide oxidizes the luminol by stripping the protons (H) off of the nitrogen. The electrons then enter a triplet-excited state, which means that the electrons are spinning in the same direction. Then, within the system the electrons settle to a singlet-excited state with opposite spins and finally, in order to once again become stable, one photon of light is emitted causing luminescence (8).
Techniques at a Crime Scene

Luminol is not a definitive test, it simply confirms or dispels the presence of blood in an area. In order for the technique to be effective, crime scene investigators follow a step-by-step process to use a crime scene to its full potential. The first step is to determine if blood even exists at a scene. At times, bloodstains will be obvious – A dried but fresh stain usually is a reddish-brown color with a glossy look to it. This can change, however with different surfaces such as metal, wood, etc. (5). If, on the other hand, there has been an attempt to cover up a crime, blood may not be visible at all. That is where luminol, or sometimes phenolphthalein, is used. If these qualitative tests show that blood is in fact present, the next step is to determine if the blood is human (3).

The Bordet test is no longer used as technology has increased tremendously since 1901. Currently the Precipitin Test is used by “...layer(ing) an extract of blood stain on top of human antiserum in a capillary tube” (3). If human, a cloudy ring is formed where the two liquids meet. This test works based on the fact that a human antiserum is formed in animals when human blood in injected into their bodies. This antiserum is the same used in the capillary tube to confirm the presence of human blood (3). The final step taken by crime scene investigators is the DNA test which will give detectives some idea, if not complete confirmation, as to who the prime suspect (or even the victim) might be (3).

Pros and Cons of Luminol

Nothing in this world is perfect and luminol is definitely falls into that category. The first problem with the use of luminol is that the scene must be completely dark (5). This is not always possible which makes the phenolphthalein test and other options more appealing. Another problem is that different surfaces produce different reactions. In a study done for the Journal of Forensic Sciences, it was found that blue denim, fabric, and paper towels are difficult surfaces to detect blood on (9). Finally, and possibly most importantly, it has been found that bleach (which is used very often when criminals are trying to clean up stains) falsely causes luminol to glow even without the presence of blood (2). Obviously, there are a few conditions that need to be taken into consideration when using luminol.

On the positive side, many of the problems with luminol have simple remedies. For instance, as far as the different surfaces are concerned, a study was done for the Journal of Forensic Sciences that found that by using 1,2-diaminoethane along with the luminol mixture, the bleach/luminol reaction was inhibited therefore giving more accurate blood stain areas (10). This is possible because the amines in 1,2-diaminoethane react with the hypochlorite ions in bleach and block chemiluminescence from occurring. Because of this study, the use of 1,2-diaminoethane is now recommended for use with luminol in all instructions for the chemical (10). Also, as far as bleach interference goes, a simple way to prevent interaction is by simply waiting a few days for hypochlorite ions to evaporate off and then testing with the luminol/hydrogen peroxide solution (10). The study done that found difficulties testing on different surfaces also had good things to say about luminol and its sensitivity. Although testing on denim, fabric and paper towels is difficult, luminol was the most effective test against six competitors (9).
Even to a dilution of 1:200 (which is still enough blood for DNA testing to be possible), luminol proved to be the most sensitive and positive qualitative test for the presence of blood (9).

Predictions

We have come very far in our knowledge of trace evidence, blood work, DNA and all other things that can link a criminal to his/her crime. It would almost seem that there is not much left that can possibly be discovered – but I am not going to say that. I think that more and more molecules are being discovered, more and more chemicals are being given new uses and it seems to me that there is no possible way to know when we have learned all that there is to know. Luminol and luminescence in general are used for many things. Luminescence is found everywhere from lightning bugs to inside the body being used for medical purposes. I believe that luminol itself has a few problems that will cause it to eventually be replaced by another chemical. One that is not as dilute and will not cause smearing, one that is so strong it can maybe be seen in normal light, and one that determines, without a doubt, that the substance glowing is in fact blood and not bleach or some other reactive substance. But that maybe is not where the emphasis needs to be placed at all. I believe that more time should be spent trying to figure out how to prevent the crimes before they need to be investigated. If a little more dedication was put forth by the judicial system to try and figure out what laws need to be revised in order to decrease the crime rate then maybe they could beat the ever thriving chemist who is trying to find a new chemical with new properties to assist in helping on a crime scene that perhaps could have been prevented from even existing. Then, and only then, would luminol have officially met its match and no longer be able to discover the hidden secrets of a crime scene.

Conclusion

In conclusion, blood has been around since the beginning of time and almost as early, its tie to crime has been recognized. Criminals keep trying to outsmart justice but justice, somehow, just keeps getting smarter and more precise. With the use of luminol and other chemicals, hidden blood can be discovered and tested in order to tie a suspect to their crime. Luminol has its problems, but it also tests very well when put in competition with other blood identifiers. All in all, luminol is a very effective chemical and will continue to be used until a possibly less dilute and more precise alternative is found. Until then, bloodstain evidence will just keep on glowing.
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INHALED INSULIN: Exubera®
By: Gertrude Chan Ocubillo
April 2003
Abstract

The long awaited modern delivery of insulin is finally on the verge of Food and Drug Administration approval in its third phase of clinical trials. Aventis and Pfizer have collaborated to introduce Exubera® inhaled insulin for treatment of Type I and II diabetes. Exubera® promises improved glycemic control and freedom from discomfort of subcutaneous injections through delivery of insulin in an affable, noninvasive, pulmonary mechanism.

Introduction

The search for treatment of Diabetes Mellitus can be traced back before the early 1900s with Opium as the commonly used drug for the disease. In the 1920s, subcutaneous injections of animal insulin and more recently, human insulin, have been developed and used to treat Diabetes Mellitus; however, despite a few attempts to develop different formulations, intensive insulin therapy with multiple daily injections has not gained widespread clinical acceptance. Inhaled insulin may be an effective, well-tolerated, noninvasive alternative to subcutaneous regular insulin.

Pulmonary delivery takes advantage of the only highly permeable “port of entry” into the body for macromolecules, the alveoli. Most peptides and proteins cannot be given orally because they are broken down by the digestive system or are too large to pass through gastrointestinal tract into the bloodstream. The large sizes of these molecules also prohibit nasal and transdermal delivery without penetration enhancers, which could also be perilous. Pharmacologic doses of peptides and proteins are now known to pass easily from the alveoli into the bloodstream, without penetration enhancers. Currently in clinical development is a dry powder insulin formulation that is delivered by an aerosol inhaler.

Aventis and Pfizer have teamed up to develop this innovative insulin inhaler, namely Exubera®, for potential management of Diabetes Mellitus. Phase III studies are ongoing to assess long-term safety and efficacy of inhaled insulin therapy versus subcutaneous insulin therapy. Currently, the initial findings of these studies show increased effectiveness, lesser side effects, and patient preference of the new mechanism.

Challenge

Diabetes is more and more becoming an epidemic, causing the diabetes drug market to increase rapidly each year. Insulin is the leading type of drug for management of this disease. This is essential for Type I diabetics, who have inadequate production of insulin resulting from the autoimmune destruction of pancreatic β cells, and Type II diabetics, who have insulin resistance and impaired insulin secretion.

Neuropathy, nephropathy, retinopathy, and even death are results of uncontrolled blood glucose levels.
Chemical Properties

Equivalent recombinant human insulins HR1799/HMR4006 are used in manufacture of the insulin powder for inhalation.

Molecular Formula: \( C_{257}H_{383}N_{65}O_{77}S_6 \)
Molecular Weight: 5807.69
Number of Amino Acid Residues: 51

Amino Acid Sequence:

Isoelectric Point: Approximately 5.5

The dry powder dosage form for inhalation was developed as an alternative to multiple daily injections of regular human insulin, i.e. Humalog®. The dry powder dosage was chosen because of the excellent chemical and physical ability of dry powders during storage in room temperature. The insulin powder for inhalation is packaged into individual blisters with strengths of either 1 mg or 3 mg. The formulation is comprised of 60% insulin with compendial grade excipients including mannitol, glycine, and sodium citrate. The sodium citrate is comprised of sodium citrate dihydrate, with trace amounts of citric acids and sodium hydroxide for pH adjustment during compounding. Humalog® is synthesized in a special strain of genetically altered Escherichia coli bacteria consisting of glycerin, m-cresol, dibasic sodium phosphate, zinc oxide, trace amounts of phenol and water for injection.

Storage conditions for insulin powder for inhalation (blisters) are between 2°C and 30°C in foil overwrap and should not be used beyond 3 weeks when opened. The pulmonary inhaler and its components should be stored between 15°C and 30°C.
Clinical Trials

The feasibility of administering insulin by the aerosol route has been demonstrated in humans. About 10% to 30% of the insulin inhaled was absorbed into the circulation, and the aerosols appeared to be well-tolerated.

Compared to subcutaneous regular insulin, in subjects with Type I and Type II diabetes, inhaled insulin was absorbed more rapidly, which is consistent with observations in healthy subjects. The bioavailability of inhaled insulin relative to subcutaneous regular insulin is approximately 10%. The estimated dose equivalence of 1 mg of inhaled insulin is roughly equal to 2 to 3 units of subcutaneous regular insulin.

In a recent study, 26 patients having type 2 diabetes for an average of 11 years replaced the subcutaneous insulin regimen with insulin inhaled before each meal. They also injected ultralente insulin at bedtime, for maintenance through the night. Each milligram of inhaled insulin delivered the equivalent of 3 units of subcutaneous insulin. Hemoglobin A1c levels decreased from 8.67% at baseline to 7.96% after 12 weeks. While 69% of patients experienced mild to moderate hypoglycemia, none had severe hypoglycemia.

A phase III study of 335 Type I diabetics of ages 12 to 65 was formulated to evaluate if an insulin regimen that included Exubera® could provide glycemic control similar to 2-3 injections per day. Glycated hemoglobin (HbA1c), used to measure average blood glucose levels over an eight to 12 week period, decreased correspondingly with patients taking Exubera® with 1 injection and patients who received 2-3 injections per day. The recommended treatment goal of less than 7 percent of HbA1c was the same in both groups. Furthermore, greater decreases in fasting plasma glucose concentration and two-hour post-prandial glucose levels were shown with patients taking Exubera® in comparison to those who had insulin injections only. The overall report showed improvements in overall treatment satisfaction with fewer hypoglycemic events and more patient preference.

Dosage and Administration

For delivery of the dose, an individual blister is opened in place within the inhaler. The powder is then dispersed by the inhaler (with an air-assis: mechanism) into a discrete aerosol cloud, which is captured in a holding chamber. The volume of the holding chamber is a small fraction, <20%, of a deep breath. The patient inhales the aerosol bolus at the beginning of a deep breath and make-up air pushes the bolus deep into the lungs. The dose of insulin is controlled by the number of blisters that are, one at a time, dispersed and inhaled (1 blister = 1 inhalation).
The aerosol delivery system is designed to optimize alveolar deposition of insulin and minimize deposition in the mouth, throat, and upper airways. This is accomplished through the use of a holding chamber that encourages a slow deep inspiration of an aerosol cloud that contains particles that have the right size for alveolar deposition (mean particle diameters approximately 3\(\mu\)m). The insulin powder formulation dissolves in the surface fluid lining of the lung. The ciliated, mucous-covered epithelium of the airways is not thought to absorb insulin as efficiently as the nonmucous-covered, non-ciliated, alveolar epithelium.  

Inhaled insulin powder that deposits in the ciliated airways will be removed from lungs via the mucociliary escalator, which transports trapped material back out of the
lungs into the throat where it is swallowed and destroyed in the gastrointestinal tract. Unlike the epithelium of the gastrointestinal tract, nose, and airways, the alveolar epithelium is quite permeable to macromolecules.

**Safety**

The primary adverse effect of insulin administration regardless of route of delivery is hypoglycemia. Symptoms include sweating, dizziness, palpitation, tremor, hunger, restlessness, inability to concentrate, headache, drowsiness, blurred vision, slurred speech, depressive mood, irritability, abnormal behavior, unsteady movement, and personally changes. Severe hypoglycemia shows signs of disorientation, unconsciousness, and seizures.

Cough is a common respiratory adverse event with the use of inhaled insulin. Incidence of chest pain was more common in inhaled insulin than in subjects treated through subcutaneous insulin. No significant differences were observed in regard to peripheral edema, congestive heart failure, or myocardial infarction.

Increased percentage of detectable insulin antibodies were found in subjects switching to inhaled insulin during phase 2 and 3 of clinical trials than subjects who continued with the subcutaneous insulin regimen.

**Exubera® Advantage**

In addition to a noninvasive alternate to injection, fewer systemic side effects, and rapid drug absorption in the bloodstream, inhaled insulin administration offers more to patients with Diabetes Mellitus. The convenience and ease of delivery of insulin is the number one attraction among people suffering from the discomfort and pain of subcutaneous injections of insulin. Second is the ease of management. Third is the flexibility at mealtimes, and fourth is the ability of the body’s metabolite levels to return to the baseline sooner. Inhaled insulin works more like the natural functions of the body than injected insulin. Furthermore, greater control of blood sugar levels is achieved with the 3-4 dose administrations of rapid acting-insulin per day.

**Personal Opinion**

Diabetes Mellitus has affected millions including my family. My grandfather is one who is suffering from continuous painful injections, not to mention countless instruments to go with it. With the new inhaled insulin, he will not have to deal with the discomfort of subcutaneous injected insulin.

Very limited information was accessible for inhaled insulin because it is not out in the market yet. It is still under trials for its long-term effects before the Food and Drug Administration will approve and have it commercially available. However, given the
effectiveness and safety of the drug, it is only going to be a matter of time until its approval by the FDA and availability to the public. *Exubera®* has proven to be noninvasive and patient-friendly and will only get more popular with the users.

This is truly a “biotechnology revolution” that will give people suffering from Diabetes Mellitus, including my grandfather, a breath of fresh air.
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Topical Analgesics

Prepared for
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April 25, 2003
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Abstract

Topical analgesics are medications that can be rubbed into the skin to provide quick relief from pain. They come in a variety of creams, lotions, and sprays which are easy to apply and generally very safe. Although these products aren't likely to banish pain for good, they can provide temporary relief for an inflammation. There are several ingredients of topical analgesics, the three most common use capsaicin, salicylates, or a form of counterirritant.
I. Introduction

It is estimated that there are over 40 million people in the United States who suffer from various inflammation-related disorders such as arthritis, tendonitis, carpal tunnel and bursitis.\(^1\,4\) This number does not include individuals suffering from backache, strains and sprains; the millions of individuals who are subject to sports injuries- muscle cramps, soreness, or inflammations of the joints as a result of their active participation in sports or exercise-related activities; and in addition, those individuals affected by the inevitable consequences of day-to-day living and working. It is safe to say that almost everyone at sometime feels some sort of pain and seeks relief from their pain and inflammation. Typically the first attempt at relief consists of purchasing an analgesic product, either topical or oral. Is has been debated whether topical analgesic cream can realistically conceal the pain.\(^1\,2\) Not only the over the counter analgesic market, but also the prescription drug market for non-steroidal, anti-inflammatory drugs (NSAID's).

For many patients oral analgesics are not a viable option to contain their inflammation. The diverse group external analgesics are often times seen as the only valid option. They affect the epidermis only superficially, by creating a warmth, cooling, or irritation that counters or masks the pain originating from deeper tissues.\(^2\) There are three common ingredients that that may be used alone or as a combination in medications to relieve pain. The first is capsaicin, which is normally found in prescription strength medications.

Another ingredient that is very common among over the counter medications is salicylates. The last group known as counter irritants in mostly found in counterirritants

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Brand Name</th>
<th>OTC/ Prescription</th>
<th>Usual Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>Zostrix</td>
<td>Prescription</td>
<td>Four times per day for 3 – 4 weeks</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Capzasin-P</td>
<td>Prescription</td>
<td>Four times per day for 3 – 4 weeks</td>
</tr>
<tr>
<td>Salicylate</td>
<td>Aspercreme</td>
<td>OTC</td>
<td>As pain persists, not to exceed four times daily</td>
</tr>
<tr>
<td>Salicylate</td>
<td>Sportscreme</td>
<td>OTC</td>
<td>As pain persists, not to exceed four times daily</td>
</tr>
<tr>
<td>Menthol</td>
<td>Mineral Ice</td>
<td>OTC</td>
<td>As pain persists, not to exceed four times daily</td>
</tr>
<tr>
<td>Menthol/ Salicylate</td>
<td>ArthriCare</td>
<td>OTC</td>
<td>As pain persists, not to exceed four times daily</td>
</tr>
</tbody>
</table>

Figure 1\(^3\)
Figure 1- Shown is a sample of some available products on the market today. Many carry out the basic treatment of pain and applications are for the most part identical.

II. Classes

Capsaicin

Figure 2

IUPAC: Trans-8-Methyl-N-vanillyl-6-nonenamide

Capsaicin, as seen in figure 1, is a highly refined and concentrated form of the active ingredient of capsicum peppers. Capsaicin was dates back to 1492 when Christopher Columbus discovered its power after his arrival to the Americas. The native Americans were the first to harness the power of capsaicin. This native to North and South America was like nothing Columbus and his men had ever seen. The native Americans used capsaicin in many things, such as food, rituals, and even medicinal purposes. They managed to form a topical form of capsaicin when they mixed cayenne peppers together with a cold mud. The Indians had harnessed the power of the capsaicin and were able to use its power for good.

Capsaicin works by depleting the amount of a nero transmitter called substance-P, which is believed to be send messages of pain to the brain. When nociceptors (neurons that transmit information regarding tissue damage to pain-processing centers in the spinal cord and brain) come in to contact with capsaicin, the neuron becomes excited, and there becomes a perception of pain, and then a local release of inflammatory mediators. The nociceptors become excited by increasing permeability of the plasma membrane to cations, however, the molecular mechanism explaining this phenomenon is unclear. Capsaicin is being used in an analgesic agent in the treatment of severe pain disorders. It has been used in cases of severe pain because it can cause long-term loss of responsiveness because it kills off the nociceptor, or it destroys the peripheral terminals.

Ointments containing capsaicin are the most potent form and are used in cases of extreme inflammatory problems. They are commonly prescription strength medications, such as Axsain, Capsaicin-P, Zostrix, and Dolorac. Capsaicin ointments are currently being evaluated with a variety of conditions including HIV neuropathy pain, postherpetic neuralgia (shingles pain), CPRS (complex regional pain syndrome), diabetic neuropathy pain and some peripheral neuropathy. In a typical over the counter medication, the cream contains a maximum of 0.075 % capsaicin. Several research settings have reported using 7-10% creams and, and with it more success. The problem however, is that at the high percentage cream itself is very painful after it is applied. The
researchers are trying new techniques and using nerve blocks to make the treatment with the capsaicin cream tolerable. For less severe cases, it is recommended that creams containing capsaicin, (0.025% to 0.075%) be applied 3-4 times daily since a burning sensation may develop if it is used less frequently. The efficacy of topical preparations of capsaicin, therefore, depends more or less upon continuous use.

**Salicylates**

**Figure 3**

A salicylate (shown in Figure 3) is any of a group of analgesics, or painkilling drug that is derivative of salicylic acid; the most popular acetyl salicylic acid, is better known as aspirin. Today it is more often made synthetically; however, they were originally derived from salicin. The reaction mechanism used in the production of aspirin is shown below.

**Figure 4**

![Reaction mechanism of aspirin production](image)

Figure 4 - This is the reaction that is used to create aspirin

The basic ingredients in aspirin have been known about for over a thousand years. It is thought that as long ago as the fifth century B.C., Hippocrates, the father of modern medicine, is said to have used ground willow bark to ease aches and pains. Willow bark contains salicin. Salicylates also occur naturally in many plants that we use every day as a source of food (e.g., strawberries, almonds, tomatoes). By the late 1800s, salicylates had become the standard drug for the treatment of arthritis. Originally developed by German chemist Felix Hoffmann in 1897 as a treatment for his father's arthritis, however, the treatment was very harsh and irritating to the stomach. Hoffman, setting out to create a less-irritating medicine for his father, synthesized acetyl salicylic acid and aspirin was born.
Counterirritants

For many years, there was two ways to treat pain without the use of drugs, and that was through the use of heat, and or ice. Ice was added to decrease swelling, and heat was added to decrease muscle pain and stiffness. Counterirritants are creams that try to mimic this type of therapy. Most often ingredients such as such as menthol, oil of wintergreen, camphor, eucalyptus oil, and turpentine oil, are used to fool pain by creating of cold or heat. The role of counterirritants is to stimulate nerve endings on the skin by creating a feeling of hot or cold. This type of therapy is somewhat effective for victims of arthritis, but very effective for athletes.

Figure 5

Menthol

Chemical Formula: $\text{C}_{10}\text{H}_{20}\text{O}$

Figure 6

Figure 6- Sample Spectra from a topical analgesic preparation
IV. Conclusion

Pain is a concept that is different for every person, because each person can withstand different integrals of pain. For pain such as arthritis, I see a real future for this drug in the market of pain relievers. Arthritis is a very serious condition that affects millions of people everyday in the United States. Arthritis isn’t something that just happens to old people, it affects young people, old people, men, and women; it is very unbiased in the victims it posses. It is a disease that keeps people form everyday activities, that we who don’t suffer take for granted ever day. For this purpose alone I think that topical analgesics is a good market. Topical analgesics have proved their effectiveness in pain management, however they do nothing to fix the pain. It is very important to note that they do not heal, they conceal. There is a place in the medicinal for topical analgesics because they do work. Topical analgesics, like any other drug do have their misuses that can be very harmful if misuse persists. Topical analgesics are not to be used with a heating pad, in a sauna, in the shower etc. It is very important to use topical analgesics alone as an external mediator. There have been known cases of problems to neurons and receptor cites due to misuse. 

Sports Injuries

Sports injuries fall into two main categories. The first is trauma due to a constant repetitive motion such as tennis elbow, which is caused by straining the same area over and over again. The second type is a direct trauma resulting from a fall or accident, such as a sprain or muscle bruise. These types of injuries don’t always necessarily happen to just athletes. The hustle and bustle of everyday life can contribute to anyone of these injuries, or even muscle soreness.

Muscle pain and joint pain are frequent problems among athletes. These types of maladies can reduce the intensity of a workout for a serious athlete, or discourage individuals who are new to a training program. Sometimes it is not as easy to sit around at work or school with a heating pad or ice to nourish the condition. This is where topical analgesics can prove to be affective for athletes. Once applied an athlete can co about their daily routine while still feeling the soothing sensation applied to the epidermis. Topical analgesics are so wonderful for athletes because they can be applied before during, or after a workout. They are also the athletes “Wonder Drug.” They help in most ailments that an athlete comes across.

As a student athlete I understand a lot about sports, and a lot about winning for that matter. Somewhere in the last century the emphasis of sports being a game became skewed to sports being a way life. Sports take precedence over anything and everything for a lot of athletes. It is not uncommon to see an injured athlete consume 10-15 aspirin to conceal pain before a competition. Winning is what matter, winning is everything. While athletes get so caught up in their drive for glory, they never really step back to ask themselves if what they are doing is right. Not just morally, but medically. Is it wrong to use a substance to conceal an underlying pain that lingers? Morally? Yes. Medically? Yes.

What we have done is found a way for people to win. We have also found a way
for people to destroy their bodies. As an injury occurs the proper treatment should be administered until the ailment has passed. Concealing the pain only leads to further problems; especially later on down the road when those muscles and joints aren’t quite as young as they used to be. Topical analgesics have a purpose laid out for some people, and for others it is still unclear. It is in my opinion that topical analgesics have no place in Sports Medicine. I believe that this type of product will lead to too many serious injuries later on down the road, and the use of it in the Sports Medicine field will decline. I think that in the long run good old heat and ice will prevail in this battle.
References

Toprol-XL® (metoprolol succinate)

By: Jacob Power
Course: CHM 236
Instructor: Hank Mancini, Ph.D.
Date: 4/19/03
Abstract

With the advent of Toprol-XL, (metoprolol succinate), people with the heart conditions of angina, hypertension and heart failure can now receive 24 hour protection with just one daily dose. Lopressor, (metoprolol tartrate), became available before Toprol-XL but is different in that it is an immediate release medication. What is the significance of an extended release metoprolol for the above mentioned heart conditions? Is the extended release form of metoprolol more effective than the immediate release? And what is the specific molecular difference in the mechanism that allows Toprol-XL to be extended release? This paper will attempt to answer these questions and give an overall look at what metoprolol is.

Background

Beta₁-selective adrenoceptor blocking agents such as metoprolol have been found effective in treating various types of heart conditions as mentioned above, but how do they work? A beta blocker competes with neurotransmitters such as catecholamines (see below), which bind to sympathetic receptor sites found in the heart and vascular smooth muscle. When the sympathetic receptor sites are blocked the benefits are decreased exercise and resting heart rate, decreased cardiac output and a decrease in both systolic and diastolic blood pressure. To achieve decreased heart rate, cardiac output and blood pressure; beta blockers reduce the sympathetic outflow from the CNS and suppress the release of renin from the kidneys. Thus through many mechanisms, beta blockers achieve the desired results. The structure and functionality of metoprolol is very similar to that of catecholamines. This similarity allows the metoprolol to compete for the sympathetic receptor site.

Structure and Synthesis of Catecholamines
Metoprolol Succinate

Metoprolol succinate or (±) 1-(isopropylamino)-3-[p-(2-methoxy ethyl) phenoxy]-2-propanol succinate (2:1) salt is a white crystalline powder with a molecular weight of 652.8. Metoprolol succinate is soluble in water, methanol, ethanol and dichloromethane. Metoprolol succinate is not very soluble in ethylacetate, acetone, diethylether and heptane. Here is a structure of metoprolol succinate. Later on in the paper, the significance of the succinate salt will be explained.

Stereochemistry

(S)-(−)-Metoprolol

(R)-(−)-Metoprolol

Metoprolol is a racemic mixture of both R and S enantiomers. "Metoprolol is B₁ selective aryloxypropanolamine adrenergic antagonist used extensively in the treatment of a variety of cardiovascular disorders and is administered as a racemic mixture. (S)-(−)-metoprolol has been reported to be significantly greater B₁-adrenergic receptor affinity by >25-fold than (R)-(−)-metoprolol." Even though both R and S enantiomers of metoprolol are present in the active ingredient of Toprol-XL, the S enantiomers seem to be the one that does most of the blocking at the receptor site.
Pharmacokinetics

Absorption of metoprolol in the human body is quick and complete. Approximately 50% of the drug is metabolized immediately leaving the other half to affect the body. Metoprolol is not blocked by the blood-brain barrier and can be found in the CSF in relatively large concentrations.\(^2\)

Toprol-XL has shown to be just as effective as the immediate release metoprolol. Toprol-XL, in comparison to conventional metoprolol has lower peaks in the plasma concentrations, longer time to peak and significantly lower peak to trough variation.\(^2\) Toprol-XL 50mg actually has been proven to be more effective over a 24-hour period in reducing blood pressure than conventional metoprolol. The true benefit of Toprol-XL is with the patients themselves. A person is much more likely to receive the correct dose if it is to be taken once a day as oppose to twice a day because there is less room for error.

In a study comparing the effectiveness of once daily metoprolol (MS) and twice daily metoprolol (MT), patients were randomly chosen to take either of the two metoprolol forms. The patients were either started on 6.25mg MT or 25mg MS and were gradually given increasing doses up to 50mg. The results of the test showed that there were significant benefits from taking metoprolol and that either form was effective.\(^5\)

The above study exhibits the point that MT and MS both produce similar hemodynamic and clinical effects acutely and chronically despite the fourfold greater starting dose of MS. However if MS were to be administered at a more rapid and readily available dose, it could possibly present an advantage of MT in treating chronic heart failure.\(^5\)
Synthesis

A closer look at one of the methods used to synthesize metoprolol shows the simple compound of phenol as the starting material. The synthesis is an eight-step procedure.
Extended Release Mechanism

Lastly, the extended release mechanism of Toprol-XL will be discussed to explain how it is different from the immediate release. Metoprolol succinate is an extended release medication because of two main reasons: the fact that the tablet itself contains little coated pellets and because of the molecular structure of the salt used in conjunction with metoprolol.

First the tablet has contained within it little coated pellets. "Toprol-XL has been formulated to provide a controlled and predictable release of metoprolol for once-daily administration. The tablets comprise a multiple unit system containing metoprolol succinate in a multitude of controlled release pellets. Each pellet acts as separate drug delivery unit and is designed to deliver metoprolol continuously over the dosage interval."

The uniqueness of metoprolol succinate comes in the succinate part of the medication. The succinate salt when compared with the tartrate salt of the immediate release metoprolol has a slight difference in molecular structure but a large difference in solubility. Succinate contains ethyl groups between the acid groups (COOH); metoprolol contains hydroxyl groups between the acid groups. The solubility of a hydroxyl group is much higher than that of an ethyl group. Thus the ethyl group on the succinate salt allows the metoprolol compound to remain in the body longer and is metabolized much slower.

![Chemical structure of Metoprolol and Succinate Salt](image)

The tartrate salt used in the immediate release metoprolol is all but the same except for the CH₂ groups (ethyl groups). The tartrate salt contains OH⁻ groups (hydroxyl groups). Alcohols in general are very water soluble because of the hydroxyl group. "The succinate, rather than the tartrate salt, was chosen for Toprol-XL because of differences in solubility. The tartrate salt is very watersoluble (>700mg/mL at 37°C) and, therefore, is released relatively quickly from the tablet. The succinate salt is less soluble in water (270 mg/mL at 37°C) and provides
relatively constant drug delivery over an extended period of time. As a result, Toprol-XL produces relatively stable plasma metoprolol levels, unlike conventional metoprolol tablets, which demonstrate high and rapid peaks in plasma levels.7

Conclusion

This paper has given a brief outlook on the benefits of Toprol-XL as opposed to Lopressor.
Bibliography


Shital Rajyaguru

Chemisty-236

Actiq

(oral transmucosal fentanyl citrate)
Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It can be fast or slow. Acute pain is felt within seconds and is not felt within the deeper tissue of the body. Chronic pain is transmitted by different fiber types and can occur in the skin or any deep issue. Chronic pain is prevalent in cancer patients. This pain can originate from the pressure of the growing tumor, or from the infiltration of the tumor into neurons, blood vessels and other organs. These two states of pain can be treated with Actiq.

Actiq is a solid formation of fentanyl citrate, fentanyl is a short acting synthetic opioid with a 30 years history of safety and efficacy as a parentally administered analgesic and anesthetic agent, intended for oral transmucosal administration. Actiq is formulated as a white solid drug matrix on a handle that is radiopaque and is fracture resistant under normal conditions when used as directed. Actiq is designed to be dissolved slowly in the mouth in a manner to facilitate transmucosal absorption. The handle allows the Actiq unit to be removed from the mouth if signs of excessive opioid effects appear during administration.

Active Ingredient: Fenanyl citrate, USP N- (1-Phenethyl-4-piperidyl) propionanilide citrate (1:1). Fenanyl is a highly lipophilic compound (octanol-water partition coefficient at pH 7.4 is 816:1) that is freely soluble in organic solvents and sparingly solubel in water (1:40). The molecular weight of the free base is 336.5 (the citrate salt is 528.6), the pKa of the tertiary nitrogens are 7.3 and 8.4, the compound has the following structural formula.

\[
\begin{align*}
\text{CH}_3\text{-CH}_2\text{-CON} & \quad \text{HO-C-COO} \quad \text{HO-C-COO} \\
\text{CH}_2\text{CH}_2 & \quad \text{H}_2\text{C} \quad \text{H}_2\text{C} \\
\hline
\end{align*}
\]

Inactive Ingredients: Sucrose, liquid glucose, artificial raspberry flavor, and white dispersion G.B. dye.

Fentanyl is a highly potent and clinically widely used narcotic analgesic. Due to its high potency and generally favourable pharmacological profile a large number of its analogues has been prepared so far including acyclic compounds.
Analgesic activity of the anilidopiperidines is greatly enhanced by the presence of a substituent in the position 4 of the piperidine ring. The chemical nature of the substituent apparently has little influence on the activity, where the analgesic potency would depend entirely on the steric factor.

1. MgMgI, Et₂O → 85%.
2. NH₄Cl, H₂O → 72%.
3. Et-CN, ~0 °C, conc. H₂SO₄.
4. 1.5 eq. Me₃Si-I, Cl-(CH₂)₂-Cl, ~7 h, 80 °C.
5. H₂O, ~100% crude.
6. MgCN, K₂CO₃, 3-6 h, 20 °C, 78%.
Above reactions show the synthesis of 4-methyl fentanyl, which was started by converting N-benzyl 4-Piperidione to carbamate using ethyl chloroformate. Then it was reacted with MeMgI to yield alcohol. With this alcohol and under the conditions of Ritter reaction and after dry flash chromatography reaction 3 was yielded. The amide of reaction 3 were first N-metalated using KH, diglyme, 20 degree for 30mins then was treated with triphenylbismuth carbonate, which is highly efficient phenylating reagent for enolate anions. The phenylation of N-metalated amide was effected with diphenylidonium chloride. After dry flash chromatography N- (1-methyl-cyclohexyl)-N-
Phenyl-acetamide and the amide 4 were isolated in ~70 and 40-50% yields respectively. In the last step of the synthesis, the carbamate moiety in amide 4 was removed quantitatively, using Me3SiI in boiling dichloroethane. The number of deprotection procedures caused complete decomposition to effect the cleavage. The ethyl carbamate group was stable towards both a strong nucleophile (MeMgI) and in concd sulfuric acid. The intermediary secondary piperidine was isolated without purification and smoothly alkylated with phenethyl iodide to afford 4-methyl fentanyl. The 4-methyl fentanyl was precipitated as mono-oxalate salt and tested for analgesic activity using rat tail withdrawal test and fentanyl citrate as a standard. As a synthesis of 4-methyl fentanyl there are several other synthesis and pharmacological evaluation of fentanyl.

Fentanyl produces pharmacological effects characteristic of opiates. It is highly lipid soluble and due to their high lipid solubility, fentanyl reaches the brain quickly to provide very fast onset action. Fentanyl, acts primarily through interaction with opioid mu-receptors, which is located in the brain, spinal cord and smooth muscle. All opioid mu-receptor agonists, including fentanyl, produce dose dependent respiratory depression and the risk of respiratory is less in patients receiving chronic opioid effects. Opioids increase the tone and decrease contractions of the smooth muscle of the gastrointestinal tract. Also the analgesic effects of fentanyl are related to the blood level of the drug, if proper allowance is made for the delay into and out of the central nervous system. Other opioid effects may include hypoventilation, bradycardia, postural hypotension, pruritus, dizziness, nausea, diaphoresis, flushing, euphoria and confusion or difficult in concentration in clinically relevant does.

The absorption pharmacokinetics of fentanyl from the oral transmucosal dosage form is a combination of an initial rapid absorption from the buccal mucosa and a more prolonged absorption of swallowed fentanyl from the GI tract. Both the buccal fentanyl profile and the bioavailability of fentanyl will vary depending on the fraction of the dose that is absorbed through the oral mucosa and the fraction swallowed. About 25% of the total dose of Actiq is rapidly absorbed from the buccal mucosa and becomes systemically available. The remaining 75% of the total dose is swallowed with the saliva and is slowly absorbed from the GI tract. About 25% of the total dose escapes hepatic and intestinal first-pass elimination and becomes systemically divided equally between rapid transmucosal and slower GI absorption. Because of all these a unit doses of Actiq, if chewed and swallowed, might result in lower peak concentrations and lower bioavailability than when consumed as directed. Actiq comes in four different doses. Dose proportionality among four of the available strengths of Actiq has been demonstrated in a balanced crossover design in adult subjects. Mean serum fentanyl
levels following these four doses of Actiq are shown in the table. The curves for each
dose level are similar in shape with increasing dose levels producing increasing serum
fentanyl levels.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters in Adult Subjects</th>
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<tr>
<td>Receiving 200, 400, 800, and 1600 mcg Units of Actiq</td>
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<table>
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<tr>
<td>Tmax minute</td>
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<tr>
<td>Cmax ng/ml</td>
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<td>Mean (% CV)</td>
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<tr>
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<td>Mean (% CV)</td>
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<td>40</td>
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<td>(20-120)</td>
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<td>193</td>
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<td>(48)</td>
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</table>

Fentanyl is highly lipophilic and data showed that fentanyl is rapidly distributed
to the brain, heart, lungs, kidneys and spleen followed by a slower redistribution to
muscles and fat. Fentanyl is metabolized in the liver and in the intestinal mucosa to
norfentanyl by cytochrome P450 3A4 isofrom. It was not found to be pharmacologically
active in animal studies. It is a synthetic narcotic analgesic and its metabolites have been
detected in horse and greyhound urine samples during normal post-race screening. The
metabolism of fentanyl in the horse has been well established with the description of the
despropionyl metabolite. Fentanyl is primarily, more than 90%, eliminated by
biotransformation to N-dealkylated and hydroxylated inactive metabolites. Less than 7% of
the dose is excreted unchanged in the urine, and only about 1% is excreted unchanged
in the feces. The metabolites are mainly excreted in the urine while fecal excretion is less
important. And the terminal elimination half-life after OTFC administrations about 7
hours.

ActiQu is intended to be used only in the care of cancer patients only by
oncologists and pain specialists who are knowledgeable of and skilled in the use of
schedule II. Elderly patients have been twice sensitive to the effects of fentanyl compared
with the younger population. A formal study evaluating the safety profile of ActiQu in the
elderly population has not been performed. No difference was noted in the safety profile in this group compared to younger population, though they did titrate to lower doses than younger patients did. There was no clinically relevant gender differences were noted either in dosage requirement or in observed adverse events. Actiq is a category C drug and there was no evidence of teratogenic effects has been observed after administration of fentanyl citrate to rats. There were no adequate and well-controlled studies in pregnant women. Actiq can be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. And it is not recommended for use in labor and delivery. Fentanyl is excreted in human milk; therefore it should not be use in nursing women because of the possibility of sedation and respiratory depression in their infants. Patients and their caregivers must be instructed that Actiq contains a medicine in an amount that can be fatal to a child. Therefore, patients and their caregivers must be instructed to keep all units out of the reach of children and should be discard opened units properly in a secured container. Actiq should be store at 20 degree C with excursions permitted between 15 degree C and 30 degree C until ready to use. Actiq should be protected from freezing and moisture and also should not be use if the foil pouch has been opened.

Actiq is indicated only for the management of breakthrough cancer pain in-patients with malignancies who are already receiving and who are tolerant to opioid therapy for their underlying persistent cancer pain. Because life-threatening hypventilation could occur at any dose, this drug must not be used in opioid non-tolerant patients. Actiq is intended to be used only in the care of cancer patients and only by oncologists and pain specialists who are knowledgeable of and skilled in the use of schedule II opioids to treat cancer pain.
References:

1) Actiq package insert.
2) Duragesic package insert
4) http://www.rhodium.ws/pdf/4-methyl-fentanyl.pdf
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Montelukast
Heidi Riley
CHM 236
Dr. Mancini
April 25, 2003
Abstract:
A new type of asthma treatment is available. They are called leukotriene receptor antagonists, and by stopping leukotrienes from binding with their receptors, many of the symptoms of asthma can be prevented. The leukotriene receptor antagonist focused on here is montelukast sodium (Singulair®), which is a receptor antagonist of LTD₄.

Asthma:
Asthma is a condition that affects millions of people in the United States every year: it causes them to miss work, school, and other daily activities. It is the third highest reason for preventable hospitalization,¹ resulting in half a million hospitalizations and 1.2 million emergency room visits per year.² It is also the cause of 5,000 deaths in the United States per year.³ Despite popular belief, only one out of four children “outgrows” asthma.³ In fact, there seems to be an increase in the incidence of “tighter” buildings that let in less fresh air.³ This increases the amount of contaminates that people are forced to breath in their homes and offices, including nitrogen dioxide, from gas stoves; formaldehyde, from insulation and carpeting; and cleaning products.³ “Tight” buildings also cause buildup of dust and pet dander, which are asthma-inducing, even to adults with no previous history of asthma.³ Conversely, some believe that asthma is becoming more prevalent because we are living in a cleaner environment than earlier in history.⁴ Also, some believe that the increase in vaccines is giving the immune system a break so that it is not working as well as it should.⁴ It is no wonder that the pharmaceutical industry is constantly at work trying to find new ways to prevent asthmatics from having symptoms. As the number of cases of asthma in the country increases, so do the number of drugs treating it.

Symptoms of Asthma:
Asthma is condition that causes difficulty breathing. It has three main symptoms: airway obstruction, inflammation, and hyperirritability. Airway obstruction is caused by narrowing of the bronchial tubes by smooth muscle constriction and is therefore called bronchoconstriction.⁴ It is caused by a parasympathetic mechanism and is involuntary. These narrowed airways are the cause of the characteristic wheeze.⁴ Also, the bronchial tubes of asthmatics are hyperirritable, meaning that they are very sensitive and constrict at the slightest stimulus.⁴ Finally, in asthmatics, the airway becomes inflamed.⁴ Inflammation causes a red, swollen appearance.⁴ It is caused by a variety of biological and chemical reactions in the intra- and extra-cellular space of the bronchial tubes.⁴

Cysteinyl Leukotrienes:
A characteristic of asthmatic airway inflammation is the presence of mast cells and eosinophils in the airways. These can produce cysteinyl leukotrienes, and in the presence of these leukotrienes, the symptoms of asthma are produced. Leukotrienes were discovered in 1938 during studies of snake venom.² They found a non-histaminergic substance causing anaphylaxis, the contraction of smooth muscle.² Leukotrienes were originally given the name “slow-reacting substance” because their effect is slower to take effect, but longer in duration than histamine.⁵ In fact, leukotrienes are at least several thousand times more potent in causing bronchoconstriction than histamines.¹ Cysteinyl leukotrienes and their receptors have now been linked to asthma: they cause airway edema, smooth muscle contraction, and inflammation.⁶ They have been
proven to be part of the asthmatic response because they are found in the urine, serum, and plasma after an asthmatic episode.\(^7\)

The Leukotriene Pathway:

Where do these leukotrienes come from? They are the result of a chemical cascade beginning with arachidonic acid. Arachidonic acid is a twenty-carbon fatty acid \(^2\) with four double bonds, unconjugated with a \(cis\)-configuration,\(^6\) that is found esterified to membrane phospholipids in the sn2 position on inflammatory cells such as eosinophils and mast cells.\(^7\) Its chemical formula is: \(\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_3\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}\).\(^8\) Arachidonic acid is used in the formation of eicosanoids, examples of which include:

![Leukotriene Pathway Diagram](image-url)
prostaglandins (the chemicals that cause pain) and leukotrienes. When an enzyme called phospholipase A2 is active, arachidonic acid is cleaved from the cell membrane and becomes a substrate for 5-lipoxygenase. This is the first enzyme in the pathway to making leukotrienes. 5-Lipoxygenase adds O2 to arachidonic acid at C5 by “radical mechanisms,” shifting the double bond in the 5 position to the 6 position, making 5-hydroperoxyeicosatetraenoic acid. 5-hydroperoxy-eicosatetraenoic acid becomes leukotriene A4 (LTA4). This is done by removing a proton from C10 and a hydroxyl group from C5 in a dehydration reaction to make an epoxide between C5 and C6 and three conjugated double bonds between C7 and C12. The subscript “4” in LTA4 denotes the number of double bonds in the molecule. LTA4 is an unstable epoxide which is prone to hydrolysis, which would form dihydroxy acids. LTA4 is a substrate for the enzymes leukotriene C4 (LTC4) synthase and LTA4 epoxide hydrolase. LTC4, an allylic epoxide, is converted to LTC4 by adding glutathione to the C6 position of LTA4 by the LTC4 synthase. It does this by causing the “conjugation of the tripeptide glutathione (glutamic acid-cysteine-glycine) to the C6 position of LTA4 via a thioester link.” Up until this point, all of these reactions have been inside the cell. Outside of the cell LTC4 is changed to LTD4 by removing a glutamic acid moiety. This is then converted to LTE4 by dipeptidases. For these leukotrienes to have effect on receptors they must have the correct stereochemistry at C5 and C6. There are names for each of these leukotrienes: leukotriene C4 is glutathione, LTD4 is cysteinyll glycine, and LTE4 is cysteine. LTC4, LTD4, and LTE4 are known as cysteinyll leukotrienes. These leukotrienes are the cause of some symptoms of asthma.

The Role of Leukotrienes:

Leukotrienes were given their name because they are produced by leukocytes (eosinophils and mast cells) and they contain conjugated trienes. Their chemical structure was discovered in 1979. They are unsaturated acids that act as hormone mediators. They cause many of the same symptoms as histamines, but to a greater extent. They increase vascular permeability, causing edema of the airway membranes. They stimulate mucous production and prevent ciliary effectiveness, which inhibits the mucous from being expelled, leading to more breathing problems. Also, they attract eosinophils to the airway, causing more leukotrienes to be formed. All of this information shows that blocking leukotrienes from taking affect helps in the prophylaxis of asthma.

Cysteinyll Leukotriene Receptors and Their New Antagonists:

Cysteinyll leukotrienes (LTC4, LTD4, and LTE4) bind to cysteinyll leukotriene receptors (CysLT1 and CysLT2) in the airway. Stimulating these receptors causes smooth muscle constriction. Of the two types of cysteinyll leukotriene receptors, CysLT1 has been more closely studied and is the most likely to be involved with asthma in humans. After studying this receptor, scientists discovered that stopping the leukotrienes from binding with this receptor would prevent asthmatic symptoms. The way to keep these receptors from being activated by leukotrienes is to introduce an antagonist into the body that would selectively attach to the receptors without activating them. This would block the leukotrienes in a way that is like musical chairs: if the antagonist gets to the receptor, or chair, first, then the leukotriene has nowhere to “sit.” This has to be done by choosing an antagonist that matches the fit for the receptor just enough to keep it there without activating it. Some antagonists to the leukotrienes have been found. In fact there are many “chemically distinct, selective, specific receptor antagonists” for CysLT1 that have been identified. They have been given the suffix “-lukast.”
Agents that alter the leukotriene pathway are the first form of new asthma treatment to be introduced in twenty years.⁷ “One of the primary reasons for the development of antileukotriene therapy was the need for a chronic asthma treatment that could be given orally and that would effectively reduce asthmatic airway obstruction, asthma symptoms, and airway inflammation.”⁷ There are four medications available that work against the leukotriene pathway: three (ending with -lukast) are antagonists of the action of cysteinyl leukotrienes at the CysLT₁ receptor, and one (ending with -leuton) stops the catalytic action of 5-lipoxygenase.⁷ These medications are montelukast, zafirlukast, pranlukast, and zileuton. The focus here is on montelukast.

Montelukast Sodium:
The chemical formula of montelukast sodium is: [R-(E)]-1-[[[1-[3-[[2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropanacetic acid, monosodium salt.⁵ Its empirical formula is C₅₇H₃₅ClN₂O₄S.⁶ Its molecular weight is 608.13 grams per mole.⁶ The structural formula is:

Montelukast sodium is optically active.⁶ It is also hygroscopic, meaning that it is capable of retaining water.⁶ It is freely soluble in ethanol, methanol, and water, but practically insoluble in acetonitrile.⁶ It has a half-life of 2.7 to 5.5 hours.⁶ Montelukast is excreted mainly through the bile,⁶ which is a fluid secreted by the liver into the duodenum, where it emulsifies fats, increases peristalsis, and retards putrefaction. The mean oral bioavailability (amount absorbed into the blood) is 63% when taken with food and 73% when taken without food.⁵

Ingredients of Montelukast Sodium:
Each 10-milligram (mg) tablet of montelukast sodium contains 10.4 mg of montelukast sodium, which is the molar equivalent of 10 mg of free acid. Inactive ingredients include microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, hydroxypropyl cellulose, and magnesium stearate. The film coating consists of hydroxypropyl methylcellulose, hydroxypropyl cellulose, titanium dioxide, red iron oxide, yellow iron oxide, and carnauba wax.

The History of Montelukast:

Montelukast was in the making at Merck for nineteen years. It was discovered at the Merck Frosst Centre for Therapeutic Research in Montreal. As stated before, the slow-reacting substance of anaphylaxis (SRS-A) was discovered in 1938 by Charles Halliley Kellaway. In 1979, Bengt Samuelsson found that the active substances in SRS-A are LTC₄, LTD₄, and LTE₄, and he suspected that these substances were a factor in asthma. This discovery caused Merck to become interested. They began working on two different forms of medicines: one that would inhibit synthesis of leukotrienes and one that would prevent the leukotrienes from activating their receptors. After ten years of research, they decided that the best approach was to work at the level of the receptor. "Any compound capable of blocking the action of leukotrienes on tissues and cells was analyzed, chemically manipulated and tested again by the scientists until they had created a workable antagonist." This included the studies of over 14,000 compounds, seven of which were put into human trials. The substance finally chosen was quinoladin, which blocked LTD₄. While other companies began dropping out of the search for leukotriene receptor antagonists due to disappointing clinical trials, Merck pressed on, and in 1988, they found MK-517. This compound was very potent. Unfortunately, when it was tested on humans, it was found that it caused peroxisome proliferation in the liver, which was unacceptable. However, when they began looking at MK-517 again, they say that it had an enantiomer, which did not cause peroxisome proliferation, but it did cause an increase in liver enzymes, which was again unacceptable. Scientists went back to synthesizing and analyzing new chemicals. In 1991, they produced L-706,631, which became MK-476, and was finally called montelukast sodium. "Mont" in montelukast came from its site of discovery, Montreal. Montelukast is a intricate compound, and its synthesis involves twenty-three steps. After much testing, Merck sent for the Food and Drug Administration, and in 1998, montelukast sodium was approved for use. It is now approved for use in more than seventy five countries.

How Montelukast Sodium Prevents Binding to Receptors:

CysLT₁ is a receptor found in the respiratory tract of humans. Montelukast is a leukotriene receptor antagonist, which means that it prevents binding of LTD₄ to CysLT₁ receptors without activating them. Montelukast binds to the CysLT₁ receptor selectively. It selects against other airway receptors, such as the prostanoid, cholinergic, and beta-adrenergic receptors. Researchers still know little about these receptors. What is known is that they are glycosylated protein-coupled receptors. When activated (bound to) by leukotrienes in someone who is not treated by montelukast or another leukotriene receptor antagonist, the leukotriene causes the receptor the concentration of calcium in the receptor increases. This influx of calcium causes a chain reaction resulting in the contraction of smooth muscle. Calcium combines with calmodulin to form an enzyme called myosin light chain kinase. This phosphorylates light myofibrils called myosin. This causes myosin and another type of muscle fiber to interact, resulting in the contraction of the muscle. This contraction is completely involuntary, as it is
caused by smooth muscle that lines the bronchioles of the respiratory system. If these receptors are not activated, this contraction does not occur.

Effectiveness in Types of Asthma:

There are three types of asthma, and all three can be treated with montelukast. Exercise-induced asthma has long been treated with beta-agonists, however this type of medication is only effective for a little while before the effect fades. Montelukast’s effect does not fade; it is just as effective in the twelfth week of treatment as it is in the first week. Singulair® inhibits 47.4% of bronchoconstriction due to exercise-induced asthma. Another type of asthma, allergen-induced, has two phases, the early and late phase responses. Montelukast inhibits 50% of bronchoconstriction in late phase responses. Studies in late-phase allergen-induced asthma are more varied, with montelukast blocking 25-90% of bronchoconstriction. Finally, for the most part, leukotriene receptor antagonists, such as montelukast, are effective for aspirin-induced asthma. Montelukast is only effective for mild to moderate asthma. Those with more severe asthma need other treatment in combination with montelukast.

Role of Montelukast in Asthma Treatment:

Although montelukast is very effective in blocking the effects of leukotrienes, leukotrienes are not fully responsible for bronchoconstriction caused by cold-air, exercise, or allergen-induced asthma types. Therefore, it is still necessary to keep medications such as corticosteroids and beta-receptor agonists in an asthma-treatment regime. Corticosteroids are still the strongest asthma treatment, working to block inflammation. Those taking montelukast are sixty percent more likely to have asthma symptoms than those on corticosteroids. Corticosteroids have many side effects, unlike montelukast. These side effects include: atrophy of the adrenal cortex when used for long periods of time, glaucoma, excessive hair growth, and imbalance of calcium, potassium, nitrogen and sodium in the body. Another type of medication, beta-receptor agonists, such as albuterol, relieve sudden asthma attacks and are often referred to as rescue inhalers. These drugs can come in metered-dose inhalers or in pill form, in which they are considered long-acting. The effects of beta-receptor agonists in the inhaled form, however, diminish over time. Many people rely on these rescue inhalers because they give them fast, quickly-seen results. After time, however, the body builds up a tolerance to these drugs so that when an asthmatic is in need of fast treatment it is not there. Many times when this occurs, they are not on an anti-asthmatic drug that, taken over time, decreases the symptoms of asthma altogether. Montelukast is one of these drugs. Results might not be seen immediately, but eventually they are more effective than beta-receptor agonists. This does not mean that beta-receptor agonists should not be taken; they have their own role in asthma treatment.

Montelukast can be used in addition to these drugs so that they are not abused, and when they are really needed in cases of exercise-induced asthma, they will be ready and effective for the patient. A similar drug to montelukast, zafirlukast, which is also a leukotriene receptor antagonist, works nearly the same way as montelukast, but montelukast has a benefit over zafirlukast: it is approved for use by children, whereas zafirlukast is not.

One main issue that physicians have to be concerned with when prescribing montelukast is that since it is so effective, it enables users to discontinue corticosteroid use. The patient may stop taking the corticosteroid because they have many bothersome effects, whereas montelukast does not. The patient ceases taking the corticosteroid, getting rid of the side effects, and still
experiencing relief from asthma due to the montelukast. This may sound like a good thing, and it is in most instances, but it is a concern for those with Churg-Strauss syndrome. Asthmatics may not even realize that they suffer from this condition until they stop taking corticosteroids because corticosteroids treat this syndrome. Symptoms include allergic granulomatas, vasculitis, eosinophilia, cardiac problems, and fever. This condition is very rare, occurring in less than one-tenth of one percent of asthmatics, but it is still a concern because it is so severe.

Montelukast may be prescribed in combination with several other drugs. In fact, many studies suggest that montelukast efficacy is greatly increased when used with other types of asthma treatment. Other medications, such as salmeterol or budesonide, are needed in some cases because montelukast does nothing to block the effects of histamines, which are another important aspect of the asthmatic response. The right combination of these drugs is different for everyone. For example, montelukast can be taken in combination with loratadine, a histamine receptor antagonist, for effective treatment of allergy-induced asthma. Also, allergy-induced asthma can be treated with montelukast in combination with budesonide. Exercise-induced asthma can be treated with montelukast and salmeterol.

Conclusion and the Future of Montelukast:

Montelukast is a leukotriene receptor antagonist that prevents the inflammatory response and bronchoconstriction that leads to asthma symptoms. It works by preventing leukotrienes, which are eicosanoids formed in the leukotriene pathway from arachidonic acid, from connecting with cysteiny1 leukotriene receptors. Montelukast is most effective when used in combination with other drugs that block other symptoms of asthma. The demand for new treatment has been growing due to increased prevalence of asthmatics in the United States. Montelukast is from a group of drugs that are the first new asthma treatment in years. Although it appears that it is a "wonder drug," there is still a great deal of research to be done before it takes a permanent place in asthma treatment. The emergence of the Churg-Strauss syndrome is just one example of an unforeseen effect of using montelukast. Another effect that might not be seen for years is the effect on the immune system. Since leukotrienes are part of the immune response, they may hold another important role in the body that is unknown.
Bibliography


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19 New guidance on asthma. Chemist and Druggist Feb 2003;24.


The Effect of Folic Acid

Prepared by
Jenny Sanchez

April 28, 2003
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ABSTRACT

This paper will address the importance of the effects of folic acid, especially in pregnant women. This paper will provide an overview of the biochemistry, source of folic acid, risk of deficiency, and signs and symptoms of it. In addition, it will provide information about different studies and the benefits of taking folic acid.
Folic Acid Benefits

I. Introduction:

In 1822 the physician Combe, in Edinburgh discovered a case of anemia produced by the deficiency on the digestive track. In 1845 the physician, Thomas Addison called this type of anemia pernicious anemia, and around 1943 scientists discovered that this was due to the deficiency on vitamin M (now know as folic acid) and vitamin B12. In addition, Folic acid was named because it was found in leaves of green vegetables (folium means leaf in Latin) by Mitchell in 1941. The folic acid has older names such as B9, folinic and vitamin B<sub>9</sub>.

Folic acid is important for people’s health especially for women. Studies prove that in the United states one of the common deficiency in the body is the folic acid. Therefore, People should became more aware of it. Most people believe that folic acid is just for pregnant women, but this is a myth because people are not informed about its benefits. People are not aware that folic acid is in the body and many people have a deficiency which causes problems. Over the years scientists have discovered major benefits for all people.

This article will focus on the benefits of folic acid for women. The government is putting more attention on the positive benefits of folic acid, this is why folic acid is added to products such as grains. The scientists were not able to prove the exact amount that should be taken daily, but they have a close estimated of .400 milligrams.

II. Biochemistry

Folic acid has a molecular formula of C<sub>9</sub>H<sub>19</sub>N<sub>7</sub>O<sub>6</sub>. The molecular weight is 441.40 daltons. Folic acid forms in yellow-orange crystals. It has other names like folate and pteroyglumamic acid (PGA). The folic acid is compound of a para-aminobenzoic acid with one pteridine ring attached to one glutamic acid (see figure 1<sup>1</sup>). The biological forms of folic acid occurs as tetrahydrofolic acids: N<sub>5</sub>-formyltetrahydrofolic acid, N<sub>10</sub>-formyltetrahydrofolic, N<sub>5</sub>-N<sub>10</sub>-methenyltetrahydrofolic acid, and formiminotetrahydrofolic acid, which are found in natural products. Ascorbic acid in the liver and kidney help to catalyzed folic acid. Folic acid enzymes carry 1-carbon fragments and it is important for the synthesis of purines and pyrimidines, which are components of nucleic acids are part of what is known as DNA and RNA. Folic acid also help with the metabolism of some amines like glycine, serine, histidine, and glutamic acid, and phenylalanine<sup>5</sup>. There are some of the reasons why it is important for human bodies.

The major function of folic acid is to transfer one carbon group. The procedure occurs when a “5-Methyltetrahydrofolate donated a methyl group to homocysteine, which is an amino acid in the body that can affect the walls of the arteries in causing higher levels, in the conversion of homocysteine to L-methionine. The enzyme is catalyzes the reaction is methionine synthase. Vitamin B12 is a cofactor in the reaction.” This is an important reaction for the regulation of serum homocysteine and this is the only reaction where the B12 and folic acid are co-participants.
The L-methionine produced in this reaction participates in the synthesis of proteins and A-adenosyl-L-methionine (SAMe). "5-Methyltetrahydrofolate also donated the one-carbon for the methylation of the deoxuryrdyl acid to form the DNA precursor thymidilic acid."6

![Folic Acid](image)

Diagram of Folic acid reproduced from PDR for Nutritional Supplement data from reference 6

the synthesis of folic acid is realized by a Waller reaction. This procedure consists of the reaction of three-component mixture of 2,4,5-triaminopyrimidin-6(1H)-one, 2-3-dibromopropionaldehyde, and p-aminobenzyol-αS-glutamic acid in water at pH 4 resulting in a crude folic acid. This process can change one, two, or all three reacting groups with related compounds. Another form of prepared folic acid is the reaction of 1,1,3-trichloroacetone with nickel chloride producing a high pure folic acid. In addition, there is another form to produce folic acid but with similar mechanism, only the groups and reagents are different. 7

Folic acid has a low solubility in water and in most solvents like methanol, dimethyl sulfoxide, acetic acid, and pyridine. In order to increase the solubility of this product, base or acid and temperature should be increased. In addition, the solubility can increase in trifluoroacetic acid. Folic acid forms gel in N,N-dimethyl acetamide, but it is recrystallized from water or 5% hydrochloric acid by lowering the temperature to about 70°C. The degradation of folic acid increases by the increase in temperature and acid strength. 7

Folic acid has a unique form of intermolecular association. "In aqueous solution the H-NMR studies indicate that folate ions are intermolecular associated in a vertical stacking arrangement in which the hydrophilic ends of the molecule alternated in orientation with respect to the hydrophobic portion of adjacent molecules."7 The following graph will show the interaction of folic acid: see graph:
Graph 2: Intermolecular activated of folic acid
Reproduce from folate and pterins data from reference 7

In addition, folic acid is absorbed by the body in a complex process. Food folate is absorbed from the proximal third of the small intestine. The majority of folate is present in polyglutamate form. Consequently, it has to split in side chain vitamin molecules by enzyme conjugates. The product is detected by the brush border conjugase to form monoglutamates. These become converted to reduced form, methylated or formulated. Afterwards, the folate is transported through the enterocytes without modification. During this process the majority of malabsorption problems start, but the reason is unknown. Next, the folic acid is transported from intestine to circulation in a faster process if reductive form of folic acid is present\(^8\) (see graph 3\(^9\)) The scientists still investigate how the mechanism of folic acid works because they still have many questions about it.
Mechanism of absorption of folic acid by human.
Reproduce from modern nutrition in health and disease see data reference 9

III. Benefits
A. Preventing Birth defects:

The neural tube, which is responsible of embryo’s neural system, can be affected by a deficiency of folic acid. This deficiency can affect unborn children with problems such as spinal bifida, which is an incomplete closure of the spinal cord. Additional effects can be missing a large part of the brain called anencephaly. The low levels of folic acid is associated with problems of development of skull. Therefore, doctors recommend that women between 25-35 years old who may become pregnant take 400 milligrams daily. However, new studies disagree and recommend higher levels. For example, Dr. Arkin in his book Vita-nutrient solution proposes that women should take more folic acid to prevent 75% of the common birth defects. Dr. Arkin explain that people usually eat “junk food” that is consists of some folic acid. However, if they keep
the same diet, they need to eat six pounds of “junk food” to receive enough folic acid. Enriched food are those that mandatory added folic acid to them. This law was passed in 1996 by the food and drugs administration (FDA), which required the fortification of bread, cereals, flour, corn meals, pastas, rise, and other grain products. The fortification program ordered that .140 mg/100 g be added. In 2001, the studies proved the decrease of neural tube defects (NTD) by 20%, but not 50%-70% as was expected. This data prove the food should have higher levels of folic acid.

One study that combined 13 studies to quantify the correct amount of folic acid for women in order to prevent birth defects proved that increasing the amount of folic acid would result in prevention of birth defects. This study collects the data of all studies (13 studies) on folic acid and organize them into a cohort study. The studies tried to prove that when the intake of folic acid increases it results in higher prevention against NTD. “The results of this study prove that women planning to became pregnant should take 5 mg instead of 0.4 mg now recommended.” This study also proved that women between 25-35 will increase their folate by level by 0.1 mg/day when they take 0.94ng/ml folic acid. This will increase double in women between 40-65 when they take the same amount (See graph 2).

![Graph showing the relationship between dose of folic acid and change in serum folate](image)

**Cohort study of doses of folic acid and effects in the serum folate**
Reproduced from data of reference 14.

The National Center of Birth Defects and Develop Disability collected data to prove that neural tube decline after fortification kids with birth defects out of 30.5 live births out of 100000 compared to 37.5 live births out 100000 before the passing of the
law. The prevention of spinal bifida fell by 23% and 11% in anencephaly. However, the Committee on Medical Aspects of Food and Nutrition Policy (COMA) recommended UK to increase the level of folic acid on fortification food to .240 g/100g to reduce the effects by 41% 15, but this has not been revised by the US government.

Studies done in 80 hospitals in Boston, Philadelphia, and Toronto prove that the consumption of folic acid antagonist will increase the risk of birth defects. These studies show that women with birth defects took different antagonists at least 3 months before pregnancy or during the first month of pregnancy. Some of the antagonist that they took are: trimetrophen, triamledene, sulfasalazine, phenytoin, phenobarbital, primidone, and carbamazepine. This study also show that during 22 years from 3870 women who had kids with cardiovascular abnormalities, 63 took some type of antagonist at least one to three months prior to pregnancy. Also from 1962 kids with oral clefts, 36 mothers took antagonist, and from 1100 with urinary tract defects, 16 women took the antagonist.16 The conclusion of this study is that if women increase level of folic acid while taking antagonist, they can reduce the risk of birth defects.

The FDA created a controversy because the majority of studies prove that the public should increase their intake levels of folic acid and food should have higher levels of folic acid than recommend now. One controversy is that if folic acid is added to bread, it can mask effects of the B12 in elder people17. Studies show that folic acid can correct anemia associated with B12 deficiency, but it will not correct the changes in the nervous system resulting in B12 deficiency if it is not treated18. Nevertheless, some specialist recommended the intake of B12 to solve this problems. Another controversy with the FDA is that it has not authorized higher levels of folic acid intake daily. The government does not allow the intake more than .800 mg daily, discharging the good benefit; Consequently, this is “an example of how public health can be harmed by excess regulation”19.

The studies prove that folic acid is beneficial in the treatment of some mental retardation. Folic acid can be used by kids who have a fragile x-syndrome, which is an inherited cause of mental retardation. One study made in 1986 demonstrated that the intake of 10 milligrams of folic acid daily by kids with fragile x-syndrome can improve many of their behavioral abnormalities. The only problem was that kids should be younger than 13 years of age to feel the effects. This study proves that it does not been work in older kids.20

B. Folic acid and multiple births:

A controversial about folic acid effects is the association of multiple births with the increase of folic acid intake. Some studies argue that the possibility of increase multiple births, which can contribute to health problems for the children, is due to the increase of intake of folic acid. However, one study done by Peking University Health Center in China on a quarter million women proved that was a false statement. During the study, the women were monitored to take folic acid during three different periods: before ovulation, around time of fertilization, and after conception. The showed just 0.62% of the women had multiple births. The rate of multiple births was 0.59% of women who took folic acid against 0.65% of women who do not take any folic acid. This proved that there is no association between folic acid and increase of multiple births.21
C. Folic acid and spontaneous abortion:

One study published in the Journal of American Medical Association proves that folic acid does not increase risk of spontaneous abortion. The study was realized in Sweden because they do not have folic acid in any food and this permits a current result. The study proved that women with high level of plasma folate did not face the risk of spontaneous abortions. Furthermore, women with low levels of folate could increase the risk of spontaneous abortions. The study did not find the mechanism, but one theory was that the body did not produce sufficient DNA and this created a higher possibility of spontaneous abortion. This is an example of the good benefits of having a large amount of folic acid daily. These studies are clear, but public is not aware of these benefits.

D. General benefits Folic acid:

On the other hand, most people believe that folic acid is beneficial for only women who want to become pregnant, but not for the general public. Most of the public is not aware of the magnificent benefits of folic acid. Folic acid can prevent heart disease because of the decreases homocysteine in blood. The menopausal women can also use mega-folic therapy to replace estrogen therapy, which sometimes have worse side effects. Other proof of the effect can be seen in people with intestinal disorders because it helps replicate and heal the cells, while other products act as antagonist causing more problems. Folic acid provided a good protection against side effects. Folic acid can prevents some cancers such as: Colorectal, lung, esophageal, brain, cervical, breast, and throat because people with lower levels of folic acid seem to have higher risk of cancer. Folic acid is a good anti-depressant, used to threat peripheral neuropathy, helps with skin problems, and finally to prevents arteriosclerosis. Folic acid has good benefits for people who take it regardless of age or gender.

IV. Sources of Folic acid:

Natural folic acid can be found in the majority of green leafy vegetables like: spinach, kale, mustard greens, turnip, greens escarole, chard, arugula beet greens, bok choy, dandelion green, mache, radicchio, broccoli, Swiss Chard. Also, in fruits like orange, in lentils, pinto beans, garbanzo beans, asparagus, cauliflower, liver and brewer’s yeasts. However, just 50% is absorbed by the body and if they are cooked they can lose 90% of the folic acid. This is why specialists recommend adding supplements in order to one’s diet that can raise the exact amount of folic acid recommended for the bodies. Doctors also recommend taking folic acid without food, otherwise it will not be as effective as possible. In addition, doctors recommend taking folic acid with Zing to increase the absorption of oral folic acid by the body. The doctors normally recommend to take approximately .400 mg of folic acid daily, but the doses can change depending on individual need. Other sources of folic acid is the intake of cereals and grains, which are fortified with folic acid. The following table shows the amount of folic acid in each product:
Table of Food Sources of Folate

<table>
<thead>
<tr>
<th>Food</th>
<th>Micromg Dietary Folate</th>
<th>%DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready to eat cereal, fortified with 100% of the DV, 3/4 c</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>Beef liver, cooked, braised, 3 oz</td>
<td>185</td>
<td>45</td>
</tr>
<tr>
<td>Cowpeas immature, cooked, boiled, 1/2 c</td>
<td>105</td>
<td>25</td>
</tr>
<tr>
<td>Breakfast cereals, fortified with 20% of the DV, 3/4 c</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>Spinach, frozen, cooked, boiled, 1/2 c</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>Great Northern beans, boiled, 1/2 c</td>
<td>90</td>
<td>25</td>
</tr>
<tr>
<td>Asparagus, boiled, 4 spears</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Wheat germ, toasted, 1/4 c</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Orange juice, chilled, includes concentrate, 3/4 c</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>Turnip Greens, frozen, cooked, boiled, 1/2 c</td>
<td>65</td>
<td>15</td>
</tr>
<tr>
<td>Vegetarian baked beans, canned, 1 c</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>Spinach, raw, 1 c</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>Green peas, boiled, 1/2 c</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Broccoli, chopped, frozen, cooked, 1/2 c</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Egg noodles, cooked, enriched, 1/2 c</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Rice, white, long-grain, parboiled, cooked, enriched, 1/2 c</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>Avocado, raw, all varieties, sliced, 1/2 c sliced</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>Peanuts, all types, dry roasted, 1 oz</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Lettuce, romaine, shredded, 1/2 c</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Tomato juice, canned, 8 oz</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>Orange, all commercial varieties, fresh, 1 small</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Bread, white, enriched, 1 slice</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Egg, whole, raw, fresh, 1 large</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Cantaloupe, raw, 1/4 medium</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Papaya, raw, 1/2 c cubes</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Banana, raw, 1 medium</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Broccoli, raw, 1 spear (about 6 inches long)</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Lettuce, iceberg, shredded, 1/2 c</td>
<td>15</td>
<td>4</td>
</tr>
</tbody>
</table>

* DV = Daily Value. DVs are reference numbers based on the Recommended Dietary Allowance (RDA).

The graph was reproduced from the Facts About Dietary Supplement reference 11.
V. Risk of Deficiency:
There are multiple reasons for the decreases of folic acid in the body. First decrease is associated with pregnancy and lactation because the body is reproducing DNA and RNA for the new offspring and uses more folic acid. Additionally, alcoholics have deficiency of folic acid because the inadequate intake as well as to ethanol’s impairment of folate absorption of its easy solubility in alcohol. Another reason is a disease called Malabsorption syndromes including Crohn’s disease, lymphoma or amyloidosis of the small intestine, diabetic enteropathy, tropical sprue, and non-tropical sprue. In addition, this deficiency is seen in people with kidney dialysis and liver disease. The Megablastic anemia, which is caused by B12 deficiency can lead to decrease of folic acid. One of the most common reasons of folic acid deficiency is the reaction of folic acid with antagonist drugs as mentioned before27.

VI. Signs and Symptoms:
The following list the most common signs and symptoms of deficiency of folic acid:28
* Diarrhea
* Loss of Weight
* Loss of appetite
* Sore throat
* Headaches
* Heart Palpitation
* Irritability
* Anemia
* Weakness
* Shortness of breath
* Cramps
* Atrophic glossitis

VII. Conclusion:
Folic acid is an important supplement for the body. In near future, specialists may find more beneficial results of folic acid for the body in order to prevent different illnesses. The major concern of scientists is figuring out the appropriate amount of daily intake of folic acid for people. The majority of the studies focus on the benefits, but do not agree on the exact amount of daily intake. One of the major concerns of increasing the level of folic acid is the masking of B12; however, the solution is to take B12 to give the necessary equilibrium to the body. The public only takes folic acid in some of the enriched foods, which is not enough. Also, The FDA should be more aware of the importance of increasing the amount of folic acid in different foods. The government also should try to fortified healthier products not just “junk food.” Finally, the public should be educated about it in order to have a healthier population. Folic acid is a great instrument to prevent health problems around the world.
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(8) Shils, M., Olson, J., Shike. (pp 406-407).
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(27) Hendler. Sheldon, S. (pp 158)
(28) Hendler. Sheldon, S. (pp 158)
Analysis of an Insomniac:
Ambien

CHM236
Michelle Nicole Smith
April 25th, 2003
Abstract

Ambien®’s chemical composition to which the body’s reaction manifests the drug in the biochemical process of receptor activation followed by metabolic conversion to inactive metabolites and elimination from the body via renal excretion. The properties of this hypnotic make it highly effective as a sleep inducer due to its quick impact on one of the main inhibitory neurotransmitter receptor sites of the CNS, followed by elimination prior to waking; thus reducing the drowsiness and other negative CNS impact on the subsequent day’s activities. One of the special properties of Ambien® is its selective binding to a specific site on the GABA$_A$ receptor that makes it a better insomniac than most benzodiazepines because it has fewer side effects. The dissertation that follows will explain the chemical itself, the mechanism that causes onset of sleep, contraindications, side effects, and clinical trial information.

Introduction

Ambien® (zolpidem tartrate) is a Schedule IV controlled imidazopyridine hypnotic intended for the short-term management of insomnia. It is available in 5-mg and 10-mg strength tablets for oral administration, the latter being the highest recommended dose. The mechanism of zolpidem tartrate involves depression of the CNS (central nervous system), therefore aiding the patient in falling asleep fast and remaining asleep for a continuous period of time. However, extended use (more than 7-10 days) is contraindicated due to the potential for rebound insomnia and a multitude of possible side effects when zolpidem tartrate is suddenly discontinued$^1$.

Zolpidem tartrate has a short half-life, thus yielding the two significant reactions in the body. First, the quick impact to the system (mechanism discussed below) makes it effective in inducing sleep shortly after oral administration. It is most effective when taken on an empty stomach, and should not be taken until just prior to the patient’s bedtime. Second, the short half-life is also helpful in reduced potential of residual daytime sedative effects and impaired psychomotor and mental performance$^1$. A major benefit of zolpidem is that has no known significant contraindications for the general, reasonably healthy public.

Pharmacodynamics:

Zolpidem tartrate has CNS depressant effects due to its interaction with gamma-aminobutyric acid (GABA$_A$) receptor complex at benzodiazepine receptor sites$^1$. GABA is one of the main inhibitory neurotransmitters of the CNS, and it has been determined that activation of GABA$_A$ receptors favors sleep$^2$. Zolpidem tartrate may bind preferentially to the BZ$_1$ (benzodiazepine) site on the GABA$_A$ receptor, which may account for the reduction of anticonvulsant, muscle relaxant, anxiolytic effects as
compared to benzodiazepines. Also, the preferential binding of zolpidem tartrate may explain the preservation of deep (stage 3 and 4) sleep at hypnotic doses in humans.

The GABA<sub>2</sub> receptor is found primarily on the Lamina IV of the sensorimotor cortical regions, olfactory bulb, cerebellum molecular layer, substantia nigra, inferior colliculus, pons, ventral thalamic complex, and globus pallidus. Nonspecific attachment to the GABA<sub>2</sub> receptor chloride channel complex is hypothesized to be responsible for the benzodiazepines' sedative, anticonvulsant, anxiolytic, and myorelaxant drug properties.

An understanding of GABA aids in the comprehension of the interaction with zolpidem tartrate. GABA participates in the regulation of neuronal excitability through interaction with specific membrane proteins (the GABA<sub>2</sub> receptors are the ones with which zolpidem tartrate interacts.) As GABA binds to these postsynaptic receptors, the chloride channel in the center of the receptor opens, which allows the entry of a Cl<sup>-</sup> and leads to the hyperpolarization of the recipient cell.

Benzodiazepines are a related class of therapeutics that displays hypnotic, anxiolytic, and anticonvulsant effects. Unfortunately, these drugs are limited by a range of side effects comprising of sedation, amnesia, alcohol and barbiturate potentiation, tolerance development, and abuse potential. As a result, there has been extensive research for receptor inhibiting agents with an improved profile with chemistries distinct from the benzodiazepines that still have the GABA receptor inhibiting effects.

Zolpidem tartrate has been one of those advances in this area as a third generation hypnotic, which acts similarly on waking and slow-wave sleep. However, the slight
decrease in paradoxical sleep during the first hours does not result from an increase of the intermediate stage. Though zolpidem tartrate and benzodiazepines have unrelated chemical structures, EEG changes are similar between the medications.

**Pharmacokinetics:**

Zolpidem tartrate is swiftly absorbed from the GI tract, and peak concentrations in the blood are reached within 3 hours. A notable property of zolpidem tartrate is its short elimination half-life of about 2.5 hours; hence its effectiveness to induce sleep and be removed from the system prior to the waking portion of the next day. Zolpidem undergoes first-pass metabolism and an absolute bioavailability of about 70%. Since Ambien must enter the brain, it is lipid soluble so that it can pass through the blood-brain barrier. Approximately 92% of the zolpidem tartrate dose is bound to proteins in the blood, and is then eliminated primarily by renal excretion. Zolpidem is metabolized primarily by the cytochrome P450 isoenzyme CYP3A4.

The medication did not accumulate in healthy, young adults following nightly dosing with 20 mg for two weeks. Elderly patients experienced up to twice the elimination time. Hepatic patients experienced an average of 9.9 hours for elimination, which could potentially result in side effects during the hours shortly after waking up if the patient did not sleep past the elimination period. Patients with impaired hepatic function should be watched carefully as well as observed for other pharmacokinetic effects.

**Warnings:**

Zolpidem tartrate shares some of the same side effects as other orally administered CNS depressant medications. In the short-term use trials (Table 1) there were some common side effects that occurred as shown in the table. In the patients that went through long-term use trials (Table 2), there was a significant increase in the number of side effects, as well as an increase in those who experienced them. The following side effects occurred in the trials.

<table>
<thead>
<tr>
<th>TABLE 1 Incidence of Treatment-Emergent Adverse Experiences in Short-Term Placebo-Controlled Clinical Trials (percentage of patients reporting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zolpidem</td>
</tr>
<tr>
<td>(n=665)</td>
</tr>
<tr>
<td>Body System/Adverse Event</td>
</tr>
<tr>
<td>Central and Peripheral Nervous System</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Drowsiness</td>
</tr>
<tr>
<td>Gastrointestinal System</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Diarrhea</td>
</tr>
<tr>
<td>Musculoskeletal System</td>
</tr>
<tr>
<td>Myalgia</td>
</tr>
</tbody>
</table>

* Events reported by at least 1% of Ambien patients are included.

As shown, the most common side effects were headache, drowsiness, nausea, myalgia, dizziness, and diarrhea. The occurrence of headache, drowsiness, nausea, myalgia, dizziness, and diarrhea increased 12%, 6%, 4%, 6%, 4%, and 2% respectively in
The long-term trials compared to the short-term. Therefore, the lowest dose that is still effective for the patient is recommended to decrease the chance of side effects.

| TABLE 2: Incidence of Treatment-Emergent Adverse Experiences in Long-term Placebo-Controlled Clinical Trials (percentage of patients reporting) |
|---|---|
| **Zolpidem** | **Placebo** |
| **Body System/Adverse Event** | **(n=153)** | **(n=151)** |
| Antinominal Nervous System | | |
| Dry mouth | 3% | 1% |
| Body as a Whole | | |
| Allergy | 4% | 1% |
| Back pain | 3% | 2% |
| Flu-like symptoms | 5% | — |
| Chest pain | 1% | — |
| Fatigue | 1% | 2% |
| Cardiovascular System | | |
| Palpitations | 2% | — |
| Central and Peripheral Nervous System | | |
| Headache | 19% | 22% |
| Dizziness | 8% | 5% |
| Dizziness | 5% | 1% |
| Lightheadedness | 3% | 1% |
| Dizziness | 2% | 1% |
| Absentmindedness | 1% | — |
| Nausea | 1% | — |
| Anxiety | 1% | 1% |
| Nervousness | 1% | 3% |
| Snore disorder | 1% | — |
| Gastrointestinal System | | |
| Nausea | 6% | 6% |
| Dyspepsia | 3% | 4% |
| Diarrhea | 3% | 2% |
| Abdominal pain | 2% | 2% |
| Constipation | 2% | 1% |
| Anemia | 1% | 1% |
| Vomiting | 1% | 1% |
| Gastrointestinal system infection | 1% | 1% |
| Respiratory System | | |
| Myalgia | 7% | 7% |
| Asthenia | 4% | 4% |
| Respiratory System | | |
| Upper respiratory infection | 5% | 6% |
| Sinusitis | 3% | 2% |
| Pharyngitis | 3% | 1% |
| Rhinitis | 1% | 3% |
| Skin and Appendages | | |
| Rash | 2% | 1% |
| Urogenital System | | |
| Urogenital infection | 2% | 2% |

*Events reported by at least 1% of patients treated with Ambien.*

The age of the patient taking the medication should definitely be taken into consideration before administration of zolpidem tartrate. The usefulness and safety of administering the medication to a child has not been determined and is not recommended until such a time. Caution should be taken when administering zolpidem tartrate to geriatric patients because they have been shown to have more side effects that seem to have a stronger effect on them. Renally impaired patients need to be carefully watched because zolpidem tartrate may not be eliminated from their body as quickly as a normal patient, so the effects may last longer than intended.

Psychotic disorders or other such conditions may cause insomnia, and each patient should be evaluated prior to prescribing Ambien. The medication will not work effectively, and could potentially have adverse effects on patients with mental health issues. Some patients have reported psychotic reactions when taking therapeutic does of
zolpidem tartrate. During nightly use for an extended period, pharmacodynamic tolerance or adaptation to some effects of hypnotics may develop such as the side effects of wakefulness during the last third of the night and the appearance of increased signs of daytime anxiety.

Addiction to zolpidem tartrate is not a general concern, however because people with a history of addiction or abuse of drugs or alcohol are at increased risk to habituation and dependence. Such patients should be under careful surveillance when receiving zolpidem or any other hypnotic drug. Overdose cases involving multiple CNS-depressant agents, including zolpidem tartrate, have resulted in more severe symptomatology, including fatal outcomes. Recommended counter actions to patient overdose of over 100 mg of zolpidem tartrate includes pumping the stomach and monitoring the patient for at least 12 hours.

Ambien® is in pregnancy category B, meaning that it is not known to be harmful to humans; however, it has only been tested on rabbits and rats. In the studies conducted on the rabbits and rats, miscarriages and incomplete fetal ossification occurred at higher rates than normal. Therefore, consumption of zolpidem and other hypnotics should be avoided unless it is absolutely necessary to prevent any undetermined birth defects.

Studies were conducted to determine the carcinogenic or mutagenic potential risk of using zolpidem. No evidence of chromosomal malformations in human lymphocytes were found, nor was there evidence of carcinogenic potential in the studies done on mice. The rates at which cancerous tumors and cells grew were comparable to those of controls in previous trials, and therefore were considered to be unrelated to the consumption of zolpidem tartrate.

**Structure Determination**

Ambien® is the trade name of zolpidem tartrate, which is in turn the common name for a chemical with the name: N,N, 6-trimethyl-2-p-tolylimidazo[1,2-a]pyridine-3-acetamide L-(+)-tartrate(2:1). Its molecular formula of \((C_{9}H_{21}N_{5}O_{2})\cdot C_{4}H_{6}O_{6}\) has a molecular weight of 764.88 grams.

![Chemical Structure of Zolpidem Tartrate](image)

Zolpidem tartrate appears white in color, as in the 10mg tablets, and Red No. 40 dye is added to the 5mg to make them appear pink in color. The tablets themselves are
thin and oval shaped, with the 5mg marked AMB 5 on one side, with 5401 engraved onto the opposite side. As for the 10mg, AMB 10 and 5421 are marked on opposite sides of the tablet. The drug is somewhat soluble in alcohol, water, and propylene glycol. The inactive ingredients in the tablets are lactose, polyethylene glycol, hydroxypropyl methylcellulose, sodium starch glycolate, magnesium stearate, and titanium dioxide.

As for stereochemistry, the only chiral carbons are on the tartrate compound, which will make creation of an isomer for zolpidem impossible, though it is possible to make a compound similar to it.

The diagrams below demonstrate the activation of the \( \text{GABA}_\alpha \) receptor by zolpidem:

![Sagittal section of receptor](image1)

![zolpidem](image2)

![Cross section of receptor and sites](image3)
Future of Ambien®

Ambien® has been a very successful insomniac since the FDA approved it in December of 1992. It has been in the top 200 dispensed prescription drugs consistently for the past few years, and has been one of the most commonly prescribed hypnotics in the United States.6 There are a few obstacles that may stand in the path of success for Ambien®. The first and foremost obstacle is a generic form being approved, which could drastically reduce the amount of Ambien® being dispensed. Its counterparts in other countries (i.e. Stilnox, Ivalal, Stilnoct, and Niotal)6 creates the obstacle of competition. Zolpidem tartrate itself will be very successful for years to come due to its mild and infrequent side effects (when taken for short periods of time or less than 11 days), and may be the drug of choice for short-term insomnia treatment rather than benzodiazepines because the decrease in side effects.
References


HIV-1 Protease and its Inhibitors
An Overview

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Abstract

This paper will introduce and define the various families of protease proteins. The molecular structure of the HIV-1 protease will be described in detail and its function outlined. Next, the development, structure and function of several types of HIV-1 protease inhibitors will be presented. Finally, several drawbacks of the current inhibitors will be discussed along with future directions of current research.

Definition and Function of Proteases

Proteases consist of a diverse class of enzymes that catalyze the cleavage of proteins. Basically, they are proteins that break down the peptide bonds (proteolysis) of other proteins. Proteases are found in plants, animals and fungi and are vital for numerous physiological functions including inflammation, infection, fertilization, cell growth and death, blood clotting, tumor growth, allergic reactions, cell signaling/migration and digestion. They are also used for many industrial applications such as the processing of wool and leather, food and beverage production and as additions to cleaning products. Proteases are highly specific and will hydrolyze only particular amide bonds depending on the identity of the amino acids before and after the scissile bond (the bond to be hydrolyzed). Proteases perform either limited proteolysis, which is the cleavage of a limited number of peptide bonds of a protein resulting in the activation or maturation of the previously inactive protein, or unlimited proteolysis, in which a protein is broken down into its amino acid constituents (5).

Proteases can be categorized as either exopeptidases, which cleave off amino acids from the ends of a protein chain, or endopeptidases, which cleave peptide bonds within the protein (3). They are further classified by their mechanism of action into 4 categories. Serine proteases have an active site consisting of a catalytic triad of Ser 195, His 57 and Asp 102. Cysteine proteases have an active site containing a catalytic triad of Cys 25, His 159 and Asn 175. Metallo proteases use a metal, usually zinc, to effect amide bond hydrolysis. Aspartic proteases have an active site consisting of 2 catalytic aspartic acid residues (2).

The HIV-1 protease belongs to the Aspartic family of proteases. The proteins that compromise the HIV virus are produced in the form of long, non-functional "polypeptides (precursors to the actual proteins and enzymes) that must be cleaved to yield the active protein components of the mature virus. If the polypeptides are not cleaved the virus fails to mature and is incapable of infecting a new cell. It is the job of the HIV-1 protease to accomplish this cleavage. Specifically, the HIV-1 protease splits the gag and gag/pol polyproteins into their essential products. The activity of the HIV-1 protease is unique in that it can cleave between a phenylalanine and a tyrosine or proline, which no human virus can duplicate. This unique cleavage pattern allows for inhibitors to be developed that will not interfere with the normal activity of human proteases.

Structure of HIV-1 Protease

Structurally, the HIV-1 protease is a homo dimeric protein consisting of (2) symmetrical 99 residue (amino acid) monomers. Aliphatic residues, noncovalent interactions, hydrophobic packing of side chains and catalytic residue interactions all help to stabilize the molecule (1). The active site is found at the dimer interface and forms several binding pockets. The sidechains of a substrate extend into these pockets and are
bound through extensive hydrogen and van der Waals bonding. It is thought that six to ten amino acid residues are involved in binding activity (2). The active site is shielded on top by two glycine-rich, flexible flaps. The specific task of the flaps is to guard the entrance to the active site as well as to secure the substrate within the binding cleft allowing for proper cleavage (6). Additionally, a water molecule is hydrogen-bonded between the two aspartic acid residues of the active site and plays a critical role in the opening and closing of the flaps, as well as increasing the affinity between the protein and its substrate. The actual cleavage of a substrate is accomplished through a general acid/base hydrolysis induced by the two aspartic acid residues and the water molecule. One of the aspartic residues acts as an acid by donating a proton to the carbonyl oxygen of the substrate, and the other aspartic residue acts as a base accepting a proton from the water molecule. A tetrahedral intermediate is formed which is thought to represent the transition state of the proteolytic reaction. Nucleophilic attack of the water molecule results in proteolysis of the substrate. The reaction is stabilized by a network of hydrogen bonds and does not involve any covalent bonds (1,2,4). The catalytic activity of the protease is highly pH-dependent with an optimum pH of 5–6, and the pKa's of the two catalytic aspartic residues are known to be substantially shifted from their expected values (11).

![Structure of native HIV-1 protease.](image)

**Figure 1.** Structure of HIV-1 protease and representation of cleavage mechanism (4).

**Transition-State HIV-1 Protease Inhibitors**

Due to the impact and significance of the HIV-1 virus an unprecedented amount of research has been performed on its function and structure. The three-dimensional
structure of the HIV protease has been determined and numerous crystal structures of the protease and its inhibitors are available. In addition, the development of HIV-1 protease inhibitors has been advanced by (and actually owes its existence to) research of the human aspartic protease renin, a protease found in the kidneys that plays a role in regulating blood pressure. There are currently (6) HIV-1 protease inhibitors that are approved by the FDA: Saquinavir, Ritonavir, Indinavir, Nelfinavir, Amprenavir and Lopinavir (4). A protease inhibitor will resemble the protein chain that the protease normally cleaves, and will act as a competitor, binding to the site in place of the protein and rendering the protease ineffective. The majority of today’s HIV-1 inhibitors are designed as transition-state analogs that mimic the transition state of the protease’s natural peptide substrate. They utilize the peptidic substrate as a template and substitute the scissile peptide bond with one that is non-cleavable. These inhibitors mimic the configuration of the substrate at the instant when it binds most tightly to the protease (the transition state) and not the final form of substrate-enzyme complex. As a result, the inhibitors bind the protease much more tightly than the natural substrate because the substrate must be distorted in order to assume its transition state configuration. Proper stereochemistry of the inhibitor is required for effective interaction with the protease (2,7,8,9).

The most common and effective transition-state inhibitors replace the scissile peptide bond with transition state isosteres such as statine, norstatine, hydroxyethylene, dihydroxyethylene or hydroxyethylamine (4). The transition-state isosteres are functional groups that mimic the tetrahedral transition-state of the amide bond but cannot themselves be hydrolyzed by the protease (2). Many inhibitors are designed to be symmetrical (due to the symmetry of the HIV-1 protease), however, most of these actually bind asymmetrically to the active site of the protease.

Figure 2. FDA approved HIV-1 protease inhibitors (4).
HIV-1 protease binds substrate-based inhibitors by interacting with both the peptide backbone and side chains of the inhibitor, and bonding is accomplished through noncovalent, reversible interactions such as hydrogen bonds, ionic or van der Waals’ forces. Substrates and inhibitors are recognized through interactions of at least six subsites lining the walls of the binding pockets and the respective side chains of the substrate/inhibitors that extend into these pockets. The subsites are numbered starting from the central aspartic acid residue as S1, S2, S3 etc. with corresponding S1’, S2’, S3’ etc. on the symmetrical side. The corresponding sidechains of the substrate/inhibitor are named P1, P2, P3, etc. outwards from the scissile bond and P1’, P2’, P3’ etc. on the symmetrical side. The nomenclature is shown in figure (4) and is according to the convention of Schechter and Burger (4). It is the above-mentioned noncovalent forces (along with the flexible flaps of the protease) that anchor the substrate/inhibitor within the binding site. Hydrogen bonding occurs between the flap residues and a water molecule, which in turn hydrogen bonds to two carbonyls in the inhibitor. It is also believed that the two catalytic aspartic acid residues play a structural role in the formation and maintenance of the binding subsites (4,10).

Other inhibitors

In addition to the transition-state isosteres, several other methods of inhibition are being utilized. One of these involves targeting the central water molecule of the HIV-1 protease. This water molecule has been shown to play an important role in the binding
process by donating two hydrogen bonds to the ligand and accepting two hydrogen bonds from the protease (12). Several classes of inhibitors, including cyclic urea and cyclic cyanoguanidines, have been designed to displace this structural water molecule. In the case of cyclic cyanoguanidines, the structural water molecule is displaced by an exocyclic nitrogen. This nitrogen occupies the same position as the water molecule between the inhibitor and the flaps of the protease. In addition, the presence of a cyano group causes one of the protease’s flaps to move away from the active site, thus enlarging the binding pocket (13). Figure (5) shows the active site binding of HIV-1 protease and a cyclic cyanoguanidine.

In addition to displacing the water molecule, the design of cyclic urea and cyclic cyanoguanidine inhibitors (as well as most other inhibitors) take into effect the substituent groups that will extend into the enzymes binding pockets. Figure (6) shows a generic cyclic urea molecule and a generic cyclic cyanoguanidine molecule. The positions labeled P1 and P1’ are where the inhibitor will extend into the enzyme’s S1/S1’ binding pockets, and are therefore targets to improve inhibitor action. A typical cyclic urea inhibitor would have unsubstituted phenyl rings at the P1/P1’ positions, but experiments have shown that several other substituents can improve the inhibitor’s binding affinity and antiviral profile. The most potent substituent found was the 3,4-ethylenedioxy group, which increased the binding affinity through increased van der Waal’s interactions and hydrogen bonding (14).

Figure (5). Hydrogen bond interactions between HIV-1 protease active site residues and a cyclic cyanoguanidine inhibitor. (13)

Figure 6. Generic cyclic urea (13).  Generic cyclic cyanoguanidine (13).
HIV-1 protease inhibitors containing heterocyclic rings that are complementary to the size and shape of the active site have also been investigated. These do not contain transition-state isosteres, thus eliminating the need for correct stereochemistry (7). Heterocycles can also improve the water solubility and oral bioavailability of inhibitors. In addition, research has been conducted on inhibitors containing tetrahydrofuran rings with bicyclic ligands (15). The binding affinity of these inhibitors is strongly affected by the ring size, ring stereochemistry and position of the ring oxygens, and binding occurs in the S2 region of the binding site. The inhibitors containing fused cyclic ethers as P2 ligands have also shown improvement in aqueous solubility and reduction in molecular weight.

Problems and Future Directions

The success of the current HIV-1 protease inhibitors has been substantial, but it is not without its drawbacks. Low oral bioavailability, poor aqueous solubility, drug interactions, drug resistance, side effects, limited ability to cross the blood-brain barrier and high production costs are all problems associated with HIV-1 protease inhibitors. In order for a good protease inhibitor to become an effective drug it must overcome all of the above barriers as well as have a low molecular weight, be highly stable to proteolytic degradation, highly selective for its specific protease and have a long lifetime in the bloodstream (2,4). One of the biggest problems with current inhibitors is that the protease mutates and becomes resistant to them. This resistance is caused by amino acid substitutions in the protease occurring either at the active site or at a remote location that affects the binding efficiency (2). One very common mutation that arises against many inhibitors is a change of isoleucine 184 to valine (184V). 184 has a side chain that is in direct contact with the inhibitor, and mutating to the smaller molecule of valine leaves a gap or cavity between the enzyme and the inhibitor (1). Usage of more than one inhibitor, along with another HIV therapy involving reverse transcriptase inhibitors, (known as a “cocktail”) has led to dramatic success in this area, but resistance is still a large problem, and HIV-1 protease inhibitors with different resistance profiles are constantly being investigated.

Perhaps the most devastating problems with protease inhibitors, from the patient’s point of view, are the side effects that can occur. Some of the side effects that people have experienced are nausea, diarrhea, kidney stones, diabetes, anxiety and depression. A further side effect that patients experience is that they may dramatically alter the way the body stores and metabolizes fat, a condition known as lipodystrophy.

As stated in the opening of this paper, proteases are involved in many physiological functions and are found in many diverse forms. The success of the HIV-1 protease inhibitors has ignited interest and research into inhibiting many other proteases. The structures for several common proteases, including those found in hepatitis C, cytomegalovirus and rhinovirus, have already been solved (8). In addition, the importance of proteases beyond viral diseases, such as in osteoporosis, inflammation, stroke and Alzheimer’s disease, is being pursued. A further direction involving HIV-1 protease inhibitors is their possible use in combating cancer. Research has recently shown that a protease involved in cancer cells shares the same cleavage site as the HIV-1 protease. Research involving one of the currently approved HIV-1 inhibitors, Saquinavir, has
shown that it does induce apoptosis in human cancer cells. This indicates that the HIV-1 protease inhibitors may be used as a new class of cytotoxic drugs, alone or in combination with radiation or chemotherapy (16). With the continuing worldwide increase in HIV infections, research into the HIV-1 protease is certain to continue and intensify. New inhibitors that more closely resemble the transition-state and those that take advantage of the physical structure and unique conformations of the HIV-1 protease are likely to be developed. Future inhibitors will also be developed to possess a higher oral bioavailability and produce fewer side affects and cross resistance.
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Christmas Cheer and Clean Hands:
Myristic Acid—The Tie That Binds

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Myristic Acid

It’s the holidays and you are warming yourself by a crackling fire. What’s that wonderful aroma that tells you this is the season of good cheer? Is it pine cones? Nooo... Is it turkey in the oven, Nooo... Is it the sweet beverage cooking on the stove? Getting warmer... It’s the distinctive smell of eggnog. What gives it that distinctive aroma? Ground Nutmeg sprinkled on top.

Nutmeg Is The Source

Nutmeg is a spice from the seed of the *Myristica fragrans*, a tropical, dioecious evergreen tree native to the Moluccas or Spice Islands of Indonesia but also grown extensively in the tiny Caribbean island of Grenada (made famous by then President Reagan’s victorious fight to save the island from the Red Menace). Wars were fought over it—including one that rendered the obscure New World island of Manhattan to the British. Around the 16th century it became so important as a commercial trade product (more on that later) that the Dutch, major powers at that time, plotted to keep its prices high, and the English and French counterplotted to obtain fertile seeds for transplantation. The nutmegs sold whole by the Dutch were dipped in lime to prevent their growth. For 500 years, this aromatic little seed is said to have cured everything from boils and backaches to strokes, rheumatism and the plague. Some in Arab and Indian cultures swear that it’s an aphrodisiac. Malcolm X smoked it in jail when he ran out of marijuana. Narcotics made from the nutmeg family are in use among the native South Americans1. Its hallucinogenic effects have been reported in the New York State Journal2 of Medicine and in the British Medical Journal3.

Nutmeg is comprised of 8 to 10 per cent volatile oil (also called *Oleum Myristicae*4 or Essential Oil), 25 to 40 per cent fatty oil (also called *Oleum Myristicae Expressum*5), 9 to 13 per cent of water, about 5 per cent ash, and the remainder nitrogenous matter, starch, gum, woody fiber. Discovered by Mary Ann Playfair in 18446, trinymristin, the characteristic constituent of the oil of nutmeg, comprises up to 84% of that oil7. This fixed oil of nutmeg is also known by a number of different names: nutmeg butter, balsam of nutmeg, oil of mace, butter of mace, and Banda soap. It is obtained by exposing the nuts to hydraulic pressure and heat or by expressing the ground nutmeg with organic solvents. The essential or volatile oil of nutmeg is obtained by steam distillation. The components of this essential oil play a key role in making nutmeg special, the details of which will come a little later in this paper.

Nutmeg, like most plant seeds are rich in compounds called triacylglycerols, the fatty acid triesters of glycerol and, for the older readers, also called triglycerides. Many seeds contain a

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3 1970, 1, 21 March 1970, page 754
4 http://www.ibiblio.org/herbmed/eclectic/kings/myristica_oleu.html
5 http://www.ibiblio.org/herbmed/eclectic/kings/myristica_olee1.html
6 http://www.ibiblio.org/herbmed/eclectic/kings/myristica.html
7 http://www.fao.org/docrep/v4084e/v4084e06.htm
wide variety of triacylglycerols based on the identity of the fatty acids present in the glycerol triester, the major triacylglycerol found in nutmeg contains a single fatty acid, myristic acid, thus its name, trinystin. The structure of this lipid is show in Figure 1. Before discussing myristic acid further, let's take a closer look at lipids.

Figure 1: Trimyristin ($C_{48}H_{98}O_{6}$)

About Lipids

Lipids can be divided into several categories: 1) Neutral lipids which are fats, oils and waxes. More on fats and oils shortly, but waxes are esters of long chain carboxylic acids with long chain alcohols. 2) Phospholipids—diesters of phosphoric acid ($H_3PO_4$); Phosphoglycerols which are diesters of carboxylic acid and an ester of phosphoric acid all on a glycerin backbone (note that cell membranes are made of these lipids); Sphingolipids—compounds made with a sphingosine backbone, found only in nervous and brain tissues. 3) Prostaglandins—a five-membered ring with two long hydrocarbon side chains found in animal tissues. 4) Terpenes—small molecules responsible for the aroma of things (also one of the building blocks of essential oils mentioned earlier). 5) Steroids—molecules based on a specific tetracyclic ring structure (also the building blocks of hormones).

Fats and oils, also called mono- di- or tri-acylglycerols are lipids that have the general structure of one, two, or three fatty acid chains joined with glycerol. They can be further divided into three categories: 1) Saturated fats where there are no carbon-carbon double bonds (myristic acid falls into this category). 2) Unsaturated fats which have one or more carbon-carbon multiple bonds. These include monoeneic fatty acids, which have one such bond and have the form cis-CH$_2$(CH$_3$)CH=CH(CH$_2$)$_2$COOH (one such is myristoleic acid that also happens to come from nutmeg) and Polyeneic fatty acids (also called polyunsaturated fatty acids, PUFA), which have 2 or more cis double bonds. Actually the lipid connoisseur would indicate that there are about a dozen other categories of exotic lipids (branched chain, ring containing, brominated, conjugated, etc.), but let's just leave it at these for this examination.

One of the characteristics of trimyristin brought to it by the myristic acid chains is its insolubility in water which is low because of the relative long hydrocarbon chain. Up to 8 carbons are considered short chain; with 14 carbons, myristic is considered medium; greater than 16 is long. Another is its melting point (54-58°C) which is midway between the short and long chain fats. By the way, this would be a good time to mention the difference between oils and fats—it all depends on what climate you live in. To Native Americans of the Polar Regions, Fats and oils are almost the same—both solids given the low ambient temperatures. In most other areas, oils are liquid at ambient temperatures and fats are solids. This melting point difference is due to the number of carbon-carbon multiple bonds and to the length of the fatty acid (more and shorter give lower, respectively).
Trimyristin is synthesized from myristic acid in nature via the biological alpha-glycerophosphate or sn-glycerol-3-phosphate pathway. In this pathway, the synthesis of sn-glycerol-3-phosphate is first catalyzed by the enzyme glycerol kinase on glycerol. It is then acylated sequentially by an acyl transferase at positions sn-1 and sn-2 to form phosphatidic acid. The phosphate group is removed by another enzyme, and the resultant di-acylglycerol is acylated one more time to form trimyristin (see Figure 2).

Figure 2: Biosynthesis of Trimyristin from Myristic Acid. Myristic acid is shown here as HOOCR, HOOCR' and HOOCR".

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\[
\begin{array}{ccc}
CH_2OH & CH_2OOCR & CH_2OOCR \\
CHOH & CHOH & CHOH \\
CH_2OPO_3H & CH_2OPO_3H & CH_2OPO_3H \\
\end{array}
\]
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Oxidation of trimyristin in nature is the same as for other lipids. The triacylglycerols are hydrolyzed to free fatty acids and glycerol in the capillaries of adipose tissue and skeletal muscle by the lipoprotein lipase. The free fatty acids are then absorbed by the cells either for continued catabolism via the beta-oxidation pathway, or for storage for future use. This process is termed beta-oxidation since it results in the sequential removal of 2-carbon units by oxidation at the beta-carbon position of the fatty acyl-CoA molecule.

In the lab these pathways are replaced by a few short reactions. Synthesis of trimyristin (an ester) from myristic acid is carried out typically through a dehydration reaction, often referred to as Fischer esterification. It can also be done with Williamson Ether Synthesis. The reverse reaction, acid- or base-catalyzed ester hydrolysis, converts trimyristin into its component myristic and glycerol parts by adding water. Acid-catalyzed hydrolysis is just the reverse of Fischer esterification. Base-catalyzed hydrolysis is called saponification, but when followed with acid application, a free carboxylic acid is given.

**Synthesis of Myristic Acid**

Myristic Acid, also known as tetradecanoic acid, is a medium chain fatty acid with empirical formula of C_{14}H_{29}O_2 and a formula weight of 228.37 grams. It has a low density as would be expected from a simple organic substance of 0.858; a melting point that is in-line with trimyristin’s (54-55°C) and a boiling point of 309.0°C that positions it as a medium long chain unsaturated fatty acid. It has a somewhat circular conformation (as shown in Figure 3: Myristic

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8 http://www.lipid.co.uk/infores/Lipids/TAG/
9 Of particular interest is the fact that having three of the same fatty acids means the order and stereochemistry of this lipid is not as sensitive as that of mixed fatty acid lipids. And interesting discussion can be found at the following web site: http://www.lipid.co.uk/infores/Lipids/TAG/
Acid (C₁₄H₂₇O₃) when free (as opposed to being part of a triacylglycerol or embedded in another molecule) is due to van der Waals forces between different segments of the long hydrocarbon chain. Note that when it combines with glycerol, it takes on the classic long straight tail shape of unsaturated fats due to steric hindrance.

Being a carboxylic acid, myristic acid is very reactive. Its pKₐ is around 4.9[^16]. The proton on the polar end of the fatty acid can easily be removed bases to form a carboxylate ion. This ion is stabilized by the resonance of the carbonyl bond and by the electronegativity of oxygen. These resonance structures are shown in Figure 4: Resonance structures of the Myristate Ion.

There are a number of ways of preparing myristic acid in the lab: oxidative cleavage with KMnO₄ of an alkene with double bond at C14; oxidation of a tetradecanol with CrO₃ in aqueous acid or with Ag₂O; hydrolysis of Tetradecanonitrile with acid; carboxylation of the Grignard Reagent, CH₃(CH₂)₁₃MgBr. There are no laboratory mechanisms, however, to catabolize myristic acid into smaller units. In nature, enzymatic reactions are used to both synthesize and breakdown myristic acid. The synthesis pathway is similar in chemistry but different in specifics from that of oxidation pathway. The chemical similarity is that both pathways involve the change of two carbon units at a time. The differences are that the two pathways occur in different cellular compartments with different cofactors: Synthesis takes place in the cytoplasm and involves oxidation of NADPH, whereas oxidation takes place in the mitochondria and involves reduction of FADH² and NAD⁺.

Figure 5: Starting step of Myristic Acid synthesis

\[
\begin{align*}
\text{bicarbonate} & \quad \text{acetyl-CoA} & \quad \text{malonyl-CoA} \\
HCO_3^- & \quad H_3C-C-SCoA & \quad \text{ACC} \\
& \quad \text{OOC-CH}_2-C-SCoA & \\
\text{ATP} & \quad \text{A} & \quad \text{Pi} \\
& \quad \text{A} & \quad \text{P} & \quad \text{i} \\
\end{align*}
\]

For the biochemistry buffs here a brief description of the biosynthesis of myristic acid. It starts with the synthesis of malonyl-CoA in a reaction catalyzed by acetyl-CoA carboxylase (ACC) as shown in Figure 5. The attachment of the product of this reaction, malonyl-CoA, and a second acetyl-CoA to the acyl carrier protein, ACP, allows them to enter the fatty acid synthesis cycle. This transfer to the ACP moiety is directed by a transacylase enzyme. Acetyl ACP is further modified by another enzyme into acetyl-synthase making it ready for reaction with malonyl-ACP. The reaction of malonyl-ACP and acetyl synthase is a Claisen Condensation with acetyl as the

[^16]: [http://www.people.virginia.edu/~fac6g/19_01_09.pdf](http://www.people.virginia.edu/~fac6g/19_01_09.pdf), [http://chemistry2.csudh.edu/rpendarvis/carbacid.html](http://chemistry2.csudh.edu/rpendarvis/carbacid.html)
acceptor and malonyl as the donor. Once formed, acetoacetyl-ACP enters the elongation cycle for fatty acid synthesis which involves its reduction and dehydration to yield butyryl-ACP. This cycle is directed by the enzyme FAS in animals (Fatty Acid Synthase). After this first round where a four carbon chain is synthesized, elongation continues by returning to step one of the cycle and transferring the butyrate moiety from ACP to the enzyme ACP synthase. This enables it to react with a new malonyl-ACP. Figure 6 has a schematic that pictorially depicts this cycle. When the fatty acid chain reaches 16 carbons, the ACP synthase cannot accommodate the large fatty acid tail and elongation ends with hydrolysis of palmitoyl-ACP to yield palmitic acid and free ACP. In the case of myristic acid, elongation ends one round before reaching palmitoyl.

Figure 6: The Elongation Cycle

Pharmacology Of The Essential Oils

Although not directly a product or precursor to myristic acid, the essential oil compounds of nutmeg are nonetheless closely related to myristic acid since the consumption of nutmeg involves the consumption of both the oil of nutmeg and the essential oil. The essential oils are made of a number of compounds. The terpenes, Pinene, Camphene, and Dignentene (see Figure 7 for their structures), constitute the largest percentage of the oil and all have the same empirical formula \( (C_{10}H_{16}) \). But the aromatic ethers, though small in volume, are purported to cause the psychotropic qualities of nutmeg when taken in large dosages. The following, for instance, is a report from D. J. Panayiotopoulos and D. D. Chisholm of the Ross Clinic, Aberdeen, UK, published in the British Medical Journal:

An intelligent 19-year-old female with a hysterical personality took one ounce of nutmeg in water and orange juice. She had five days previously taken LSD with very little effect. She had also experimented with cannabis, but the only noticeable effect of this was that she developed a dry mouth. In contrast to this the effects of nutmeg were marked. At first she felt no effect, but after four hours she felt cold and shivery. Six to eight hours later she was vomiting severely. She saw faces and the room appeared distorted, with flashing lights and loud music. She felt a different person and everything seemed unreal. Time appeared to stand still. She felt vibrations and twitches in her limbs. When she shut her eyes she saw lights, black creatures, red eyes and felt sucked into the ground. Her mood was one of elation. She was taken by her friends to be seen by one of us (D.P.) as an emergency. She was admitted and quickly fell into a sound sleep. For the next week,
however, she felt that she was walking in a cloud and complained that her thinking was confused and she found it difficult to follow what people were saying. Her concentration seemed poor and lapses of attention were noticed.\textsuperscript{11}

Also, in the personal notes of E. Callaway, Chief of Research, The Langley Porter Neuropsychiatric Institute, San Francisco, California (April 6, 1964), he maintained that jazz musicians "have known about nutmeg for some time but will not discuss it except with friends". He goes on to say that most bohemians, addicts and students who try the spice probably are equally secretive.\textsuperscript{12} Those were the 60's for you.

**Figure 7: Structure of Terpenes Pinene, Camphene, and Dipentene.**

The primary aromatic ethers of the essential oil of nutmeg are Myristicin, Elemicin, Safrole, Eugenol and IsoEugenol. Each of these has a structure similar to amphetamine, lacking in most cases only a molecule of ammonia. Table 1 shows this remarkable resemblance.

**Table 1: Comparison of Structures of Aromatic Ethers from Essential Oil of Nutmeg with Structures of Amphetamines**

<table>
<thead>
<tr>
<th>Myristicin</th>
<th>MMDA</th>
<th>MDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Myristicin" /></td>
<td><img src="image2" alt="MMDA" /></td>
<td><img src="image3" alt="MDMA" /></td>
</tr>
<tr>
<td>The most studied individual compound found in nutmeg, especially its pharmacological properties.</td>
<td>3-Methoxy-4,5-methylenedioxy amphetamine</td>
<td>MDM; Adam; Ecstasy; 3,4-Methylenedioxy-N-Methylamphetamine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eugenol</th>
<th>Safrole</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image4" alt="Eugenol" /></td>
<td><img src="image5" alt="Safrole" /></td>
<td><img src="image6" alt="MDA" /></td>
</tr>
<tr>
<td>Used in the manufacture of vanillin, in perfumery and as a dental analgesic.</td>
<td>Used in perfumery and as an antiseptic.</td>
<td>3,4-Methylenedioxyamphetamine; Hug Drug; Pill of Love</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Elemicin</th>
<th>TMA</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image7" alt="Elemicin" /></td>
<td><img src="image8" alt="TMA" /></td>
</tr>
<tr>
<td>Valued in the pharmaceutical industry and an important flavoring agent.</td>
<td>3,4,5-Trimethoxyamphetamine</td>
</tr>
</tbody>
</table>

\textsuperscript{11}http://www.ewrowid.org/plants/nutmeg/nutmeg_faq.shtml
\textsuperscript{12}http://www.unodc.org/unodc/bulletin/bulletin_1966-01-01_4_page003.html
Although similarity in structure is an interesting phenomenon, the proof of whether or not these chemicals are truly related in form is whether or not they can be made to have the same effect. Well, of course, budding organic chemist just had to try (including the US Government through the 1960s). The following is an excerpt from one chemist’s diary of the initial steps in his protocol to convert Oil of Nutmeg into one of the amphetamines listed above:

This is one of many ways to begin making MMDA… (from Oil of Nutmeg) The careful distillation of Oil of Nutmeg (or the Oil of Mace) allowed the isolation of a number of compounds in varying degrees of purity. The fraction that boiled in the 110-115 °C range at about 1.0 mm/Hg was myristicin (3-methoxy-4,5-methylenedioxyallylbenzene). It constituted some 7% of the original oil of commerce and, in its original isolated form, was obtained with a purity of 87%. The major contaminant was elemicin (3,4,5-trimethoxyallylbenzene). A solution of 100g myristicin in 100g absolute EtOH was treated with 200g solid KOH and heated on a steam bath overnight. Removal of the volatiles under vacuum, flooding the residue with H₂O, and extraction with 3x100 mL CH₃CO₂H gave, after removal of the solvent from the combined extracts, a residue of crude isomysristicin (a mixture of the cis- and trans- isomers). This product was distilled, and the fraction boiling at 125-130 °C at 1 mm/Hg gave 63 g of isomysristicin as a pale yellow oil that spontaneously crystallized. The mp was 41.5-42.5 °C. Part of the losses associated with the purification of these solids was due to formation of the cis-isomer of isomysristicin, which was an oil.¹³

No wonder the Dutch and the English were so keen on controlling the trade of nutmeg from obscure and almost inaccessible islands off the southern mainland of Asia.¹⁴ It was an early form of a legal drug cartel as later generations did with tobacco, coffee and opium. Keep this in mind the next time you drink eggnog or eat that spice cake.

How We Use Myristic Acid: Macroscale

Although myristic acid in nutmeg is only consumed in small amounts, we use or consume it in large quantities through other means. For instance, it is a major component of many soaps. Myristic acid is the right fatty acid to make soap because, as a medium length fatty acid, it tends to create good lather foam and the bar or liquid soap itself is soft and creamy feeling. Shorter chain fatty acids make the soap too brittle with their strong defatting action and longer chains make the soap too brittle.¹⁵ Another use for myristic acid is in homeopathic medications. For instance, Jojoba Oil from the Sonoran Desert’s second most economically valuable native plant, *Simmondsia chinensis*, has become important since the 1970s when whaling was banned, since jojoba oil has almost the same properties as the oil obtained from the sperm whale. Like spermaceti, it too contains myristic acid. As myristic acid is purported to have an anti-

¹⁵ [http://www.snowdriftfarm.co.uk/fatproperties2.html](http://www.snowdriftfarm.co.uk/fatproperties2.html)
inflammatory effect, Jojoba is often recommended by herbalists as a good oil for use by those who have arthritis or rheumatism.16

And then, of course, there is food. As the fats of whole milk contain varying degrees of myristic acid, depending on their source, we have consumed different levels of this acid during different stages of our life. As babies, we breast feed on human milk whose fat contained 7.4% myristic acid. As children and adults, we increased that level slightly to 8.9% by drinking cow’s milk. And then when we decided to travel to far away lands, we increased it even further by drinking either sheep’s milk or goat’s milk (9.7% and 12.3%, respectively).17 In our cooking, we generally consumed minute amounts in the cooking oil unless we used coconut oil (18.5%) or Palm kernel oil (16.2%).18 While myristic acid has positive effects when applied to the skin, the jury is out on whether it has positive effects when consumed. Numerous trials conducted over the past 35 years have demonstrated that increasing MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids) at the expense of SFA (saturated fatty acids) helps lower serum total cholesterol and LDL cholesterol, key coronary heart disease risk factors. Myristic has been found to be the most hypercholesterolemic while the effect of palmitic is somewhere between lauric and myristic. The C18 Stearic acid has been found to have little or no cholesterol raising effect, possibly due to its rapid conversion to mono-unsaturated oleic acid.19

How We Use Myristic Acid: Microscale

Although myristic acid does not comprise a large amount of the lipid material in our bodies, it is nonetheless quite ubiquitous. It is found in all cells as either an anchor for proteins (being hydrophilic, proteins can’t easily anchor themselves to the phospholipid-bilayer) or as a conformational change agent on proteins. This is where the story really gets interesting from a chemistry and physiology standpoint.

1) Signal Transduction

Signal transduction is the process whereby information from outside the cell is conveyed into the cell, often involving internal messenger systems. The most popular second messenger system (cyclic AMP) uses G-proteins to aid in the mechanism. G-proteins are constructed from three different sub-units, α, β, and γ. The α subunit is where the G-protein is covalently bonded to myristic acid and is able to bind with GTP and cause the other subunits to dissociate. This step, for instance, is key in binding the G-protein with adenylate cyclase which initiates its messenger role of instructing the accumulation cAMP. If the binding were to become defective in some way, as when Cholera toxins interrupt the signaling, bad things, like the uncontrollable discharge of water and sodium—causing severe dehydration and diarrhea—can occur.20

2) N-Myristoylated Proteins

Myristic acid is one of the two most common fatty acids in cell membranes that are used to covalently bind to proteins with the other being palmitic acid. The bonding occurs at the polar carboxylic end of the fatty acid with the non-polar tail firmly anchoring itself in the lipid region of the phospholipids layer. The linkage occurs post-translationally and always occurs on the inside layer of the membrane facing the cytosol. These proteins typically have a glycine as the N-terminal amino acid. The glycine is attached to the myristic acid through an amide linkage, a

17 http://class.fst.ohio-state.edu/FST605/lectures/Lipidstructure.pdf
18 http://www.bcs.uni.edu/shil/HTMLpages/ResearchResources/StatisticalData/PrintTable4.htm
carboxyl group of the fatty acid bonded to the α-amino group on the glycine (see Figure 8). Sometimes proteins have a cysteine N-terminal in which case they attach through a thioester linkage (see Figure 9). These protein attachments are referred to as myristoylation. An example of this arrangement is shown in Figure 10.

The acylation of the peptide occurs as the peptide begins to emerge from the ribosome by the action of Myristoyl-CoA:protein N-myristoyl transferase (NMT). Generally, N-terminal acylation is believed to be an irreversible modification because of the strong chemical nature of amide bonds. The N-myristoylated lipoproteins constitute a large family of essential eukaryotic and viral proteins presumably with many different functions, and they are located either in the cytosol or in the cytosolic (inner) membrane of cells, or both. They are involved in regulating protein activity, perhaps by modifying their conformations, and in targeting and anchoring otherwise soluble proteins to the membranes and to appropriate receptors.

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21 http://www.lipid.co.uk/Infores/Lipids/protlip/file.pdf
Figure 10: The Myristic acid is shown embedded in the lipid layer of the cytoplasmic side of the lipid bilayer. It is attached to a glycine residue via an amide bond which in turn is connected to the rest of the protein whose C-terminal is shown as COO⁻.

Extra cellular side

Cytoplasmic Side

3) HIV Protein Myristoylation: Nef and MA

The human immunodeficiency virus (HIV) is unique in its capacity to produce chronic disease in almost all infected hosts and evading destruction by pharmacologies. One of the ways it does this is through a protein that down-modulates the epitope density on the surface of infected cells. By reducing the number of epitopes, the antigen determinant on the surface of the cell that would reveal its true nature to HIV antibodies, it reduces the chance that the CD8 (or cytotoxic) cells will destroy them (i.e., they can hide). To do this, they use an N-myristoylated protein, the Nef (or negative factor) protein. Interruption of the myristoylation process would reduce Nef's efficacy and thereby HIV's ability to evade destruction. It would also hurt another of Nef's capabilities: disruption of the pro-apoptotic pathways (preventing Hari-kari by the infected cell when it realizes that it has been co-opted by HIV).²³

An equally if not more provocative example of myristoylated proteins serving in their targeting and anchoring role is with the Gag (group antigen gene) precursor. One of the key dependencies of infectious virion assembly is NMT, which adds myristic acid to the N-terminus of Gag. The Gag protein is first synthesized as a precursor protein, Pr⁵⁵Gag, which forms the core of the virus particle. Either during or shortly after assembly of the virion, the Gag precursor is eventually cleaved into four proteins by viral protease yielding three enzymes (protease, reverse transcriptase, and integrase) and a forth segment that is further split into a number of proteins including Matrix (p17-MA, which forms the scaffolding of new virion and targets the next Gag precursor to the plasma membrane through its myristoylated N-terminal), Capsid (p24-CA), and Nucleocapsid (p7-NC)²⁴. See Figure 11 for the location of these proteins. Current research indicates that disruptions to viral assembly can occur as a result of disruptions to N-myristoylation of MA:

Targeting of the human immunodeficiency virus type 1 (HIV-1) Gag precursor Pr⁵⁵Gag to the plasma membrane, the site of virus assembly, is primarily mediated by the N-terminal matrix (MA) domain. N-myristoylation of MA is essential for the stable association of Pr⁵⁵Gag with membranes and for virus assembly. We now show that single amino acid substitutions near the N-terminus of MA can dramatically impair assembly without compromising myristoylation. Sub-

²³ http://www.bentham.org/chivr1-1/collins/collins.htm
²⁴ cumicero2.cpmc.columbia.edu/Micro_Files/2604.pdf
cellular fractionation demonstrated that Gag membrane binding was compromised to a similar extent as in the absence of the myristyl acceptor site, indicating that the myristyl group was not available for membrane insertion. Remarkably, the effects of the N-terminal modifications could be completely suppressed by second-site mutations in the globular core of MA. The compensatory mutations enhanced Gag membrane binding and increased viral particle yields above wild-type levels, consistent with an increase in the exposure of the myristyl group. Our results support a model in which the compact globular core of MA sequesters the myristyl group to prevent aberrant binding to intracellular membranes, while the N-terminus is critical to allow the controlled exposure of the myristyl group for insertion into the plasma membrane.\textsuperscript{25}

Figure 11: Location of key proteins and myristic acid within the mature HIV virion.

From a myristoylation standpoint the key protein is the matrix protein, p17. It forms the outer shell of the core of the virus, lining the inner surface of the viral membrane. In addition to directing viral assembly via targeting signals that direct the gag precursor protein, Pr\textsuperscript{55K}, to the host cell membrane, it also interacts with the transmembrane protein, gp41, to retain the env-encoded proteins in the virus. It also plays a post assembly role by directing the pre-integration complex to the nucleus of infected cells, thus enabling the virus to turn productively non-dividing cells into little virion factories.\textsuperscript{26} And the key to all of this is the myristic acid chain attached to the protein’s N-terminal.

\textsuperscript{25} cumicro2.cpmc.columbia.edu/Micro_Files/2604.pdf
\textsuperscript{26} http://www.ccrms.ox.ac.uk/idc/structures/p17/
As Pr55gag is synthesized on ribosomes in the cytoplasm, it also becomes cotranslationally modified by the N-terminal attachment of a myristyl group, which increases its affinity for membranes. Myristoylated Gag precursor molecules are targeted to the cytosolic side of the plasma membrane, where they coalesce into a patch. Through the continuous lateral addition of N-myristoylated Gag molecules, the patch grows into a spherical structure that increasingly protrudes from the cell surface and eventually pinches off, releasing an immature virus particle into the extracellular environment. An example of this is shown in Figure 12. If mutations that prevent the myristoylation of MA invariably block particle assembly and virus replication, then clearly pharmacologies that do the same thing should have the same effect. As study done by O. Wolf Lindwasser and Marilyn D. Resh entitled “Myristoylation as a target for inhibiting HIV assembly: Unsaturated fatty acids block viral budding” seems to indicate just that.

Figure 12: Budding of HIV virion from external cellular wall

Just when it all seemed too good to be true, reality hits again. In this case, the warnings that this is not the Holy Grail cure, but just another step along the way of understanding how to weaken HIV. Continued studies of this topic have indicated that while myristoylation of the Gag precursor (from which the MA protein will be derived) is a must for a nascent virion, the existence of MA in a mature virion is not necessary for replication. But every little bit helps.

Other Functions for Myristic Acid

While the connection to virology is enough for a lifetime of research, there are other ways in which one can pursue a study of myristic acid. These range from a study of myristic acid in aquatic life and its connection to cancer cures to micro-computing with myristic acid as raw materials in the year 2014. The following is a quick survey of some of the more interesting topics.

- **Whole-Cell Biocomputing**: Advancement in micro-scale engineering and manufacturing naturally leads to the desire to manipulate systems on the molecular scale and to the design of molecular-scale machines. As nature has already designed such machinery, it

27 cumicro2.cpmc.columbia.edu/Micro_Files/2604.pdf
29 http://hiv-web.lanl.gov/content/hiv-db/REVIEWS/GOTTINGER2001/Gottlinger.html

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is only prudent to use and mold these working machines to address our needs. Significant work has been done in this area and interestingly, myristic acid plays a key role in the function of the whole cell microelectronic components. Called Bioluminescent Bioreporter Integrated Circuits (BBICs), these whole cell circuits contain the enzyme luciferase and are capable of being programmed to emit light on cue. Figure 13 below, one mechanism designed by a firm of this new bio-"technology" is shown. As can be seen, this mechanism piggybacks on the fatty acid synthesis mechanism discussed earlier in this paper.

Figure 13: Biosynthesis of light using Myristic Acid as one input

![Diagram of biosynthesis of light using myristic acid]

Figure 13 shows the biochemical reactions responsible for light production luminescent prokaryotic cells. This reaction uses myristyl aldehyde as the enzymatic substrate, FMNH₂ (an electron carrier similar to FAD) and O₂ as co-effectors, and light, FMN, H₂O and myristic acid as products.

- **Jurkat Cell Stimulation:** Jurkat is a helper T cell line that produces Interleukin 2 (IL-2) upon activation. This stimulation of IL-2 production is due to an increase in the transcription of the IL-2 gene. Research has indicated that myristic acid blocks anti-CD3-induced Ca²⁺ traffic in Jurkat cells by interfering with the regulation of Ca²⁺ mobilization. This double negative indicates that myristic acid helps the flow of Ca²⁺ ions which, from physiology, we know means it helps to activate the cells functioning.

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30 [URL](http://64.58.76.136/search/cache?p=luciferase+BBIC&ei=UTF-8&vm=i&n=20&fl=0&url=--myvpV7S3BMc:embibemu.edu.cn/view/0108/616.pdf)

31 Luciferases are enzymes that emit light. A diverse group of organisms use luciferase-mediated bioluminescence to startle predators or to attract prey or mates. The luciferase from the North American firefly releases green light during the oxidation of its chemical substrate, luciferin. Other organisms, including plants, that express the luciferase (luc) gene will also glow faintly green when supplied with luciferin.

32 [URL](http://www.biochemj.org/bj/283/bj2830113.htm)
• **Bengamide Enhancer**: Twenty-four natural bengamides have been isolated, principally from *Jaspis* sponges found in coral reefs near the Fiji Islands and Australia. These compounds have been shown to demonstrate anti-proliferative characteristics against tumorous cells. Bengamide B (bearing a myristate ester) has especially shown inhibition promise against cancer growth in human breast cells implanted in mice. Because isolation of this compound from sponges is a lengthy, low-yielding process, a synthesis protocol has been developed that involves 30-32 steps for bengamide B. The structure of this compound is shown in Figure 14.

![Figure 14: Structure of Bengamide B compound](image)

- **Myristoylation as Tool**: Studies to increase the efficiency of association of tumor necrosis factor (TNF), a hydrophilic model protein, with liposomes, resulted in an N-myristoylation signal sequence being linked to the N-terminus of TNF by gene fusion. In vitro indications are that affinity levels increased.

- **Designer Drug Applications**: A dual action prodrug concept is being developed wherein a myristic acid analogue is coupled via an ester group to FLT or AZT (two anti-HBV nucleosidal agents). Subsequent intracellular cleavage of the prodrug ester would simultaneously release FLT or AZT that could inhibit reverse transcriptase and the myristic acid analogue that could inhibit myristoyl-CoA:protein N-myristoyl transferase (NMT). The current data suggest that this prodrug concept offers a potential drug design approach to design dual acting anti viral agents, with superior pharmacokinetic and biodistribution, reduced cytotoxicity and/or increased efficacy.

**Myristic Acid Research**

There are a number of research efforts underway to crack the secrets of myristic acid and its connection to human conditions and pathologies. The following is a sampling of such efforts:

33 [http://journals.iranscience.net:800/Default/pubs.acs.org/journals/orlef7/asap.cgi/orlef7/asap/html/o1026101eb00001#o1026101eb00001]

34 [http://journals.iranscience.net:800/Default/pubs.acs.org/journals/orlef7/asap.cgi/orlef7/asap/html/o1026101eb00001#o1026101eb00001]


36 [http://www.ualberta.ca/~cpsp/PPS1(3)/E-Knaus/hepatitis.htm]
- Vincent RIOUX and Philippe LEGRAND have written a report about their research efforts on myristic acid in Vol. 8, Issue 2, March - April 2001, Oléagineux, Corps Gras, Lipides. In that report they examine the recent data describing the metabolism and function of myristic acid including its origins (endogenous or dietary), uptake by the cell, incorporation into lipids, beta-oxidation rate, conversion to other fatty acids by elongation and desaturation, and the acylation of proteins.37

- George W. Gokel, Ph.D. and Prof. J. I. Gordon of the Department of Molecular Biology & Pharmacology, Washington University School of Medicine, are studying the fatty acid binding site in myristoyl-CoA: protein myristoyl transferase, NMT. They have prepared numerous analogs of myristic acid that aid in this research. As they have determined that NMT uses the coenzyme A derivative of myristic acid rather than the fatty acid itself, they seek to understand the formation of CoA derivatives and how they are bound.

- Rajiv S. Bhatnagar, Klaus Futterer, Thalia A. Farazi, Sergey Korolev, Clare L. Murray, Emily Jackson-Machelski, George W. Gokel, and Jeffrey I. Gordon & Gabriel Waksman published an article in the Nature Structure Biology Form and Structure Magazine, December 1998 Volume 5 Number 12 pp 1091 – 1097 entitled “Structure of N-myristoyl transferase with bound myristoyl-CoA and peptide substrate analogs” in which they described their approach to reveal structural features that define the enzyme's substrate specificities and regulate the ordered binding and release of substrates and products. They propose a catalytic mechanism involving deprotonation of the N-terminal ammonium of a peptide substrate by the enzyme's C-terminal backbone carboxylate.38

- Among the major topics of research for Prof. Jehoshua Katzhendler, Ph.D., Department of Medicinal Chemistry and Natural Products at the Hebrew University School of Pharmacy, Jerusalem, Israel, are analogues of myristic acid. Specifically he is researching the connection between myristic acid and CD4 derivatives with anti-HIV activity.

- Dr Nicholas Gay, Director of Studies for Biochemistry at Christ's College, Cambridge University and UCT biochemist, Dr Monde Ntwasa, are collaborating in research aimed ultimately at combating fungal diseases. They are using mutant fruit flies to study NMT. According to Dr. Gay, "'Myristoylation', as it's called, is critical for a wide variety of important signalling processes, which are essential for the growth and development of all cells. The process has adopted medical importance because it's a target specifically for anti-fungal therapies. When people get infected with fungi, such as didiasis (thrush) or Aspergillus (farmer's lung), NMT is seen as a possible target for producing drugs that are specific to the fungi."39

**Summary**

Myristic acid is more than just a good smell at Christmas. The next time you wash your hands with liquid soap or use cream or milk, remember that myristic acid is a key ingredient. Also remember that when you make that cup of eggnog not to sprinkle too much nutmeg on it or you may be experiencing more Christmas cheer than you expected. On a more serious note, this benign fatty acid plays a pivotal role in the functioning of eukaryotic and viral proteins and

understanding it is the subject of research at many institutes. Its role in viral replication and virulence alone should warrant a significant research focus. Add to that the varied ways in which it can be utilized as a tool, both medically and commercially, one can imagine a booming industry in the unraveling and use of its secrets. Continued education about myristic acid and its associated mechanisms in colleges and universities will help lay the ground work for the discoveries that will have major impacts on our health, economic well-being and longevity.

Abstract
Myristic acid is a saturated fatty acid that assumes many roles in our lives. Best known for being the main fatty acid in nutmeg (from whence it gets its name), it is also a major component of milk and soap. Recently, it has been found to be ubiquitous in the functioning of our cells and in the processes involved in viral replication. Because of its connection to Nutmeg, myristic acid has a colorful past and a bright future.
Sodium Etching

Cory Thompson

April 17, 2003
Abstract:

Polytetrafluoroethylene, or more widely known as PTFE, has been such a remarkable discovery within this century that the applications for which it has been used have become endless. However, because of its lubricity there arose a problem. The problem was that virtually nothing could adhere to the surface. The industry developed a new process called sodium etching, a process that enables PTFE to be bonded with other organic compounds. From the implementation of this process, the applications are now not as dangerous and continue to expand.
History:

Before addressing sodium etching, it is necessary to first explain the history of PTFE. Many of us have seen how remarkable it is when we are shown in the "infomercials" the amazing properties of Teflon® coated pans. Nothing sticks to them. It has also been used in the chemical processing industry as lining to pipes and transporting vessels. PTFE has also been useful in the biomedical field. It can be used to make prosthetic joint replacements among other things. The list could go on and on.

"In order to take advantage of any of the other remarkable properties of PTFE, it sometimes has to sacrifice its lubricity in order to be bonded with or laminated to another material."(Sodium Etching of Fluoropolymers, 1) Thus arises the need for a process called sodium etching. There are a number of products in the market today that use this process in order for other materials to be bonded to PTFE. These include NATREX® and TetraEtch® etchants.

The early days in the development of this process were extremely dangerous. A number of "quite toxic and very dangerous substances were involved such as tetrahydrofuran (THF), anhydrous ammonia and of course the raw sodium itself."(2) Sodium metal is an oxidizer and is highly reactive with water. "Anhydrous ammonia is a hydroscopic compound, which means that it seeks water from the nearest source."(Anhydrous Ammonia, 1) Tetrahydrofuran is an ether which is especially dangerous because it will form explosive peroxides in storage if it is in contact with air. As one can imagine from the compounds and metals named it could easily be seen that the process could end in catastrophe if not carried out in very regulated and/or isolated conditions. To lower the chance of a mishap, there were changes made to make the process less dangerous. However the metallic sodium is still the "most reliable, readily available chemical ‘abrasive’ for members of the fluoropolymer family."(2)

The medium that was originally used to carry the sodium in solution was liquid ammonia. But due to the low portability and very low boiling point of liquid ammonia (-36°C) further solvents were sought as the medium for the etching process. Sodium, naphthalene and THF were tried together but without success. As stated above, there were particular hazards in working with sodium metal and tetrahydrofuran.

A group of solvents called the glycol diethers were subsequently developed and eventually became the solvents in which the sodium naphthalene complex could easily be stored and more safely handled. Glycol ethers do not contain any functional groups. They are aprotic compounds that are rather inert chemically. They are complete miscible in both water and hydrocarbon solvents. Like other oxygen containing solvents, they tend to solvate cations. This leaves the anions active, so that for reactions involving basic reagents, the use of glycol ethers as solvents and reaction medium can greatly enhance reaction rates. Ethylene glycol dimethyl ether (H₂C-O-CH₂CH₂-O-CH₃), monoglyme for short, "has the greatest affinity for sodium and, therefore, is the easiest solvent with which to complex sodium naphthalide."(3) It is not very conventional because of its low flash point (-1°C) and high viscosity. In addition to this, above 0°C it starts to consume
its active ingredients to form methyl vinyl ether. Diethylene glycol dimethyl ether (H₂C-O-CH₂CH₂CH₂CH₂-O-CH₃), or diglyme “dissolves less sodium naphthalene in complex, has a much higher flash point (57°C...) and makes an etchant with the viscosity of about that of water.”(3) The decomposition of diglyme into methyl vinyl ether is only 10% of that of monoglyme. The current practice, therefore, is to use diglyme, sodium and naphthalene as a complex in which the process of sodium etching is performed today.

Reactions involved in sodium etching:

The process of sodium etching still presents significant safety hazards. However the mechanism and the conditions under which it is performed are more controlled. The enumerations of the health risks involved will be done later in this report. The mechanism in which the sodium naphthalene/diglyme complex is made is:

\[ \text{Naphthalene} + \cdot \text{Na} \xrightarrow{\text{DME}} \text{Sodium naphthalide (a radical anion)}\]

(Wolf, 23-51)

This is apparently the most stable medium in which the best results are obtained. The color of this mixture is a green black color with the odor of that of moth balls. There are many fluoropolymers with which the process of sodium etching is used. For an example, when a wire coated with PTFE is introduced into the liquid, there is no visible reaction. However there is much more going on under the surface of the liquid than meets the eye. The fluorine atoms at the surface of the fluoropolymer is literally being ‘rubbed’ off by the reaction with sodium in the solution. Consequently the reaction of this happening is:
The surface of the PTFE is now what is called ‘wetable’ or ‘bondable.’ “Chains deprived of fluorine are electron deficient and readily bond to oxygen and water vapor when the fluoropolymer surface is removed from the etchant bath and exposed to air.” (How Etchants Work, 1) It may not seem that a significant change has occurred. However, the change seems to be very remarkable when viewed under high magnification. The surface goes from looking like this:

![Virgin PTFE](image)
to this:

![Etched PTFE](image)

(1)

The resulting surface of the PTFE is much more conducive to bonding with other organic compounds. Small or large areas can be etched. Thus this process is not limited in its applications. For example, to waterproof a plug or switch with solder connections, the wires need to be etched so that the compound which is used, called potting, will stick to all surfaces including the wires. Thus provide a water tight connection. To actually perform the process described may seem very mundane but is very important because it expands the uses for which fluoropolymers can be used.

**Safety:**

The use of this chemical is not without its risks. However if the necessary precautions are made then the operator performing the etchant process will be within the safe parameters. The use of neoprene gloves is required when performing the operation. If the etchant comes in contact with skin it “will cause irritation and possible burns.”(Pritchard, 2) The material will also be absorbed through the skin. If it comes in direct contact with your eye, burns are very possible. Irritation to the eyes will occur from the vapors of this solution. Burns will occur in “the mouth, esophagus and gastrointestinal tract”(2) if ingested. “Breathing difficulty, respiratory irritation, dizziness, drowsiness and nausea”(2) will occur from the inhalation of the vapors. As one can gather from the difficulties that can be had while using the etchant solution, it pales in comparison to the dangers that some without doubt faced in the earlier days of the etchant process.
The sodium naphthalene/diglyme complex has longer lasting effects that may be experienced after repeated handling of the product. The studies performed on male mice "with the glycol ether have shown anemia, bone marrow damage, hemolysis, immunosuppression and testicular atrophy."(5) The organs and the organ systems that the glycol ether may affect over the long term are skin, eyes, digestive tract, liver, kidneys, blood, central nervous system and reproductive systems. Much care should be taken when working with a solution that can have so many acute and chronic effects.

Conclusion:

Sodium etching has expanded the marketability of fluoropolymers insomuch that the applications and uses are nearly limitless. There have been formidable technical obstacles to overcome trying to develop it into a technology that is somewhat safe and affordable. However, there are risks still involved with the process. But to consumers and many in the industry, the benefits greatly outweigh the risks that are inevitably involved.
Works Cited:


Abstract

The subject of this project paper will report on the toxic chemical agent that is known as Sarin Gas. An attempt will be made to identify why this is used as a lethal weapon. This paper will review the production and synthesis of this compound, biological and chemical reactions, properties, and possible military applications.

Sarin: What is Sarin?

The chemical agent Sarin gas, in spite of the fact that it would not be found “lying around the house” has become somewhat of a well-known substance due to the discord in the world. The United States is currently at war with the regime that governs Iraq, partly because they possess Sarin, among other chemical and biological weapons. Also known as Methylphosphofluoridic acid, 1-methylene ester, Isopropylmethane-fluorophosphonate, and isopropyl methylphosphonofluoridate, Sarin gas is infamous for being a nerve agent used in chemical weapons. In terms of its chemistry, this substance is identified as a toxic fluorinated organophosphorous compound and has a chemical formula that consists of C₅H₁₀FO₅⁵.

The actual structure that makes up Sarin is fairly simple, with the phosphorus being the central atom. There is only one double bond present, connecting one of the two oxygen atoms to the phosphorus atom. The second oxygen atom is also bonded to the phosphorus, in this case by a sigma bond and it is joined to the isopropyl group of the chemical. The compound also has a fluorine atom that is bonded to the phosphorus, and it is rounded out with a final methyl group. The structure of Sarin would appear as CH₃-P(=O)(-F)(-OCH(CH₃)₂) were it written in the text or for visual structure refer to the illustration 1.⁴

Physical Properties of Sarin

Having established the structure of Sarin and the atoms that constitute its parts, one is able to explore the well-documented physical characteristics. Although Sarin is usually regarded as a gas, mainly because of its use in chemical weaponry, it can most commonly be found in the liquid state. Possessing a molecular weight of 146.10 grams/mole, it has a higher than average boiling point of 147⁰C and exists mostly as a liquid in the average laboratory environment.⁵ When this volatile, colorless compound is released freely into the air it gives off an equally colorless vapor that actually resides in the air as a fine suspension of liquid, not a gas.
In discussing any nerve agent or gas that is used as a weapon, one of the most important physical properties that affects the use or production of a compound is its persistency. Persistency is very important when a chemical agent is used because of concern of the military over exposure to civilians and its own troops and serviceman. Sarin is actually considered a non-persistent compound due to its relatively short life span as an effective agent when released into an open environment. This nerve agent is only “effective” for about a half an hour under common summer conditions of 15°C or more. When compared to the 3 days that the more recently synthesized VX gas will persist, Sarin renders an area poisoned for a very short time.[3] While this might seem militarily unfavorable, it actually has its upside for troops that would advance into a recently contaminated locale.

Under winter conditions, the persistency of Sarin does vary somewhat. Colder environments, those that are at least -10°C, allow it to remain at toxic levels for up to eight hours or longer. Being highly volatile at these lower temperatures and having a density of 1.09 g/cm³ at 25°C, this very soluble substance will remain longer than eight hours if there is sufficient humidity in the air, even if the temperature increases slightly. The persistency can also be increased if it is mixed with certain oils and petroleum products. In its pure form, Sarin is a very stable substance.[3]

### Physical Properties of Common Nerve Agents

<table>
<thead>
<tr>
<th>Property</th>
<th>Tabun</th>
<th>Sarin</th>
<th>Soman</th>
<th>GF</th>
<th>VX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular weight</strong></td>
<td>162.1</td>
<td>140.1</td>
<td>182.2</td>
<td>180.2</td>
<td>267.4</td>
</tr>
<tr>
<td><strong>Density g/cm³</strong></td>
<td>1.073</td>
<td>1.089</td>
<td>1.022</td>
<td>1.120</td>
<td>1.008</td>
</tr>
<tr>
<td><strong>Boiling-point °C</strong></td>
<td>247</td>
<td>147</td>
<td>167</td>
<td>92**</td>
<td>300</td>
</tr>
<tr>
<td><strong>Melting-point °C</strong></td>
<td>-50</td>
<td>-56</td>
<td>-42</td>
<td>&lt; -30</td>
<td>-39</td>
</tr>
<tr>
<td><strong>Vapour pres. mm Hg</strong></td>
<td>0.07</td>
<td>2.9</td>
<td>0.3</td>
<td>0.06</td>
<td>0.0007</td>
</tr>
<tr>
<td><strong>Vapour pres. mg/m³</strong></td>
<td>600</td>
<td>17,000</td>
<td>3,900</td>
<td>600</td>
<td>10</td>
</tr>
<tr>
<td><strong>Solubility in water %</strong></td>
<td>10</td>
<td>Complete</td>
<td>2</td>
<td>~2</td>
<td>3 (oo &lt; 9.5 °C)</td>
</tr>
</tbody>
</table>

*= at 25°C **= at 10 mm Hg[3]

### Origin of Sarin

The desire to manufacture a good pesticide was the original driving force that led to the eventual synthesis and use of Sarin as a chemical weapon. In the early part of the 1930’s it was discovered that organo-phosphorus compounds were toxic and it was subsequently hypothesized that one of these materials could possibly operate in that role. Therefore in 1934, in Nazi-ruled Germany, the chemist Dr. Gerhard Schrader and
colleagues found themselves assigned the tedious and dangerous task of synthesizing a viable pesticide. After laboring for two years, Snrader produced a compound that had an exceedingly high toxicity to living organisms. This substance, known as Tabun, was a precursor that led to the development of Sarin.

After reporting the vicious nature of the newly discovered material, they were commissioned to continue their work; this time, with designs on creating something to be used as a weapon. After creating upwards of 2,000 new organo-phosphorus compounds, the team synthesized the most toxic in 1938. It was to be known as Sarin, deriving its name from the chemists that participated in its creation, Shrader, Ambrose, Rüdiger, and van der Linde.\textsuperscript{[6]}

**Synthesis of Sarin**

The following is the traditional synthesis for Sarin\textsuperscript{[1]} or isopropyl methylphosphonofluoridate:

**First stage**

\[
\begin{align*}
\text{Cl} \quad & \quad \text{P} & \quad \text{Cl} \\
\quad & \quad \text{Cl} & \\
\quad & \quad \text{Cl} & \\
\end{align*}
\quad + \quad \\
\text{H} & \quad \text{C} & \quad \text{O} & \quad \text{H} \\
\quad & \quad \text{H} & \\
\quad & \quad \text{H} & \\
\quad & \quad \text{H} & \\
\rightarrow & \quad \\
\text{P} & \quad \text{O} & \quad \text{O} & \quad \text{CH}_3 \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\rightarrow & \quad \\
\text{P} & \quad \text{O} & \quad \text{O} & \quad \text{CH}_3 \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\end{align*}
\]

During the first stage, care must be taken as hydrochloric acid is produced. The starting products are not particularly dangerous. PCl\textsubscript{3} is toxic if inhaled as hydrochloric acid would be formed. Methanol is extremely flammable.

**Second Stage**

\[
\begin{align*}
\text{P} & \quad \text{O} & \quad \text{O} & \quad \text{CH}_3 \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\rightarrow & \quad \\
\text{P} & \quad \text{O} & \quad \text{O} & \quad \text{CH}_3 \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\quad & \quad \text{OH} & \\
\end{align*}
\]

Simple heating will result in this transformation shown; however the product shown below is also formed simultaneously.

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{P} & \quad \text{O} & \quad \text{O} \\
\quad & \quad \text{CH}_3 & \quad \text{OH} & \quad \text{OH}
\end{align*}
\]

(byproduct)
Third Stage

\[
\begin{align*}
\text{H}_3\text{C} - \text{P} & \equiv \text{O} + 2\text{Cl} \rightarrow \text{H}_3\text{C} - \text{P} & \equiv \text{O} + \text{PCl}_3 + \text{CH}_3\text{Cl} + \text{HCl} \\
\end{align*}
\]

During this stage care must be taken as chlorine is required as well as the toxic PCl₃ used in the first stage. Yet again HCl is produced so care must be taken. The product can be separated from the other products by distillation.

Fourth stage

\[
\begin{align*}
\text{H}_3\text{C} - \text{P} & \equiv \text{O} + 2\text{HF} \rightarrow \text{H}_3\text{C} - \text{P} & \equiv \text{O} & 2\text{HCl} \\
\end{align*}
\]

Yet again hydrochloric acid is produced during this stage and the hydrofluoric acid used is extremely dangerous.

Fifth stage

\[
\begin{align*}
\text{F} & \text{H}_3\text{C} - \text{P} & \equiv \text{O} + \text{H}_3\text{C} - \text{P} & \equiv \text{O} \\
\text{F} & \text{H}_3\text{C} - \text{C} & \equiv \text{C} - \text{CH}_3 \rightarrow 2 \text{H}_3\text{C} - \text{P} & \equiv \text{O} + 2\text{HCl} \\
\end{align*}
\]

Military Synthesis of Sarin

The synthesis of Sarin as performed in a laboratory generally includes the five stages listed above. In the past the military followed these same procedures and stored the pure substance they synthesized. While the military still remains the main known producer of Sarin in the United States, this is not the current way in which the nerve agent is produced for weaponry purposes. Due to the danger of soldiers transporting and handling weapons filled with such a toxic substance, the last stage of synthesis is usually delayed until a missile or other delivery system is in flight to its destination.

As is shown in illustration #2[^3], there are separate substances that are initially packaged into the missile. In the case of Sarin, the missile would contain methylphosphoryldifluoride and isopropanol. Isopropylamine would also be included in with the isopropanol so that the hydrogen fluoride produced could be removed as the reaction moved forward. A disc inside the cylinder of the missile routinely separates the reactants involved in forming the Sarin. This rupture disc serves to divide the components before breaking up at a pre-
disposed point during the flight. The elimination of the barrier allows the final stage of synthesis to take place as the components mix.

In spite of all the planning and technology, problems still arise for the military as they try to devise ways to use Sarin and other chemical agents as weapons. The conditions and environment that a missile or bomb encounters during its flight can vary greatly, affecting the yield of a desired product. They also have to deal with the fact that these projectiles are closed systems and any undesired products from a side reaction will not be removed. Nevertheless, new and better delivery systems are being devised that will ensure the most pure and lethal product will be yielded and delivered to a target.

**Why is Sarin so dangerous?**

Sarin has been around for well over half a century. While there have been other powerful biological, germ, and nerve gas weapons created, it is still one of the most dangerous. Along with other nerve gases, the damage that can be done to a population of soldiers or civilians is immense. They are regarded as some of the most dangerous materials that are known to man. It is 26 times more toxic than cyanide and the lethal dose for an average human is about 0.5 milligrams. A decent sized drop would be enough to kill several people. All that considered, one asks the question: Why is Sarin, together with the other nerve gases, so dangerous?

Nerve gases are known as such because of the devastating effects that they produce on the central nervous system of a person, during even extremely limited exposure. A person usually comes in contact with Sarin through contact with the skin or eyes and through respiration. Even with only a miniscule amount of Sarin brought into the body, the individual will immediately have difficulty breathing as their airways become obstructed. Their pupils begin to enlarge and dilate while a feeling of nausea begins to set in. This is usually accompanied by drowsiness and headache and usually takes place in less than two minutes. These changes are being brought on chiefly because of the ability of Sarin to inhibit the enzyme acetyl-cholinesterase.

![Diagram of nerve cell and muscle cell with acetylcholine, receptor, acetylcholinesterase, and Sarin](image)

Acetyl-cholinesterase is responsible for the hydration and breakdown of acetylcholine after it has delivered its chemical message to the muscle cell. It removes the
acetylcholine, virtually instantaneously, so that the receptors of the cell are able to receive the continuous message that the nerve is trying to deliver. When Sarin is absorbed by the body it attacks the areas of the nerves that receive the messages and restricts the ability of the enzyme, acetyl-cholinesterase, from removing and breaking down the messages that have been sent. The result is disastrous for the individual. Convulsions and spasms result as the movements of involuntary muscles become repetitive. Paralysis sets in as the Sarin has left no effective enzyme to break down the acetylcholine or chemical message produced by the nerves. Breathing becomes shallow until it ceases. Death ensues.\[1\]

**Chemistry of Acetyl-cholinesterase Inhibition**

The following is the chemical reaction as it takes place at the junction of the synapses and the nerve cells without the introduction of Sarin:\[1,2\]

1. Acetylcholine molecule and active site of enzyme shown together without having undergone any reaction.

2. Acetylcholine combines with the enzyme to form a substrate-enzyme intermediate.

3. The ester link in the acetylcholine has been broken and free choline has been formed.

4. The acyl group has become detached from the enzyme leaving: choline, acetic acid, and the enzyme returned to its original state.

In the above illustrations the removal of the acetylcholine from the receptor site of the muscle cell is almost instantaneous. Chemical messages or “action potential” is transferred from the synapses and then are turned off again without delay. When Sarin is introduced into the body of the individual, whether it be from inhalation or exposure through the skin or eyes, and makes its’ way to the nervous system of the individual it wreaks havoc on this process.
The following illustrations demonstrate the chemical reaction that takes place as Sarin inhibits the chemical message or “action potential” from being hydrolyzed by the enzyme as it makes its way from the synapse to the receptor of the cell.  

1. Sarin binds its phosphorus atom to the active site of the enzyme as the synaptic molecule attempts to deliver its message.

2. The hydrogen is removed from the synaptic molecule as Sarin bonds to its nitrogen.

3. The synaptic message is removed from the receptor of the muscle cell without undergoing hydration and is now free to accumulate in the nervous system. This accumulation results in the symptoms of Sarin exposure such as blurred vision, convulsions, labored and restricted breathing, and in many cases, death.

Conclusion

Sarin is one of the most toxic substances to be synthesized by man. The capability that it has to affect the human organism and cause damage to the central nervous system is alarming. The average chemist could easily synthesize Sarin, since the reagents needed to produce it are readily available. In a utopian world effort would be made to monitor and control the people and organizations that have access to the chemicals used to make Sarin. However, many countries, including the United States and Iraq, possess Sarin and other chemical weapons.
Bibliography


The Study of A Reaction Mechanism of the Artemisinin: an Antimalarial Agent
(3R,5aS,6R,8aS,9R,12S,12aR)-Octahydro-3,6,9-trimethyl-3, 12-epoxy-12H-pyran[4,3-j]-1,2-
benzodioxepin-10(3H)-one

By

Candace H. Vo

Paradise Valley Community College
Spring 2003
Introduction

Malaria continues to be to one of the major health problems and challenge of the world to date. The disease has infected approximately 300 to 400 million people worldwide representing 40 percent of the world’s population. It also causes over one million deaths annually. Although malaria is found throughout the tropical and sub-tropical regions of the world, ninety percent of malaria-related deaths occurred in the sub-Saharan Africa. These infected individuals are mostly children under the age of five. According to the World Health Organization (WHO) the children mortality rate caused by severe malaria is about one African child in every 30 seconds. Moreover, those children who survived through an acute malaria episode may suffer from learning impairment and brain damage. Pregnant mothers and their fetus are also particularly vulnerable to malaria and is the major cause in perinatal mortality, low-birth weight, and maternal anemia. In addition to children and pregnant mothers, those immunocompromised individuals such as HIV/AIDS patients also find themselves vulnerable to the disease (15, 16, 17).

Despite extensive control efforts, the incident of malaria has not been decreased in most malaria-endemic regions of the world but, in some regions, it has rather been increased. This is due to the problem of the multi-drug resistance occurred among the Plasmodium species, particularly the P. falciparum from Southeast Asia since 1970’s. At the present, most of the conventional antimalarial chemotherapy agents such as chloroquine and other alkaloids, sulfaamides, and diaminopyrimidines are less effective in treating severe malaria infections such as cerebral malarial caused by the P. falciparum species. In most cases infection of cerebral malaria has often resulted in death. The consequence of lacking the potentially efficacious and safe antimalarial agents raises the possibility of epidemics to occur to those populations that have little or no immunity to malaria (16, 17).

In the 1970’s, the search for an alternative class of non-alkaloid antimalarial compounds began with an isolation of the cyclic 1,2,4-trioxane artemisinin from the tealeaves of Artemisia Annua. Also known to the Chinese as Quinghaosu, a remedy to treat fever since 281-340 A.D., artemisinin was found to have chemical and biological properties of a fast-acting and high antimalarial agent ideally for combating the disease and its multi-drug resistance problem (9). Since the findings of artemisinin, several of its semi-synthetic derivatives have been synthesized and developed clinically for treating malaria in the endemic areas. Among these compounds, artemether, artemether, arteunate, and artelinate have been given in millions of doses in Asia, Africa, and Central America (4, 10).

Since its inception, the Special Programme for Research and Training in Tropical Diseases (TDR) has coordinated research efforts from worldwide collaborations in the attempt to improve existing methods and develop new and cost effective approaches for combating malaria and its multi-drug resistant problem in endemic regions. Among these international initiatives has been the on-going research to develop clinically a second generation of the more fast-acting, efficacy, safe and cost-effective antimalarial
artemisinin. During the past decades, numerous studies have been conducted on the design, structure, and synthesis of artemisinin analogues. According to the researchers, the findings from these studies have shown promising therapeutic leads and have been used to develop a chemical understanding of artemisinin and its derivatives (4). A current review on scientific literatures of the antimalarial artemisinin suggests for a consensus among the researchers that the artemisinin molecule reacts with the ferrous ion found in heme during the hemoglobin degradation of the infected erythrocyte generating a cascade of potentially cytotoxic intermediates (4,7,10). A further structure-activity relationship (SAR) review indicates that the reaction is mediated by the iron (II) reduction of the peroxide linkage on artemisinin’s cyclic trioxane to form a sequence of potentially lethal oxygen-center free radicals, carbon-centered free radicals, high-valent iron oxo species, and reactive electrophilic alkylation species such as epoxides and dicarbonyl compounds. Consequently, any of these intermediates and/or a combination of them is believed to be responsible for intervening and disrupting vital biochemical process of the invading parasite (3,10,12).

The purpose of this study is to investigate and to outline the mode of reaction of artemisinin purported by the medical researchers to be highly antimalarial. Due to the absence of a characterization of relevant biological adduct(s) from such reaction, the study primarily aims at the step-wise chemical degradation process of artemisinin and its analogues in a unified scheme that responsible for killing the Plasmodia. In particular, an analysis is based on the reaction of a simplified cyclic trioxane of an artemisin-type antimalarial, 4β-methyl-3-phenyltrioxane, and iron (II) bromide ion.
Artemisinin and Its Derivatives

![Molecular Structures of Artemisinin and Derivatives](image)

**Figure 1.** Molecular Structures of Artemisinin and Derivatives

The general chemical structure of artemisinin and its derivatives is consisted of the tricyclic 1,2,4-trioxane. The corresponding molecule(s) is an unusually stable sesquiterpene lactone with a peroxide linkage. The presence of the peroxide linkage is essential for the ferrous-mediated univalent reduction reaction during which a cascade of potentially cytotoxic intermediates are generated. It has also been suggested that these radical species are responsible for killing malarial parasites through alkylation and/or oxidative processes (4,10). (A study of a possible mechanism scheme proposed by Posner and others and the characterization of an artemisinin compound are illustrated in the following section of this report.)

**Artemisinin** (C₁₅H₂₂O₅) compound is a white crystalline powder. It is generally hydrophobic and has a melting point between 150-153 °C. The compound is soluble in ethanol and hexane. Since its discovery there have been numerous synthesizes of water or oil-soluble artemisinin analogues such as dihydroartemisinin, artesunate, artemether, artelinic acid and arteether. Artemisinin acts as a blood schizontocide and therefore kills malarial parasites during the human erythrocytic stage of the malarial infection (21).
Artesunate (C₁₉H₂₈O₉) is a slightly water-soluble compound. Artesunate is very soluble in dichloromethane, ethanol, and acetone. It has the melting point of 144°C. Artesunate is synthesised by the reaction of dihydroartemisinin (C₁₅H₂₄O₅) and succinic acid anhydride (C₄H₄O₃) in an alkaline medium. This type of reaction invariably yields an ester linkage in alpha configuration (21).

Artemether (C₁₆H₂₆O₅) is insoluble in water and very soluble in dichloromethane, acetone, ethyl acetate and dehydrated ethanol. The compound has a melting point of approximately 84 - 86 °C. It is synthesised from dihydroartemisinin using methanol and a catalyst in acidic medium with a predominantly β-artemether is observed. The α-epimer is also produced but the compound is difficult to purify. The pure alpha product a melting point of 100°C whereas the β-epimer has a melting point of 84-86°C. Both the α and β-epimers are active antimalarials (21).

Arteether (C₁₇H₂₈O₂) chemical properties are similar to that of artemether (21).

Dihydroartemisinin (C₁₅H₂₄O₅) is colorless or white, crystalline powder. It exists as a mixture of α and β epimers. The compound is insoluble in water and soluble in acetonitrile, ethanol, and dichloromethane. Its melting point is about 140 °C. Dihydroartemisinin should be kept in a well-closed container, protected from light and stored in a cool place (21).

Reactive Mechanism of Artemisinin: An Analysis of the Chemical Degradation Process and the Structure-Study Relationship (SAR)

According to Posner and others at John Hopkins University's School of Medicine, the mechanism of artemisinin and artemisinin-type analogues such as the 4β-methyl-3-phenyltrioxane illustrated in this study is a chemical degradation process during which a chain of reactions is initiated with the reduction of the oxygen-2 of the peroxo linkage of the trioxane molecule by a ferrous (II) ion, generating the oxygen-centered radical intermediate (refer to Fig. 2). This reaction is also known as the Fenton reaction (19). In the second step of the chain reaction, the oxygen-centered radical formed from the last step abstracts a secondary C₄ hydrogen atom via a 1,5-H shift of the McLafferty rearrangement forming a highly-reactive carbon radical intermediate, a C₄ radical. Following formation of the C₄ radical intermediate, the chain reaction continues to undergo with either (1) the β-scission of a high-valent iron oxo radical species [Fe^{III}-O- ← Fe^{IV}=O] and the rebound epoxidation to an epoxy intermediate and liberate the ferrous ion (Fe^{II}), or (2) direct epoxidation to liberate the ferrous ion and to generate an epoxy intermediate. Protonation of the epoxide formed from the prior step produces a penultimate C₄-hydroxy epoxide. Rearrangement of the C₄-hydroxy epoxide generates aryl ketone at the C₄ in this mechanism scheme (4,10). Of the two alternative epoxidation steps mentioned above, the β-scission step is considered critical for antimalarial activity since it produces a strong oxidizing and possible cytotoxic high-valent iron oxo species [Fe^{III}-O- ← Fe^{IV}=O] (10).
Additional review on the structure-activity relationship study (SAR) suggests that the 1,5-H shift (McLafferty Rearrangement) pathway leading the C₄ radical center and the β-scission helps to identify the restriction on the carbon-center radical in antimalarial activity. According to Posner and others, artemisinin-type analogues with the C₄ substitution stabilizes the resulting tertiary radical and therefore increases antimalarial activity over the C₄ unsubstituted system because it encourages the formation of the C₄ radical. In comparison with C₄-substituted system, the C₄α-substitution decreases efficacy relative to its parent system. On the contrary, the C₄β-substituted analogues stabilizes the resulting tertiary radical more than simple alkyl such as the C₄-((trimethylsilyl)methyl)trioxanes. However, C₄-phenyl substitution is found to have lower malarial potency compared to that of the C₄-unsubstituted systems according to the researchers (4). In general, any system that intercepts a C₄ radical following by the elimination of radical species other than the high-valent iron oxo species [Fe^{III}-O⁻ ↔ Fe^{IV}=O] would lower antimalarial according to the researchers. Derived from the studies of Posner and others suggest that the C₄ radicals and the high-valent iron oxo radical species are responsible for killing the parasite (4, 6, 10).

![Reaction Scheme of 4β-methyl-3-phenyltrioxane with Fe(II) bromide](image)

**Figure 2.** Reaction Scheme of 4β-methyl-3-phenyltrioxane with Fe(II) bromide

Recent Developments: International Collaboration on Antimalarial

Artemisinin and its derivative have been used to treat malaria throughout Southeast Asia and have been effective against acute cases such as cerebral malaria with minor toxicity. In July 2002, the United State’s FDA Anti-Infective Drugs Advisory Committee has unanimously voted for the rectal artemisunate as a new drug. According to Medicines for Malarial Venture (MMV), the rectal artemisunate has been approved for its efficacy and safety and the drug has been satisfactorily addressed and is approvable. Contingent upon a few quality issues the drug will be approved under the regulations of 21-CFR 314.510 of Subpart H – Accelerated Approval of New Drugs for Serious and Life Threatening Illnesses in the United States, and under orphan drug status, which gives this development special protection and support (19, 20).

In addition to the upcoming rectal artesunate, the Medicines for Malarial Venture’s (MMV) new drug portfolio includes an intravenous artemisinin, artemisone and other antimalarial-artemisunate combination are in preclinical and phase I clinical developments. It is also projected by the World Health Organization that these preclinical and clinical developments to be completed during the next three years in the attempt to eradicate malarial within the endemic regions of the world (20).

Figure 3. Malaria Endemic Countries
(http://www.cdc.gov/travel/diseases/malaria/index.htm)
Conclusion

Malarial continues to be a major cause of morbidity and mortality in many areas of the world, particularly the sub-Saharan Africa and Southeast Asia. The disease has infected approximately 300-400 millions worldwide and is responsible for killing over one million deaths annually. Of those, children are the most frequent victims comprising eighty-five percent of total malarial mortality. Since the 1970’s, incidents of malarial have been increasing in the endemic areas as the result of the multi-drug resistance among the Plasmodia species. Conventional antimalarial agents such as chloroquine and other alkaloids are becoming less effective in treating acute cerebral malarial caused by the P. falciparum species.

The search for an alternative non-alkaloid began with an isolation of the cyclic 1,2,4-trioxane artemisinin from the tealeaves of Artemisia Annua. The agent was found to have chemical and biological properties of a fast-acting and high antimalarial agent ideally for combating the disease and its multi-drug resistance problem. Derived from the studies of many researchers is a general consensus that the artemisinin molecule is believed to be responsible for killing the Plasmodia in a chemical degradation process during which the molecule reacts with the ferrous ion found to generate a cascade of potentially cytotoxic intermediates.

Since its findings, several of its semi-synthetic derivatives such as artesunate, artemether, and arteether have been synthesized and developed clinically for treating malaria in the endemic areas. Current formal clinical developments of new artimisinin drugs in various forms is made possible with the combined research efforts of world wide scientists under the coordination of the World Health Organization of TDR.
REFERENCES


The Effectiveness and Differences between Drugs that Treat Serotonin Deficiencies:

A Comparison of Today's Leading Antidepressants

by

Megan Weick

Professor Hank Mancini
Organic Chemistry 236
25 April 2003
Abstract

Neurotransmitter deficiency is a leading cause of depression disorders in humans. One target neurotransmitter is 5-hydroxytryptamine, also known as serotonin. There are drugs called antidepressants that focus on inhibiting the reuptake process of neurotransmitters and allow for excess levels to be produced. One class of antidepressants in particular is the SSRI group, or selective serotonin reuptake inhibitors, which will be the focus of in this research document.
Outline

Title: The Effectiveness and Differences between Drugs that Treat Serotonin Deficiencies: A Comparison of Today’s Leading Antidepressants

Thesis: Depression is a major disorder among the public and many patients are left untreated. Serotonin disorders are an underlying cause of many depressed people. Serotonin deficiencies lead to depression, anxiety, obsessive compulsive and anger disorders. Drugs have been developed to these conditions, but each has different properties and effectiveness. Four of the most common antidepressants of the selective serotonin reuptake inhibitor class will be analyzed.

I. Serotonin: Introduction: Function in body, effects of deficiency
   a. Serotonin deficiency causes depression
   b. How antidepressants work

II. Types of Antidepressants and how they work
   a. Prozac: General information
   b. Chemical makeup.
   a. Zoloft: General information
   b. Chemical makeup
   a. Celexa: General Information
   b. Chemical makeup
   a. Paxil: General information
   b. Chemical makeup
   c. Synthesis of (+)- and (-)-paroxetine

III. Overview (Conclusion)
1. The Functions of Serotonin

Serotonin was first isolated from serum as a vascular constricting factor. "Sero" translates to "serum" and "tonin" translates to "vascular" from Latin (1). After it was isolated, the molecule was purified and the chemical structure was determined. It was named 5-hydroxytryptamine and is labeled 5-HT for short.

![5-hydroxytryptamine (5-HT)](image)

Serotonin is an ancient molecule. It is a protein of 444 amino acids and has a molecular mass of 51 kDa. Serotonin is made from the essential amino acid L-tryptophan. This amino acid is obtained from dietary sources and is not synthesized by humans. L-tryptophan is the least common amino acid in natural proteins. It evolved from a common ancestral protein at least 750 million years ago. It is widely distributed and predates the formation of the nervous system. Serotonin is found in tissues of almost every mammal. In worms and snails, serotonin neurons in the ganglia are involved during learning events. Serotonin plays a central role in memory processes in snails. They use it to adjust to non-harmful stimuli. In plants, serotonin assumes a central role in phototropism and germination. It also is used in chemical defense for preservation of the species. In some species serotonin is concentrated in venom and contributes to vasoconstriction that occurs at the sight of injury. Serotonin plays an important role in invertebrate learning and controls many important functions in the mammalian brain. Milk, fruits, nuts, turkey and some other meats have tryptophan levels of abundance. Tryptophan can increase after a meal if the food contained it. The increased 5-HT levels are responsible for the sleepy feeling after that Thanksgiving turkey meal. Diets rich in tryptophan are important to depressed patients (1).

The brainstem is the oldest part of the brain; the forebrain evolved much later. The reticular neurons, large and highly branched neurons that receive information from modalities such as touch, hearing, and seeing, are the oldest cells in the brain stem. Reticular neurons are clustered along the midline or raphe region. From this region, multiple pathways branch throughout the brain and into the spinal cord. All neurons in the brain that synthesize serotonin are located in the raphe nucleus (8). There are two major groups of raphe neurons: the group that branches to the forebrain and the group that extends to the spinal cord. Vesicles are small membranes sacs that store serotonin
and are located near release sites. Less than a million neurons produce 5-HT in the human brain. Axonal projections of neurons innervate every area of the brain. They produce 5-HT and distribute serotonin to about 1000 billion neurons. Therefore, one serotonin molecule can influence about 10,000 target neurons (1).

Serotonin is manufactured in nerve cells within the raphe nuclei. After it is synthesized, it gets transported to nerve endings throughout the brain and spinal cord. Nerve cells carry information in the form of electrical impulses and cells must get signals across the gaps, called synapses, in order to communicate. Neurotransmitters like serotonin are the messengers that bridge the gaps. In vesicles, tiny sacs at the ends of nerve cells, serotonin is stored. These sacs merge with the nerve ending's outer membrane when triggered by an electrical signal. The sacs release the neurotransmitter into the synapse when they merge with the nerve's membrane. After the serotonin molecules diffuse across the gap they bind to specialized proteins called receptors on the surface of the secondary nerve cell. When enough serotonin molecules have been taken, the receptors releases the molecules which are then either broken down or reabsorbed by the first nerve cell and stored for later use (5). Every cortical neuron is in close proximity to a tryptophan bouton, an enlargement on the axon where serotonin can be released. Therefore, the entire brain is continually bathed in serotonin and firing continuously during the wake cycles of a human. When a person is asleep, the raphe neurons appear to be shut down (1).

![Figure 3. Schematic of a raphe nucleus serotonergic neuron. Receptors are illustrated with 7 transmembrane-spanning segments. 5-HT = 5-hydroxytryptamine (adapted with permission from Briley and Moret).](image-url)
Serootonin neurons fire in continuous, slow and rhythmic patterns. They are in register with muscular activity and fire constantly when a person is awake. It is believed that firing in harmony with other neurons distributed throughout the brain and spinal cord is an important characteristic for neurons to establish a long and lasting connection.

Many evolved processes regulate serotonin synthesis. Serotonin synthesis is demanded throughout the brain. Tryptophan hydroxylase is the enzyme for tryptophan and is the rate-determining step in serotonin production. Tryptophan hydroxylase activity has a rapid regulatory control that depends on phosphorylation. Phosphate ions are obtained by the action of the kinase enzyme that is activated when serotonin neurons are firing. Serotonin neurons have many branches distributed throughout the brain and can rapidly and locally regulate the synthesis of 5-HT without requiring the transport of new enzymes from the cell body area. Serotonin has immediate demands and transporting materials can take many hours to days to accomplish.

It is proposed that serotonin is involved in homoeostatic regulation of the entire brain because it has such diverse cellular targets. Strong evidence links serotonin and depression. The loss of serotonin may actually be responsible for the disease itself (1).

Serotonin Deficiency Causes Depression

Depression is a clinical mood disorder that is diagnosed by these core signs: depressed mood, diminished interest and enjoyment in activities, significant appetite or weight change, sleep problems such as insomnia or hypersomnia, fatigue, loss of energy, inability to concentrate, indecisiveness and thoughts of death and dying which typically include suicide. Depression affects nearly nineteen million, or approximately ten percent, of the population over eighteen years of age, and only thirty percent of depressed patients receive treatment according to surveys done by the National Institute of Mental Health (8).

Neurotransmitters that effect mood have been targets for depression studies. They are serotonin, dopamine. Monoamine oxidase (MAO-A and MAO-B) is an enzyme that degrades serotonin and is also a target for studies in the treatment of depression. Drugs have been developed that inhibit the reuptake process of these neurotransmitters and allow for higher levels of them to remain in the brain. These drugs are what we commonly know as antidepressants. Antidepressants are serotonin or dopamine inhibitors. Monoamine oxidase inhibitors (MAOIs) inhibit the binding of serotonin to the MAO-A and MAO-B enzymes (8).

Antidepressants were made available for the first time more than forty years ago. Half of today’s antidepressants were made available between 1988 and now (8). SSRIs are more favorable of the antidepressants because they have fewer side effects such as weight gain and sleepiness (10). There are currently three classes of antidepressants: monoamine oxidase inhibitors (MAOIs), biogenic amine neurotransmitter reuptake blockers, and serotonin type 2A receptor blockers.

Monoamine oxidase is present in mitochondria, which are found in the nerve ending. In most cells of the body, the inhibition of monoamine oxidase results in an increase in the concentration of neurotransmitter available for release at the synapse.
Biogenic amine neurotransmitter reuptake blockers are the antidepressants commonly known as specific serotonin reuptake inhibitors (SSRIs). Serotonin type 2A, 5-HT 2A, receptor blockers block the 5-HT-2A receptor from binding with serotonin. Antidepressants that block 5-HT2A receptors are believed to contribute to anxiety, sleep disturbances and sexual dysfunction disorders. The blockage of these receptors believed to help these disorders and is why some antidepressants are bi-functional with other disorders (8).

Some common antidepressants:

Antidepressants are currently marketed as racemic mixtures, pure enantiomers or as achiral antidepressants with chiral metabolites. There is a significant contribution made by either an enantiomer’s stereoselective metabolism or pharmacogenomics to a drug’s pharmacology and toxicology (3). Pierre Baumann quoted in the European Neuropsychopharmacology article, “Enantiomers’ potential in psychopharmacology—a critical analysis with special emphasis on the antidepressant escitalopram,” “Once the preserve of chemistry textbooks, enantiomers are rapidly becoming part of mainstream medicine in psychiatry and other areas of clinical practice.” Antidepressants most often present a chiral structure (3).

How SSRIs Work: The Reuptake Process

For some biogenic amine neurotransmitters, such as serotonin, norepinephrine, and dopamine, they are taken back through the nerve ending after they are released. This is called reuptake and occurs through transport proteins that scientists have been able to clone. This mechanism prevents the over-stimulation of receptors in the synapse. Blocking the transport with a drug causes over-stimulation to occur. The vast majority of antidepressants block the transport of neurotransmitters back into the cells from which they were released.

Desensitization and down-regulation can occur after long-term treatment with antidepressants. Desensitization is the loss of the sensitivity of a cell to neurotransmitters and down-regulation occurs when the cellular surface loses its receptor protein. By removing the negative feedback loops using desensitization and down-regulation of these autoreceptors, synaptic levels of serotonin are greatly increased in the continued presence of the uptake blockade. This is the theory of how SSRIs increase the levels of serotonin and other biogenic amine neurotransmitters. This is only theory, though. It has not been shown to occur in humans yet, only animals. Testing cannot be performed on humans because of the health risks. Newer antidepressants are more selective and potent than the older compounds at blocking the transport of serotonin and continue to become more specialized (8).

Treatment of depression with SSRIs must be long-term. Short-term treatment with an SSRI yields modest amounts of serotonin because the negative feedback loops prevent excessive amounts of serotonin to accumulate. Long-term treatment causes an overall increased firing rate of raphe neurons, an increase in serotonin synthesis, and an increased release of serotonin by the presynaptic autoreceptors. By removing the
negative feedback loops by desensitization and down-regulation of these autoreceptors, synaptic levels of serotonin are greatly increased in the continued presence of the uptake blockade (8).

Monoamine oxidase (MAO) type A degrades serotonin when serotonin is released. It can also be taken back into the serotonergic terminal by a reuptake protein that is located on the serotonin axon. If the reuptake action is blocked, serotonin remains in the extra-cellular space for an extended period of time. SSRIs are developed for this process and this is why they are called specific serotonin reuptake inhibitors. When serotonin is brought back in to the terminal from the extra-cellular space, it remains there to be re-released, moved back into vesicles, or degraded by MAO type A.

The precise action of mechanism for SSRIs is not yet fully understood. The most important effect is believed to be the enhancement of actions of serotonin from the blockade of the serotonin reuptake at the neuronal membrane (4).

When serotonin is in the cytoplasm of a cell is a target for monoamine oxidase. When serotonin is in the cytoplasm it can be released directly when a reuptake protein works in reverse order. Many drugs of abuse such as cocaine, methamphetamine, and ecstasy cause the reuptake protein to work in reverse order. These drugs release nonvascular serotonin in large amounts and the effects vary from hallucinations to feelings of extreme pleasure (1).

II. Types of Antidepressants and How They Work

Fluoxetine

(4)

The drug fluoxetine sells under the common name Prozac. Prozac is administered orally and absorbed from the gastrointestinal tract. Eli Lilly & Co. produces Prozac and its commercial derivatives (4).

Fluoxetine hydrochloride was the first drug of the SSRI class to be approved (10). It was approved in December of 1987 for the treatment of depression and in 1994 for the treatment of obsessive-compulsive disorder (OCD). Prozac is a racemic or (R)- and (S)-fluoxetine. R-fluoxetine is slightly more potent and was under clinical development.
originally as a second-generation SSRI, but it was soon discovered that both R-fluoxetine and S-fluoxetine contribute to its effects (7). In turn, the focus shifted from creating an enantiomer of fluoxetine to specializing one of citalopram because citalopram showed stereoselective preferences in reacting with the enzymes that degrade it.

Fluoxetine is highly protein-bound with an affinity of 94.4%. This means that 94.4% of the drug is available after a single oral dose. It bonds primarily to alpha-1 acid glycoprotein. Therefore, the drug is well distributed and crosses the blood-brain barrier. In the liver, fluoxetine is demethylated to several metabolites, the only active one being norfluoxetine. Norfluoxetine is just as effective in blocking serotonin reuptake.

Fluoxetine is metabolized to the active metabolite norfluoxetine, which is also chiral. Fluoxetine and norfluoxetine exist as enantiomers.

![Chemical structures of fluoxetine and norfluoxetine](image)

Fig. 1. Chemical structures of the analytes and internal standards. The asterisk indicates the chiral center.

(10)

**Sertraline**

![Structure of sertraline](image)

(11)

The FDA approved Sertraline in December of 1991 for the treatment of major depression. In October of 1997 it was approved for OCD and panic disorders. In 1998 it became approved for children 6 years old for the treatment of OCD and depression, one of the only antidepressants approved for youth.
Sertraline hydrochloride is sold under the name Zoloft. It is administered orally and well absorbed. The extent of absorption is increased with food. Like fluoxetine, it is highly protein bound, 98% is available after one oral dose, and is assumed to bind to alpha-1 acid glycoprotein (11).

**Citalopram**

![Chemical structure of Citalopram](image)

(2)

Citalopram is sold under the name Celexa by Forest Laboratories and administered orally in doses that depend on the condition of the patient. The FDA approved it in 1998 for treatment of depression. Citalopram has a protein binding affinity of 80% and was favored over all the SSRIs because it shows a minimal potential for drug–drug interactions and limited side effects (3). It's enantiomer, S-citalopram or escitalopram, is becoming more popular now because it is a more improved version of its parent compound.

Citalopram is structurally unrelated to any of the other antidepressants. Other SSRIs have effects on the other neurotransmitters but citalopram has little or no effect on them. It is a more selective for serotonin than the other antidepressants. Celexa quickly became one of the most popular of the SSRI class. It is presently prescribed to over 4 million patients worldwide and is approved by over 50 countries (7).

CYP2C19 and CYP3A4 enzymes N-demethylate racemic citalopram into its metabolite desmethylcitalopram. The CYP2D6 enzyme further metabolizes the desmethylcitalopram. The metabolism of citalopram is stereoselective and the enzymes CYP3A4 and CYP2C19 metabolize S-citalopram preferentially. In addition, MAO-A and MAO-B stereoselectively metabolize citalopram. The S-enantiomer is metabolized by MAO-B in whole blood (3).

**Metabolism of citalopram and its enantiomers**

(3)
S-citalopram is now marketed as the drug escitalopram under the name Lexapro and is distributed by the same company that distributes Celexa. Its popularity is increasing as Celexa patients are quickly switching to Lexapro. Escitalopram shows therapeutic benefits faster than most SSRIs. Others can take weeks to show results as escitalopram shows results in the first week of treatment (3). In previous studies done on laboratory animals, the potency and selectivity of escitalopram, R-fluoxetine, and all other available SSRIs were compared for binding affinity at the human serotonin, norepinephrine, and dopamine transporters and several select neurotransmitter receptors using radiogland and binding assays. Escitalopram was the most serotonin-selective compound (7).

Paroxetine

![Chemical structure of paroxetine]

(12)

The FDA approved paroxetine in 1992 for the treatment of major depression. In 1996 it was approved for the treatment of OCD and panic disorder. Paroxetine became a famous SSRI in 1999 when it was approved for the treatment for social anxiety disorder and sold under the name Paxil. Paxil is more potent than Sertraline and much more potent than fluoxetine in inhibiting the reuptake of serotonin in the neuronal membrane according to rat brain in vitro studies (12).

Paxil is administered orally. Its dosage recommendation depends on the need of the patient. It has a 94-95% protein-binding affinity (12).
Below is an enantio-selective synthesis of Paroxetine done by Amat Mercedes and Joan Bosch:

**Scheme 1**

**Scheme 2**

**Scheme 3**

**Scheme 4**

**Scheme 5**

**Scheme 6**

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* Reagents and conditions: (i) LHMDS, CICO₂R, PhSeBr, THF, \(-78^\circ\text{C}, 77\%\) (trans-2), 96\% (trans-3); (ii) Os, CH₃Cl, \(-78^\circ\text{C}\), then Os, 25 \(^\circ\text{C}\), (iii) R'Cu(CN)₂Li, THF, \(-78^\circ\text{C}, 64\%\) (17), 75\% (18), 64\% (19), 57\% (90); (iv) HCO₂NH, \(\text{MeOH}\), 25 \(^\circ\text{C}\), then toluene, reflux, 55.

* Reagents and conditions: (i) AlCl₃, LiAlH₄, THF, \(-78^\circ\text{C}\) to 25 \(^\circ\text{C}\), 56\% (26), 75\% (21); (ii) H₂, \(\text{CuCl}_2\)/O₂, 20\% Pd(OH)₃/CoAcOEt, 25 \(^\circ\text{C}, 88\%\) (28), 73\% (25); (iii) MeCl, pyr, 10 \(^\circ\text{C}\), then NaH, Ar-OH, THF, reflux, 66\%; (iv) TFA, \(\text{CH}_3\text{Cl}_2\), rt, 72%.

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In summary, R-phenyl glycinol reacts with S-oxopentanoate and gives major products bicyclic lactam cis-1 and trans-1. They were then converted to the cis or trans unsaturated lactams. Next, they underwent a conjugate addition when treated with lithium alkyl (or aryl) cyanocuprates and give enantiopure trans-3, 4-disubstituted 2-piperidone derivatives in high yield and stereoselectivity. Further reactions give (+)- and (-)-paroxetine and (+)-femoxetine. The mechanisms shown have been highlighted to show the syntheses of (+)- and (-)-paroxetine (6).

III. Overview

As it looks, for treatment of depression, serotonin reuptake inhibitors are the most successful and least hindering out of the antidepressants. Each have different side effects and have different drug interactions, but Lexapro seems to have the least and its popularity is growing quickly. The other SSRIs are also prescribed for disorders other than depression or combinations of disorders including depression. Each patient condition is prescribed an antidepressant that will best treat his disorder(s).
Bibliography


H₁ – Receptor Antihistamines: History to Present

Ronald Seth Williams
Organic Chemistry 236
Spring 2003
Dr. Hank Mancini
Abstract:

Antihistamines have evolved immensely since their introduction in the early 1940’s. Histamines themselves are vital to our being; therefore, occurring in most all tissues in the human body.(1) Newly developed antihistamines such as Claritin® are more specific to certain histamines than first generation antihistamines such as Diphenhydramine (Benadryl®) or Chlophentrinaire. Claritin® itself has also been further developed into Clarinex®.

Histamines:

The decarboxylation of the amino acid histadine forms an amine named histamine (Figure 1.1).(2) As previously mentioned, histamines can be found in nearly all human tissues. Histamines can be released from cells in its free, active form in three ways. When a virus destroys a cell or cellular membrane injury occurs, histamine will be released. Histamine can also be released by histamine liberators such as drugs or foreign proteins and if the cytoplasm granules dissolute, perhaps from radiation.(1) There are many histamine receptors in the human body; however, this paper will concentrate on H-1 histamine receptors.

Histamines can trigger many reactions in the human body. If the H-1 receptors on the bronchilo systems are attacked, asthma may be induced. Histamines may also cause exocrine secretions such as bronchial mucus.(2) Histamines can be inactivated in two ways. Methylhistamine can be formed from N-Methylation and Imidazole acetic acid from oxidative deamination (Figure 2.1). Drugs such as Nedocromil® or Cromolyn® inactive histamines in these ways.(5)
Antihistamines

Antihistamines compete competitively with the histamine receptor sites rather that block the release of histamines. H1 - receptor antagonists block all actions of histamine except for the secretion of hydrochloric acid by the stomach. HCl production antagonism is caused by H2 - receptor antagonists. H1 - receptor antagonists will be the focal point for the remainder of this discussion. H1 - antagonists do not only block the receptor site, they do many other actions that aid to anti-allergic action. Basophils and mast cells both release inflammatory mediators; however, H1 - antagonists do not allow this release. They also do not allow the migration of eosinophils, basophils and/or neutrophils. (1)

Since their introduction in 1940 H1 - blockers have been divided into two groups. First generation H1 - blockers include, for example, diphenhydramine hydroxyzine, and chlorpheniramine. Second generation H1 - blockers include drugs such as loratidine, desloratidine, and cetirizine. Cetirizine is the only drug in the second generation to be further classified as sedating. (2)

Chlorpheniramine

Chlorpheniramine is one the most effective and popular sedating H1 - blockers on the market. Chlorpheniramine is an H1 - blocker from the propylamine group and is more potent and less sedating than drugs from the ethanolamine group such as
diphenhydramine. The structure for both chlorpheniramine and diphenhydramine can be found in figure 3.1.

**Figure 3.1 (7.8)**

**Diphenhydramine Hydrochloride**

\[ \text{C}_{17}\text{H}_{21}\text{NO} \cdot \text{HCl} \]

**Chlorpheniramine**

\[ \text{C}_{16}\text{H}_{19}\text{ClN}_2 \]

**Mechanism and Site of Action for Chlorpheniramine:**

As previously mentioned, chlorpheniramine competes competitively with the histamines at the H1 receptor sites in the bronchial smooth muscles, large blood vessels and various other sites in the body. These antihistamines are able to competitively compete with the histamines due to the similarities in their chemical structures. A generic example of this competition can be seen in figure 3.2.

**Figure 3.2**

- The block entitled as “A” is the H1-receptor. B is the histamine and C is chlorpheniramine. As you can see both B and C are similar enough in structure
Pharmacokinetics

It is possible to give chlorpheniramine in a multitude of methods. Orally (PO), subcutaneously (subq), intravenously (IV), and intramuscularly (IM) are all possible methods to administer chlorpheniramine. As with most antihistamines the onset time is approximately thirty to sixty minutes and will reach its maximum effect in about six hours. Due to the fact that chlorpheniramine crosses the placenta and is excreted in the breast milk, chlorpheniramine is classified as a pregnancy category B. Being placed in this class simply means that chlorpheniramine is safe up till the last 2 weeks of pregnancy. The metabolism of chlorpheniramine is rapid; however, very extensive. The drug first goes through the gastric mucosa and onto its first-pass through the liver. This may be saturable. The drug is then dealkylated by N-dealkylation, which produces several metabolites and is excreted in the urine. Chlorpheniramine can stay in the body anywhere from 10 to 330 hours, depending on the age and health of the patient. Chlorpheniramine is generally given every 4 to 6 hours as needed, with a half-life of merely 3 hours.(8)

Interactions and Side Effects of Chlorpheniramine

Although the sedation effect of chlorpheniramine is not as drastic as the drugs from the ethanolamine group of H1 – receptor blockers it is still noticeable. The sedation effect is due to chlorpheniramine’s high lipid solubility; therefore, allowing the drug to cross the blood-brain barrier. The patient must also look out for CNS depression expressed as dizziness during therapy. Chlorpheniramine also is described as what they call a anticholenergic. Anticholenergic drugs prevent defecation, lacrimation (crying), urination and salivation. Due to the anticholenergic effects of chlorpheniramine, patients with respiratory such as COPD or asthma are discouraged from using this generation of antihistamines because it may result in thicker mucus production.(8)

Advantages and Disadvantages of Chlorpheniramine as Apposed to Diphenhydramine

Diphenhydramine and Chlorpheniramine are both under the same class of medications, but do have some differences. Diphenhydramine has many more uses that chlorpheniramine. Not only do they use diphenhydramine for allergic rhinitis, but also for cough, mild cases of Parkinson’s disease, and especially sleep. Diphenhydramine is the active ingredient in most over the counter sleep aids. Again due to its sedative effects, diphenhydramine is effective in mild cases of Parkinson’s disease. Another advantage to diphenhydramine is that it is available in more forms. Not only is it available as an oral
tablet or capsule, but as a suppository, liquid and a topical cream, ointment or spray. As a
topical form, it allows for a more immediate solution for allergic hives or rashes. For
patients needing an effective antihistamine, but do not need the drowsiness effect;
chlorpheniramine would be the better alternative.

**Loratidine (Claritin ®)**

Loratidine (as seen in figure 5.1) is one of the most popular and most effective
second-generation H₁ receptor antagonists on the market. The second-generation H₁
antagonists are favorable because most of them are non-sedative. In order for the
antihistamine to be sedative it must first cross the blood-brain barrier. Due to the low-
lipid solubility of loratidine this barrier cross is prevented. Sedation may be noticed in a
small population or patients. The mechanism and site of action is very similar to those of
chlorpheniramine and diphenhydramine. The only noticeable difference is the fact that
loratidine does not show significant anticholinergic effects.

In regards to pharmacokinetics, loratidine sets into action in 1 to 3 hours.
Loratidine shows its peak effect in about 8 to 12 hours after administration with duration
of action lasting more than 24 hours. If administered with food, the time to reach peak
effect is delayed due to the disruption of absorption. After ingested, loratidine is
metabolized into deschloroethyldoratidine.

The average adult dose of loratidine is 10 milligrams per day. In children, a 5
milligram a day dosage is protocol. Loratidine is available in a multitude of dosage
forms. The most popular are oral tablets and children's liquid. Schering © has also made
loratidine (Claritin®) available as an orally disintegrating tablet, which allows absorption
into the body to be much quicker.

**Desloratidine C₂₂H₂₃ClN₂O₂**

**Ethyl 4- (8-chloro-5,6-dihydro-11 H-benzo[5,6]cyclohepta[1,2-b]
Pyridin-11-ylidene)-1-piperidinecarboxylate**

![Desloratidine molecule](image-url)
Desloratidine (Clarinex®)

In 2002 Schering® introduced what they called a “revolutionary” new antihistamine called Clarinex® (desloratidine) (Figure 6.1). Desloratidine is a major product of the decarboxylation of loratidine that occurs when metabolized in the body. The decarboxylation of loratidine to desloratidine is carried out by simple hydrolysis. This reaction can be seen in Figure 6.2.

The mechanism of action of desloratidine is the exact same of that of loratidine. Schering® claims that desloratidine has duration of therapy of up to 27 hours as apposed to a claim of over 24 hours with loratidine. Desloratidine is metabolized into 3-hydroxydesloratadine and is then glucuronidated.

Clarinex is currently available in only a 5 mg tablet. Clarinex has a maximum daily dosage for adults of 5 mg, and has not been tested in children. Clarinex is classified as a pregnancy category B, and is ok in pregnant women.(6)

Figure 6.1 (6)
Desloratidine C_{19}H_{19}CIN_{2}

8-chloro-6,11-dihydro-11-(4-piperidinylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyradine

\[
\text{\includegraphics[width=0.5\textwidth]{desloratidine.png}}
\]
Comparison of Desloratidine and Loratidine

Desloratidine and loratidine do share a lot in common; however, do also share some differences. It is claimed that desloratidine is longer acting than loratidine. Through my research and speaking to pharmacists, I have found this untrue. Desloratidine is the active metabolite of loratidine. The only difference as you can see is the replacement of the carboxyl group on loratidine with hydrogen on desloratidine. It has been shown that the decarboxylation during the metabolism of loratidine is what prolongs the life of loratidine in the body. If that reaction does not occur, as in the metabolism of desloratidine, the drug simply goes in and “boom” is gone. The literature on desloratidine states that the drug can stay in the body “up to 27 hours” while in loratidine may last in the body “over 24 hours.”(s, s) These statements seem extremely close and unclear to me. I, along with many pharmacists, propose that Loratidine is actually the “better” drug, and Schering® simply came out with desloratidine when they realized that their patent on loratidine was ready to expire. Another advantage to loratidine is that it is available in a multiple forms and has been proven safe in the treatment of allergic rhinitis in children. Desloratidine; on the other hand, comes in one form, a tablet, and may only be used in adults. The only advantage I found to using desloratidine is that there was a 1 in 100 less chance that dry mouth and fatigue will occur.
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7. *Pharmacology Online/Diphenhydramine*

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BIRTH CONTROL PILL:
TRIPHASIL-28

JOANNA WOZNIAK
APRIL 11, 2003
ABSTRACT:

The purpose of this report is to give an overview of the birth control pill Triphasil-28. This paper explains the advantages and disadvantages of taking Triphasil-28, as well as it gives some insight on how the pill works in the body.
HISTORY

The practice of birth control can be dated as far back as the book of Genesis where it stated that men should perform *coitus interruptus*, i.e. what is now commonly referred to as the “withdrawal” method, but it wasn’t until 1914 when Margaret Sanger first used the term “birth control.” Sanger, an activist for birth control, used the term for the first time in her radical journal, *The Woman Rebel*. At this time in the US, there was a law in place that prohibited Sanger to even discuss contraception. This law, referred to as the Comstock Law, was an anti-obscenity act that listed contraception as obscene. The US was the only Western nation at the time to enforce laws criminalizing birth control. Despite the law, there were many different contraceptives still available. These were termed “feminine hygiene” so they could be sold over the counter, one of the most popular was the “Lysol douche,” it was simple to use and inexpensive. Many of these products were ineffective and dangerous methods of preventing pregnancy.³

In 1941, a chemistry professor Russell Marker discovered a way to make synthetic progesterone from Mexican wild yams. This discovery was a giant stepping-stone for what would be the oral birth control market. In 1952, through tests of the synthetic progesterone on rabbits and rats, Gregory Pincus confirmed that the synthetic hormone worked as an anti-ovulent. It was later the same year that Pincus had a chance meeting with the renowned Harvard gynecologist and obstetrician, Dr. John Rock. By this time Rock had already been testing the chemical contraceptive on women and demonstrating it’s effectiveness. The two men decided to work together. However, it wasn’t until the summer of 1957 that the FDA (Food and Drug Administration) approved the first birth control pill, Enovid. Enovid was used to treat menstrual disorders and was required to carry a label warning that it would prevent ovulation. Even though Enovid was a progesterone-only pill, by accident the two men had also discovered that a combination of progesterone and estrogen also reduce similar problems. These types of combination pills are still used today.
One such popular combination pill used today is Triphasil-28. The tablets include a combination of estrogen and progesterone that vary in concentration throughout the cycle. They consist of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17beta-hydroxygon-4-en-3-one), a totally synthetic progesterone, and ethinyl estradiol (19-nor-17(-pregna-1,3,5(10)-trien-20yne-3, 17-diol). When combined they act as an inhibitor for ovulation, thereby increase the difficulty of sperm to enter into the uterus and reduce the chance of implantation.²

The 28 tablet regimen consists of 6 brown tablets, each containing 0.050 mg of levonorgestrel and 0.030 mg of ethinyl estradiol. The second phase is made up of 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol. The third phase is made up of 10 light yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol. The last phase of the cycle is comprised of 7 light green inert tablets. The inactive ingredients present are cellulose, lactose, iron oxides and FD&C Blue 1, just to name a few.³
HOW THE PILL WORKS

Menstrual Cycle without the Pill

HORMONE LEVELS – 28 DAYS

- LH
- FSH
- ESTROGEN
- PROGESTERONE

At the beginning of the cycle, low levels of progesterone and estrogen cause the hypothalamus to release hormone signals to the pituitary gland to release FSH (follicle stimulating hormone) and LH (luteinizing hormone). The FSH and LH make their way to the ovaries where FSH causes maturation of the follicles. A follicle consists of an egg surrounded by cells that produce estrogen. One dominant follicle develops that releases an ovum during the menstrual cycle. During the first half of the cycle, the estrogen levels continue to increase. Due to this increase in estrogen, the lining of the uterus begins to thicken, and it slows FSH production. The constant high levels of estrogen trigger ovulation. The follicle bursts and releases the ovum. The burst follicle then develops cells that produce progesterone. For the second half of the cycle, progesterone becomes the dominant hormone. This increase in the progesterone triggers a suppression in the production of FSH and LH. If fertilization does not occur, the production of progesterone stops, the uterine lining breaks and menstruation begins. If fertilization occurs at anytime during the cycle, progesterone is produced. This is the beginning of a pregnancy.
**Menstrual Cycle with the Pill**

The difference between the cycle with the pill and without is that the pill fools the body to react as though it is pregnant. The estrogen suppresses the production of FSH so that the follicle does not mature. Since no follicle matures, there is no increase in estrogen and the uterine lining does not thicken. Constant levels of progesterone prevent the LH surge, which prevents ovulation. The inert pills trigger the release of the uterine lining.¹

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**HORMONE LEVELS - 28 DAYS**

- LH
- FSH
- ESTROGEN
- PROGESTERONE

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**Metabolism**

Ethinyl estradiol is primarily metabolized by aromatic hydroxylation but a wide variety of hydroxylated and methylated metabolites are formed. Conjugation reactions, such as glucuronidation, methylation, and sulfation are performed by the body to detoxify substances and to make them more water-soluble so that they can be excreted by the body. Conjugated ethinyl estradiol is excreted in urine and feces. About 40% of the drug is excreted in the urine and 60% is eliminated in the feces.²

The most important metabolic pathway for levonorgestrel occurs in the reduction of the Δ 4-3-oxo group and hydroxylation at positions 2α, 1β and 16β, followed by conjugation. Levonorgestrel and its metabolites are also excreted in the urine and feces.²
BENEFITS

Birth control pills are mainly used for contraception, but they are also used for relieving minor discomforts and pain that are associated with the menstrual cycle. Triphasol-28, if used correctly, meaning that it is taken at the same time each day and using a back-up method if some other medication is being taken, then there is a 0.1% chance of becoming pregnant in the first year. The pill is as effective as a male sterilization; both methods have the lowest percent of accidental pregnancy in the first year of use.³ Birth control pills have proven to lower the chances of ovarian and endometrial cancers, benign cysts of the ovaries and breasts and pelvic inflammation disease.⁴ See figure below.

<table>
<thead>
<tr>
<th>Table 1: Lowest Expected and Typical Failure Rates During the First Year of Continuous Use of a Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Women Experiencing an Accidental Pregnancy in the First Year of Continuous Use</td>
</tr>
<tr>
<td>Method</td>
</tr>
<tr>
<td>(No Contraception)</td>
</tr>
<tr>
<td>Oral contraceptives</td>
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<tr>
<td>combined</td>
</tr>
<tr>
<td>progestin only</td>
</tr>
<tr>
<td>Diaphragm with spermicidal cream or jelly</td>
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<tr>
<td>Spermicides alone (foams and vaginal suppositories)</td>
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<tr>
<td>Vaginal Sponge</td>
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<tr>
<td>nulliparous</td>
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<tr>
<td>multiparous</td>
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<tr>
<td>DEPO-PROVERA® (injectable progestogen)</td>
</tr>
<tr>
<td>NORPLANT® SYSTEM (implants)</td>
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<tr>
<td>IUD</td>
</tr>
<tr>
<td>progestrone</td>
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<tr>
<td>copper T 380A</td>
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<tr>
<td>Condom without spermicides</td>
</tr>
<tr>
<td>Periodic abstinence</td>
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<tr>
<td>(all methods)</td>
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<tr>
<td>Female sterilization</td>
</tr>
<tr>
<td>Male sterilization</td>
</tr>
</tbody>
</table>

Adapted from J. Trussell et al, Table 1, Studies in Family Planning, 21(1): Jan.-Feb. 1990.

*The authors' best guess of the percentage of women expected to experience an accidental pregnancy among couples who initiate a method (not necessarily for the first time) and who use it consistently and correctly during the first year if they do not stop use for any other reason.

* This term represents "typical" couples who initiate use of a method (not necessarily for the first time), who experience an accidental pregnancy during the first year if they do not stop use for any other reason.

** N/A-Data not available.

#This data is based on Norplant System clinical trials.
SIDE EFFECTS AND CONSEQUENCES

There are definitely some consequences and side effects to using the pill. The pill is usually known for making women gain weight while taking the pill, but there are more serious risks involved. There have been increased risks of serious conditions associated with taking the pill, such as stroke, gall bladder disease, hypertension and diabetes, just to name a few. There is even a small chance of mortality that comes with taking the pill.

This chance, dramatically increases with women who smoke and are over the age of 35, this is why women who use the pill are urged not to smoke. See the figure below.

![Circulatory Disease Mortality Rates Per 100,000 Women](image)


CONCLUSION:

The birth control pill Triphasil-28, a combination pill, can be very effective against pregnancy when used as suggested. But everything has a price; therefore there are quite a few side effects that come with using the pill. It is up to the woman to decide which decision is best for her.
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Blood Coagulation and Hemophilia

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April 25, 2003
Abstract

Hemophilia is a blood clotting disease in which sufferers can bleed to death from minor injuries if they do not receive prompt treatment. Normal blood clot formation involves many proteins working in a cascade of reactions to prevent blood loss. Treatments for hemophilia include infusions of replacement proteins and gene therapy. Hemophilia treatments are getting more efficient all the time, and hemophiliacs are living more normal lives as better treatments become available.
Background

Hemophilia is a sex-linked recessive blood coagulation disorder. A second century B.C. Jewish document, the Talmud, contains one of the earliest descriptions of hemophilia, “If she circumcised her first child and he died, and a second one also died, she must not circumcise her third child.” Hemophilia got more attention in the 1800s when Queen Victoria, a carrier for the trait, passed the gene to several of her children. The hemophilia gene eventually spread to the royal families of Russia, England, Germany, and Spain. It is now understood that the gene for hemophilia is found on the X chromosome, and because males get one X and one Y chromosome, males are much more likely to exhibit the disorder than females, with two X’s. As in Queen Victoria’s case, females can be carriers of the gene and be completely unaware of it until a son develops hemophilia.

There are different forms of hemophilia based on the specific blood protein that is missing or nonfunctioning. Hemophilia A is the most common and involves coagulation factor VIII. In hemophilia B, coagulation factor IX is the faulty or missing protein. The third version is hemophilia C, which is caused by problems with coagulation factor XI. Hemophilia is seen in one of every 4,000 live male births. Individuals with hemophilia suffer joint and muscle hemorrhage, in addition to the easy bruising and prolonged bleeding from wounds that is directly related to the clotting factor deficiency. A major concern for hemophiliacs is receiving blood contaminated with HIV or many other blood-borne pathogens. Hemophilia sufferers have lived with HIV, painful joints, and dangerous bleeds from minor injuries. The life expectancy and quality of life for hemophiliacs has room for improvement with the methods used in their treatment.

Normal Clotting Mechanisms

The pathway to coagulation, or clotting, involves many proteins and some ions working together in a cascade reaction to prevent massive blood loss. There are two pathways for clotting: the extrinsic mechanism and the intrinsic mechanism. Both pathways have the final steps in common (figure 1). The extrinsic mechanism starts when tissue injury occurs. The damaged tissue releases tissue thromboplastin (factor III or TF) and is the starting point for extrinsic clotting. Factor III activates factor VII, and the activated factor VIIa combines with and activates factor X. Next, factor Xa combines with and activates factor V. These reactions depend upon the presence of calcium ions (factor IV), and they lead to prothrombin activator production.

The prothrombin activator and calcium ions then convert prothrombin (factor II) into thrombin (factor IIa). The thrombin is an enzyme that catalyzes the reaction that fragments fibrinogen (factor I), a soluble protein. The fibrinogen pieces join together to form long threads of insoluble fibrin. Thrombin also activates factor VIII, and factor VIIIa stabilizes and strengthens the fibrin threads and cross-linkages. The fibrin threads stick to the damaged tissue and create a meshwork in which blood cells and platelets get trapped. This is now a blood clot that seals off the bleeding. The blood clot’s formation is part of a positive feedback loop and will keep strengthening and thickening the clot until the clotting factors are deactivated.
Figure 1. Blood Clotting Mechanisms (adapted from: www.indstate.edu/thcm/e/mwking/blood-coagulation.html)

The intrinsic clotting mechanism is initiated by detection of a foreign substance in the blood, which activates the Hageman factor (factor XII). The activated factor XIIa activates factor XI, and the factor XIa can then activate factor IX. Factor IXa joins with platelet phospholipids and factor XIII to activate factor X. These reactions also depend upon calcium ion (factor IV) presence, and the reactions help in the production of prothrombin activator. As seen in the extrinsic mechanism, thrombin is activated, factor VIII is activated, and a fibrin meshwork is made and forms the clot.¹

The proteins used in blood clot formation are synthesized in different locations of the body. Factors II, VII, IX, and X are synthesized in the liver. These proteins require vitamin K to convert them to active clotting factors. The vitamin K helps to modify γ-carboxyglutamate residues by acting as a coenzyme in the carboxylation of the glutamate residues. In the carboxylation, vitamin K is oxidized to an epoxide form and must be reduced to function again (figure 2). The reduction is the site of action of certain anticoagulants, like Warfarin.⁴

The other clotting factors produced in the liver are factors I, V, XI, XII, and XIII. These do not require vitamin K as an activating coenzyme. Factor III is produced by damaged tissue cells, and calcium ions (factor IV) are produced from bone or obtained in the diet. Platelets and endothelial cells release Factor VIII.¹
Figure 2. Role of Vitamin K in Activation of Factors II, VII, IX, and X (adapted from: tollefsen.wustl.edu/projects/coagulation/coagulation.html)

The activity of factor VIII depends upon proteolytic cleavage by thrombin (factor IIa) and calcium ions. Small quantities of thrombin convert factor VIII to its active VIIIa form that functions as a cofactor in the intrinsic pathway when aiding factor IX and platelet phospholipids in activating factor X. In the fibrin mesh formation step of both the intrinsic and extrinsic mechanisms, factor VIIIa functions as a highly specific transglutaminase, an enzyme that helps to form cross-linkages made of covalent bonds between the amide nitrogen of glutamine and the ε-amino group of lysine within the fibrin monomers (figure 3).

Figure 3. Transglutaminase Activity of Factor XIIIa (adapted from: tollefsen.wustl.edu/projects/coagulation/coagulation.html)

Hemophilia Treatments

Treatment for hemophilia A and B has primarily been infusion of purified blood to boost levels of factor VIII or IX. The blood is pooled from several thousand donors, purified, and packaged or freeze-dried for distribution. The clotting factors can also be genetically engineered by recombinant mammalian cells grown in a laboratory culture. Recombinant proteins avoid the possibility of infective viruses or bacteria that can be found in donated blood. As preventative treatment, injection of factor mixture must be
given every few days, depending on the severity of the person’s hemophilia. Also, factor infusions are given after an injury in hopes of clotting normally. However, in some hemophilies, the immune system attacks the “foreign” factor proteins and destroys them before they can function. Unfortunately, infusions nearly everyday are expensive, inconvenient, and increase chances of infection.

Researchers have identified the portion of factor VIII that arouses immune attack; it is the active site that normally binds with factor IX in the intrinsic pathway. Soon they hope to make synthetic factor VIII that will function normally but that won’t attract immune responses.

The method of delivering the factor XIII into the body can also be improved. Medical engineers have developed a new syringe system that will reduce the cost of injections and efficiently mix the reconstituted factor VIII. For many delivery devices, the patient must complete a multistage process to reconstitute the drug from its freeze-dried state. Within this process, there are many chances of needle-stick injuries or of denaturing the factor VIII by inefficient mixing. This new syringe design will reconstitute the drug and inject it in a simpler and safer way.

Another area in which hemophilia treatment can improve is in the gathering of factor VIII from donor blood. The current method of extracting proteins from blood plasma is only about 25% effective, but researchers at the University of California Irvine have found a more efficient way. They found that adding citric acid to blood plasma before extracting the clotting factors produced a yield of 97%; this process is called cryoprecipitation. It seems that the citric acid acts as a primer allowing easier extraction of the proteins. The researchers’ other concern is maintaining the low contamination rate of the current method. The AIDS virus, the tuberculosis bacterium, and many other bacteria and viruses are found in donated blood. The citric acid is a potent antibiotic and should kill off most of the contaminating organisms. The researchers are still looking into methods to increase the safety and purity of the extracted proteins.

A new area of research for hemophilia is gene therapy. Gene therapy has the potential to cure hemophilia without the need for transfusions. Gene replacement therapy would involve the introduction of the correct DNA sequence in place of the faulty sequence that already exists. This would allow the body to synthesize its own properly functioning coagulation factors. The vector of choice to introduce the replacement gene is an adeno-associated virus (AAV) that will infect the body’s cells with the DNA that will produce functioning clotting factors. The AAV is treated so that it does not introduce any diseases or unwanted substances, and even the untreated AAV is not known to cause any human diseases, making it a safe vector for gene therapy. There are two main methods of administering the vector: into the liver or intramuscularly. The genes must be placed in any tissue that can export proteins to the blood stream, which both the liver and muscles can do. A difference between the two methods is that higher doses are required for the intramuscular treatment.

The gene therapy via liver injection method was shown to be effective for hemophilia B in both dogs and mice by teams led by Mark Kay at Stanford University. The AAV vector was injected into a portal vein leading into the liver, and the AAV then delivered the gene to liver cells. As mentioned above, the liver is the normal site of factor IX production, and gene replacement here could maximize the benefits of this therapy. Katherine High, from the University of Pennsylvania, delivered the AAV vector
intramuscularly.\textsuperscript{2} The benefits of intramuscular injection include the ease of the procedure, it can be given as a shot, and that people with damaged livers can undergo intramuscular injections.\textsuperscript{2} Both methods of administration showed similar results, but the different methods could aid hemophiliacs in need of options in their therapy.

**Looking Toward the Future**

The men suffering from hemophilia today have a brighter outlook than those of just a few years ago thanks to newer treatment options and safer blood treatment methods. Infusion of factor VIII, IX, or XI is more efficient, and gene therapy is becoming safer and more effective. Hemophiliacs do not have as many prolonged bleeds or joint problems because preventative clotting factor infusions keep blood levels healthier. Also, donated blood is monitored more closely and is very unlikely to contain HIV. The newer treatments are improving the outlook for all hemophiliacs, especially those yet to come.

Soon, men born with hemophilia will not feel any ill effects or constraints from the disease because gene therapy will replace the faulty gene allowing the individual to have normal levels of all clotting factors. Hemophilia will become an affliction treated at birth and lived through without a second thought.
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Figure 3. tollefsen.wustl.edu/projects/coagulation/coagulation.html
HUMAN DIAMONDS

Erin L. Ziegler

CHM236
INTRODUCTION:

There is much to say about tradition and opinions about burial versus cremation, but the tangible outcome from turning them into a beautiful diamond is a convincing way to contribute to someone’s life. It is not just a diamond, but also a part of someone that you knew, which makes it a much more invaluable piece of jewelry, even when it costs more than a comparable sized diamond. Buying a flawless diamond can be purchased from anywhere, even if lost, but a diamond made from a loved one becomes something more special and unique in many ways. There may be natural flaws and a certain color that is created when the diamond is made. A deceased-person diamonds are blue in color, which is due to the element boron contained in the human body. This process is even available to create a diamond from a pet (Pela, 10 pp).

Today, the cost of cremation and collection of carbon from human cremains to be made into a diamond is less expensive than the complete cost of a funeral due to the casket, the burial plot, and fees. Someone has a tangible object, other than personal possessions, to pass down and to remember a loved one by, where as once the person is laid to rest or the ashes are spread, there is nothing to show for the expenses. It is even possible to collect carbon from existing ashes to make into several smaller diamonds, mostly less than a quarter carat in size, but it is not as easy because there is less carbon present. In the normal process, about fifty to one-hundred stones varying in size, can be made from the extracted carbon of a normal size person, and depending on how much is collected. The LifeGem The LifeGem Company has made provisions to make sure that the carbon collected is carefully marked and stored so that the diamond made from a loved one is assured to be from that person. The whole idea behind the process is to feel and to know that the diamond created is made from that person’s collected carbon from their cremains, to have a piece of them on a finger, around the neck, or as earrings to remember someone by. Company will store any unused carbon if a diamond is lost or additional stones are requested later for any reason for the price of the size of the diamond. Creating something beautiful instead of just cremating and spreading the ashes, or burying them, is an entirely new concept in dealing with, remembering, and paying respect to the loss of a loved one (Pela, 10 pp).

Because the colored, or “fancy” diamonds are the most rare and priceless diamonds available, the market seems to be slanted towards white or colorless ones. Intense, or ‘canary” yellow is the most common, with an only a handful of natural blues and reds even known to exist. The cost per carat of a colored diamond runs over one hundred thousand dollars, where some reach well over one million dollars per carat (LifeGem, education). High-quality created diamonds have been present for many years. These diamonds are created by placing carbon, the primary element of all diamonds in conditions that recreate the forces of earth. An exact source of carbon from a partially cremated body is used to create this diamond. The process of capturing the carbon from a specific human body has been discovered and placed into unique diamond presses that replicate the awesome forces of nature due to heat and pressure. In a matter of eight-weeks, the certified LifeGem memorial involves cremation, creation, faceted, and certified by the same processes as the finest jewelers use. (LifeGem, How is LifeGem Created).

EXPERIMENTAL

The age of most diamonds found in nature are 1 billion to 3 billions years old, by most accounts, in which eighty percent of them are not suitable for jewelry. Diamonds are mined by several distinct methods, depending on the way that they are presented to the earth’s surface. Erosion and rivers carry and bring them to the surface. Riverbeds are dug away and the river silt is sieved or the sandy coastal strata, is dredged. In all, the large industrial operations methodically find diamonds by
processing 250 tons of rock, sand, and gravel to yield one diamond, is an indication of their rarity. “Five billion truckloads of earth for one truckload of diamonds,” is said to be the case (LifeGem, Diamond Education).

Geologically, diamonds form at great depths within Earth and are typically billions of years old. In nature, diamond crystallizes from hot carbon-rich fluids. This crystallization requires tremendous heat and pressure—1000 to 1200°C (1800 to 2200°F) of heat and 50 kilobars of pressure. (One bar is the pressure the atmosphere exerts at sea level, equal to 1.02 kg per sq cm, or 14.7 lb per sq in; 50 kilobars is 50,000 bars.) The pressures and temperatures at which natural diamond forms only occur deep underground. Scientists believe that diamonds form at depths greater than 150 km (93 mi), and there is evidence that some diamonds formed as deep as 670 km (420 mi) beneath Earth’s surface (Diamonds, How Diamonds Form).

The deceased body is placed into a cremation container (if required by state law) and then into the cremation chamber, where it takes usually two to three hours until cremated. Any metal from bridgework or from prosthetic devices are removed. As the body returns to its essential elements during the cremation process, it passes through a “carbon phase,” where the carbon is lost in the standard process as it is reduced and released as carbon dioxide leaving the white ash consisting of calcium. The cremation process strives to make the ash as white as possible by burning out all the black carbon or removing it before giving it back to the family. To collect the carbon, the cremation is stopped about half way through the process to separate the carbonized matter into a unique carbon-curing container where it remains for the rest of the cremation. The carbon cures and becomes a black powder. It is then placed into a sealed and uniquely identified crucible and moved into the purification phase of processing. Once purified, the carbon is placed in unique diamond presses and the creation of a LifeGem begins (LifeGem, Cremation Education).

Parkview Cremations in Fond Du Lac, Wisconsin has conducted test cremations for LifeGem was the first crematorium in the United States to be certified by the LifeGem Company. There is nothing concerning the process to be in violation of state laws regulating Illinois’ 66 registered crematoriums according to Illinois Environmental Protection Agency, or no existing regulations to prohibit it. One expert opinion on death and dying indicated that survivors who scatter a loved one’s ashes sometimes have more difficulty in coping with the death, because they don’t have personal mementos to cherish, a gravestone to visit, or a vessel to hold onto. There is a strong human need to have something tangible because memories fade and float away, says Kyle Nash, a grief counselor for physicians at the University of Chicago. LifeGem officials will provide customers to view any part of the diamond-making process to counter any skepticism. A certificate from the European Gemological Laboratory in New York will be provided to identify the stone as a synthetically made diamond (Tatum, 2-4 pp).

Greg Herro, Chief Executive officer of the company and partners of LifeGem, which formed in 2001 after three years of research on the diamond making process had discussed that “We wanted something that would allow us to continue to celebrate life on a daily basis,” which would give the spiritual connection that a loved one deserved. Rusty Vandenbiesen, who is the chief operating officer of LifeGem, thought up the idea after deciding that he wanted something else for himself after he died instead of being buried in a cemetery or having his urn of ashes left on the fireplace mantle. VandenBiesen figured that if carbon could be collected from the cremation process, then a diamond could be produced from the remains of the human body. Three years of trial and error using several remains of animals and a cadaver took place in a diamond-manufacturing laboratory outside of Munich, Germany and succeeded in April of 2002. LifeGem confirms that the German laboratory created the diamonds for them from the carbon extracted from animal and human bone. The German laboratory indicated that they would continue to produce the stones as LifeGem builds its own diamond-making facility in the United States (Tatum, 3 pp).
LifeGem is working with the German Laboratory to develop isotopes, or chemical markers that can be attached to the collected carbon and identified in the finished product by an expert to ensure customers the diamond received is indeed made from their loved one’s carbon. During cremation, technicians control the oxygen levels to prevent carbon in the body from converting to carbon dioxide. The incineration is interrupted so the technician can collect the body’s carbon in the form of a dark powder. The powder is then sent to a Pennsylvania company where it is heated in a vacuum at extreme temperatures to produce graphite. Only a thimbleful size of carbon is needed to create a stone. The graphite is sent to the German laboratory and placed into autoclaves that stimulate the intense pressure and temperature needed to create the stones. To create a precious stone, the cremation must be accomplished in a special way in order to collect the carbon properly. The ashes then go back to the family and the carbon is placed into a special container to be delivered to the LifeGem lab, where it is purified to graphite and put into a special diamond press to be subjected to extreme amounts of heat and pressure. To guarantee a LifeGem, the company oversees the cremation process. To make a one stone requires little of the material, so the ashes left over fill an urn container (Tatum, p4). In nature, carbon-12 accounts for about 98.89 percent of all carbon. Carbon-13 has a natural abundance of 1.11 percent, and the amount of carbon-14 is negligible (Carbon, Isotopes).

Carbon exists in four different allotropes. Allotropes are different physical forms of the same element, such as a hard, highly structured crystal and a soft, less-structured substance. Allotropes differ in the way the atoms bond with each other and arrange themselves into a structure. Because of their different structures, allotropes have different physical and chemical properties. The three common allotropes of carbon are diamond, graphite, and amorphous carbon (examples of amorphous carbon include charcoal, soot, and the coal-derived fuel called coke). The density of diamond is 3.5 grams per cubic centimeter (g/cm³), graphite ranges from 1.9 to 2.3 g/cm³, and amorphous carbon ranges from 1.8 to 2.1 g/cm³. Diamond is one of the hardest known materials, while graphite is one of the softest. These differences arise from the differences in bonding between the carbon atoms (Carbon, Allotropes).

Atoms of the element carbon can link together in several ways to form substances with very different properties. In diamond, the atoms form a three-dimensional network that extends throughout a crystal and makes diamond the hardest naturally occurring substance. Graphite is made up of layers of carbon that can slide over each other easily, making graphite a useful lubricant. In the family of substances called fullerenes, the atoms link to form spherical or cylindrical surfaces (Carbon, Allotropes).
Allotropic Forms of Carbon

Graphite is black and slippery and conducts electricity. In graphite, the atoms form planar, or flat, layers. Each layer is made up of rings containing six carbon atoms. The rings are linked to each other in a structure that resembles the hexagonal mesh of chicken wire. Each atom has three sigma bonds (with 120° between any two of the bonds) and belongs to three neighboring rings. The fourth electron of each atom becomes part of an extensive pi bond system. Graphite conducts electricity, because the electrons in the pi bond system can move around throughout the graphite. Bonds between atoms within a layer of graphite are strong, but the forces between the layers are weak. Because the layers can slip past each other, graphite is soft and can be used as a lubricant. Diamond makers can transform graphite into diamond by applying extremely high pressure (more than 100,000 times the atmospheric pressure at sea level) and temperature (about 3000°C or 5000°F). High temperatures break the strong bonds in graphite so that the atoms can rearrange themselves into a diamond lattice. About 90 percent of the diamonds used in tools in the United States are made this way (Carbon, Allotropes).

In diamond, each carbon atom bonds tetrahedrally to four other carbon atoms to form a three-dimensional lattice. The shared electron pairs are held tightly in sigma bonds between adjacent atoms. Pure diamond is an electrical insulator; it does not conduct electric current. It is colorless and, because of its hardness, is used in industrial cutting tools. Cut diamonds sparkle brilliantly, which makes them treasured gemstones in jewelry (Carbon, Allotropes).

The high demand for diamonds has led to the development of methods for producing artificial diamonds. Artificial diamonds used in industry are generally known as synthetic diamonds; artificial diamonds used for ornamentation are called imitation diamonds. Even though the majority of natural diamonds are industrial grade, only about 10 percent of the diamonds used for industrial purposes are natural diamonds. The other 90 percent are synthetic. The two most common processes of synthesizing diamond are the high-temperature high-pressure (HTHP) and chemical vapor deposition (CVD) methods. The chemical vapor deposition method is commonly used because of the high deposition rates possible, the excellent resulting film uniformity, and the ability to process large objects and/or large quantities easily and quickly. Chemical Vapor Deposition (CVD) uses chemical reactions of gaseous
metal halide precursors and non-metal source gases as the film to be deposited. A method of Plasma Assisted Chemical Vapor Deposition is also used to enhance and speed up the process (Diamond, Synthetic and Imitation Diamond)

\[
\text{Carbon} \quad \xrightarrow{\text{High-Temperature}} \quad \text{Graphite} \quad \xrightarrow{\text{HTHP/CVP}} \quad \text{Diamond} \\
\text{High-Pressure} \quad \xrightarrow{\text{Chemical vapor deposition}} \quad \xrightarrow{\text{rearrangement}} \quad C_{33}H_{42}
\]

\[
C_{12}H_{20}
\]

Only about 0.001 percent of this total is found in living plants and animals. As noted earlier, carbon is found in elemental form as amorphous carbon (mostly coal), graphite, and diamond. Plants, animals, and other life forms make carbon-based organic molecules that range from small to enormous in size. Small molecules include acetic acid (C₂H₄O₂), which gives vinegar its sour taste; the simple sugar glucose (C₆H₁₂O₆); and common table sugar, sucrose (C₁₂H₂₂O₁₁). The three basic energy-providing nutrients of living organisms, carbohydrates, fats, and proteins, are all based on carbon. The human body is about 18 percent carbon by mass, and the biologically significant molecules (other than water) have carbon as part or all of the backbone of their structure. Cell membranes are made up of lipids, which are large organic molecules of carbon, hydrogen, oxygen, nitrogen, and phosphorous. Other large organic molecules of the body are the proteins found in blood, muscle, skin, hair, and every living cell. Ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) are gigantic carbon-based molecules that contain the genetic information, or the blueprints, for a living organism. Biochemical processes, the chemical reactions that create and sustain life, rely on the chemical reactions of carbon-based substances. These life processes involve the complex and coordinated making or breaking of carbon bonds (Carbon, Occurrence).

RESULTS:

The density of diamond ranges between 3.15 and 3.53 g/cm³, but the density of pure diamond is always very close to 3.52 g/cm³. Diamond is much denser than crystals composed of elements of similar weight to carbon atoms because the carbon atoms in diamond are packed tightly together. Diamonds are crystals composed of carbon atoms. Atoms in a crystal are arrayed in a regular repeating pattern. A crystal’s outward form, bounded by smooth plane surfaces that meet at predictable angles, reflects this internal order. Crystals tend to cleave, or split, along lines called cleavage planes between layers of atoms. In the case of diamond crystals, each carbon atom is bonded to four surrounding carbon atoms. This microscopic arrangement determines the visible shape of diamond crystals, which are generally octahedrons (solid shapes with eight faces). Individual diamond crystals therefore cleave cleanly along planes parallel to the faces of an octahedron (Diamond, How Diamonds Form).

This process resembles the natural process that takes place by the forces of the earth in creating the hardest substance or diamond that takes millions of years to be created (Asian Week, 2 pp). The LifeGem diamonds are identical in every aspect to natural diamonds, with the same brilliance, fire, and
hardness (Tatum, 4 pp). They are transformed from rough crystals to polished gems by a succession of manufacturing processes such as cleaving, sawing, and polishing. The diamond quality is then defined by the Carat, Color, Clarity, and Cut, and graded in a certificates laboratory (EGL), or the Gemological Institute of America (GIA) (LifeGem, How is LifeGem Created).

Two important properties, brilliance and fire, contribute to diamond's beauty. Brilliance is the fraction of the light that falls on a diamond that the diamond returns to the eyes of an observer—the more light returned, the higher the brilliance. Diamond's brilliance arises from its index of refraction, which determines the angle at which light is bent as it crosses the boundary between the air and the stone. Fire is the ability of a substance to split white light into rainbow colors—the greater the separation between colors, the greater the fire. Diamond's fire originates with its dispersion, which is the difference in diamond's index of refraction for light of different colors. Diamond has both a higher index of refraction and a higher dispersion value than any other natural, transparent, colorless material. Diamonds exhibit a wide range of transparency and color. Transparency is a measure of the amount of light that passes through a diamond rather than being absorbed. Colorless diamonds, known as white diamonds, are most familiar, but green, blue, red, orange, yellow, and brown diamonds also are known. Structural imperfections or dislocations and the presence of trace elements, mainly nitrogen, cause color in diamonds. Some diamonds luminesce (emit light) when exposed to sunlight or other ultraviolet-light sources. The light that diamonds emit is usually light blue, but yellow, orange, and red luminescence occurs in some stones (Diamonds, How Diamonds Form).

LifeGem manufacturers high quality, colored diamond made from the loved one's carbon, which is the building block of life and diamonds come from it (Asian Week, 2 pp). LifeGem provides six options of sizes with prices to chose from ranging from option I at $2300 for a .20 carat sized diamond to option VI at $10,000 for a .70 carat sized, and up to a 1.3 sized carat specially ordered diamond, where an order of two diamonds must be placed to create a LifeGem from your loved one. The LifeGem is produced exclusively in blue, and will include future options of yellow or red colored diamonds (LifeGem, Prices).

DISCUSSION:

Only high-quality diamonds are suitable for use as gems. In judging the quality (and therefore the value) of a cut diamond, a buyer must take into account four criteria, known as the “four C’s”: color, clarity, carat weight, and cut. Colorless stones are extremely valuable, while yellow or brown-tinted stones are regarded as imperfect. Fancy, colored diamonds, or fancies, exhibit clear, strong colors such as blue, green, red, and orange. Fancies are quite rare and highly prized. The presence or absence of internal blemishes and flaws determines clarity. Weight reflects a diamond’s size. The unit of weight usually employed for diamonds and other gems is the metric carat, which is equal to 0.2 g (about 0.007 oz). Another unit used to express the weight of diamonds is the point, equal to 0.01 carat. A stone of 82 points would therefore weigh 0.82 carat. A 5-carat stone is worth more than five 1-carat stones that are otherwise of the same quality. The final criteria that buyers use to determine the quality of a diamond, is its cut. The cut is the shape and proportion of the stone, as determined during the diamond-cutting procedure (Diamonds, Judging of Diamond Quality).

Other characteristics of diamonds are frequently useful in identifying the stones and in differentiating between true diamonds and imitations. Because diamonds are excellent conductors of heat, they are cold to the touch and are sometimes called “ice.” Most diamonds do not conduct electricity well, but diamonds do become charged with positive static electricity when rubbed. Diamond resists attack by acids or bases. Since diamonds are a form of carbon, like coal, they will burn, but only when heated to extremely high temperatures (Diamonds, How Diamonds Form).
Cremations are on the rise in the United States and worldwide, and possibly will result in a growing market for the creation of a LifeGem. The Cremation Association of America indicates that about twenty-six percent of the 2.3 million United States residents who died last year were cremated, and predicts that the rate of cremation should rise to almost forty percent by the year 2010. In Japan, cremation rate is ninety-eight percent, and veterinary offices may be a market to tap into (Tatum, 3 pp).

A LifeGem is a certified, high quality diamond created from the carbon of your loved one as a memorial to their unique and wonderful life. For a loved one, it is more than a memorial to visit; it is a way to embrace your loved one day by day. The LifeGem goes with you and stays with you in your life at all times, it is a one-of-a-kind diamond, an heirloom for family generations to come. A LifeGem is an everlasting connection and closeness to someone lost, a celebration of life with a piece of jewelry that tells a unique story and represents a new beginning (LifeGem, What is a LifeGem).
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Project Paper

*Stinky Rose*

By
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Organic Chemistry 236-Chemistry Symposium
Paradise Valley Community College
April 25, 2003
Abstraction

Stinking rose or garlic is a plant whose medical benefits were known for over 4,000 years, yet it is still an open source for many new researches. This paper briefly explains history and mechanism of garlic compounds, mainly focused on its sulfur compounds. Emphasize is on the four different ways of garlic preparations for commercial purposes, as well as the main products of each preparation. Then, it follows the medical applications of those products, based on the garlic abilities to serve as: natural antibiotic, cancer preventer and heart disease preventer. These medical properties are only small fraction of enormous potential that garlic has.
I. Introduction

At the beginning of the XXI century, the era of technology and modernism, people often forget sometimes to turn back to nature in their search for certain answers. Solutions are often lying in the balance between old uses of nature and new advanced technology. In that manner, garlic (Allium Sativum) has been recognized for its medical benefits for over 4,000 years, but just nowadays scientists have become able to more completely understand the nature and mechanism of this plant, which is so important in the human diet and disease prevention. "The Codex Ebers, an Egyptian papyrus from around 1550 BC, gives more than 800 therapeutic formulas of which 22 are garlic-based remedies for ailments which include body weakness, headaches and tumors of the throat."(1) Garlic was also well known in the other parts of the world: "Hippocrates and Indian physicians are also reported to have used dietary garlic as a method to reduce tumor growth."(2) In China, which is believed to be the cradle of this plant, garlic is thought to prolong life.(1) Even one of the greatest Greek philosophers, Aristotle, depicted garlic as a tonic.(3)

Garlic has a wide range of health benefits: healer of infections, natural antibiotic, cancer prevention, as well as high blood pressure reducer, preventer of blood clotting, reducer of atherosclerotic buildup (plaques) in arteries, cholesterol and sugar regulator, all of which are very important for improving the cardiovascular system and preventing heart diseases. On the other hand, its singular, very unpleasant smell and bad breath are highly socially unaccepted, which turns many people away from this very healthy plant. This is where technology came into play; researchers have developed many alternative solutions, such as garlic pills, oils and aged garlic extracts. However, these alternatives are not always such a great solution because of some properties of garlic compounds, which are very difficult to control and predict. "The complex chemistry of garlic makes it plausible that variations in processing can yield quite different preparations."(4)

II. Mechanism

From whole variety of different compounds from the garlic, therapeutically most important are sulfur compounds, which also make the majority of the components of garlic cloves and "about 2.0% or more of its dry weight."(5) The unique property of garlic is that its untouched bulbs hold only few active compounds of which the most important is Alliin (S-allyl-cysteine sulfoxide).

\[
\text{Alliin: } \text{CH}_2=\text{CH}-\text{CH}_2-S-\text{CH}_2-\text{CH}-\text{COOH}
\]

Alliin is found in the cell cytoplasm that also contains vacuoles (pockets) filled with hydrolytic enzyme Allinase. When the bulb is disrupted (smashed, cut, chopped...) the enzyme Allinase is released which breakdown the Alliin into volatile sulfides, with a major product of Allicin (diallyl disulfide-D-oxide).(5)
Allicin has a strong odor responsible for the garlic smell. It is considered to be antibacterial substances, but also it is very unstable and easily decomposes to numerous compounds, such as Diallyl sulfides, Ajoenes and Vinylthiins.(5)

The mechanism of the major sulfur compounds formed from Alliin is shown below:

III. Preparation

When Allicin breakdown to assortment of different compound, every compound has its own beneficial properties that vary from one to another. For example, Diallyl Disulfide suppresses the growth of colon cancer (2), Ajoene reduces platelet aggregation which makes blood less sticky (6) and so on. Therefore, if one seeks for specific beneficial properties it become scientifically important the way the garlic is preceded. Different preparations of the garlic would emphasize the different major products. Generally, garlic could be treated in following procedures:

1) Garlic Pills (Odorless or with reduced odor)

When the garlic is cut, chopped or crushed the alliin in a presence of alliinase is immediately converted into allicin, with a half-life of only about 2.4 hours. Beside the allicin, extract may contain small amounts of "several other dialkyl thiosulphinates [RS(O)SR'; where R and R' are allyl, methyl or propyl groups] and complex sulphinyl compounds."(1) Because of the Allicin's instability at room temperature and its short half-life, it cannot be preserved as it is, but rather garlic powder is carefully dried such that contains both alliin and enzyme alliinase separated. When such capsule or tablet is
consumed, pill’s enteric coat is dissolved in digestive tract so that allin and allinase now come into contact with each other and forms the allicin.\(^{(5)}\)

Although odorless garlic pills appear as a great alternative to omit horrible garlic breathe, the credibility of such pills is very hard to determine since it is difficult to precisely measure and expect that exact amount of allicin to be formed. In October of 2002 ConsumerLab tested and released the report that states following: “Not all garlic products are created equal. Good quality, non-aged garlic should yield a minimum of 3,000 micrograms of allicin—the active compound in garlic—per gram of dried garlic, or 1,000 micrograms of allicin per gram of fresh garlic.”\(^{(7)}\) About that same report Newsweek wrote that, “garlic contains the compound allicin, which at high levels—3,000 micrograms or more—may clear the arteries. ConsumerLab, a firm that regularly debunks supplement makers’ claims, tested 14 garlic supplements and found that half of them contained less than 3,000 micrograms, including Jamieson Laboratories’, which was advertised as “allicin rich.” Seven brands, like Garlinase 2000 and Nutrilite, really were allicin-rich.”\(^{(8)}\)

Another issue might be the efficiency of pill’s enteric coats that are designed to dissolve in small intestines so that the smell would be prevented. This means that coat has to resist stomach acids which may inactivate the enzyme alliinase.\(^{(5)}\)

2) Garlic Oils

Another preparation of the garlic is through steam-distillation where the garlic oil is produced. “When distilled in stem, garlic yields on oily mass consisting of diallyl, methyl allyl, dimethyl, and allyl 1-propenyl oligosulphides—all originating from the thiosulphinates.”\(^{(1)}\) Garlic oils are found to be rich in diallyl dulfides\(^{(5)}\) which possess anti-tumor and anti-oxidant effects. Limitation of the garlic oils is that it is deficient in antibiotic properties, “oil lacks the bactericidal and antithrombotic activity, with exception of diallyl trisulphide, methyl allyl trisulphide and other paraffinic polysulphides, which show antiplatelet activity in vitro.”\(^{(1)}\)

3) Aged Garlic Extracts

This is the only completely odorless form of garlic preparation. Chopped garlic is stored in alcohol for long period of time (about two years) in a process called long cold ageing. During this time no allicin is present, but more readily some major water soluble organosulphur compounds, such as S-allylcysteine, S-allylmercaptocysteine, and some sulphur containing amino acids with a manor oil soluble organosulphur compounds, such as diallyl sulphide, diallyl disulphide, ajoene and dithiins. Aged garlic has considerable controversy with different opinions. Major cause is the fact that it does not contain any allicin, which is so emphasized in all other forms of garlic preparations. But, it was also determinate that S-allylcysteine and S-allylmercaptocysteine have anti-cancer effects in animals and to protect liver from damage.\(^{(1)}\)
The table with some important garlic derived organosulphur compounds and their biological activities, taken from South African Journal of Science (February 1995), is shown:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Chemical structure</th>
<th>Biological activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alliin</td>
<td>( \text{CH}_2=\text{CH}-\text{CH}_2\text{S}\cdot\text{CH}_2-\text{CH}_2\text{COOH} )</td>
<td>Hypolipidaemic, Antimicrobial</td>
</tr>
<tr>
<td>Allicin</td>
<td>( \text{CH}_2=\text{CH}\cdot\text{CH}_2\text{S}\cdot\text{S}\cdot\text{CH}_2-\text{CH}=\text{CH}_2 )</td>
<td>Hypolipidaemic, Hypoglycaemic</td>
</tr>
<tr>
<td>Ajoene</td>
<td>( \text{CH}_2=\text{CH}\cdot\text{CH}_2\cdot\text{CH}=\text{CH}\cdot\text{S}\cdot\text{S}\cdot\text{CH}_2-\text{CH}=\text{CH}_2 )</td>
<td>Antithrombotic</td>
</tr>
<tr>
<td>Diallyl sulphide</td>
<td>( \text{CH}_2=\text{CH}\cdot\text{CH}_2\cdot\text{S}\cdot\text{CH}_2-\text{CH}=\text{CH}_2 )</td>
<td>Chemopreventive</td>
</tr>
<tr>
<td>Diallyl disulphide</td>
<td>( \text{CH}_2=\text{CH}\cdot\text{CH}_2\cdot\text{S}\cdot\text{S}\cdot\text{CH}_2-\text{CH}=\text{CH}_2 )</td>
<td>Intesticide</td>
</tr>
</tbody>
</table>

4) **Oil Macerates**

Oil macerates are prepared with garlic chopped and soaked in vegetable oil for a few days to several weeks. These extracts are especially rich in ajoene compounds.(5) Ajoenes are fairly stable, therefore it could be stored at room temperature. Their main function is prevention of blood clotting that minimize the probability of strokes and thromboses.(6)

There is also always an option of consuming raw garlic (for those who can stand their odor) which is in the same time the least expensive option and most beneficial. Yet, raw garlic may be very irritative for a digestive track.(9) Always better option is frequent use of garlic into cuisine, which may serve as a great prevention for some disease including rather often colds and viruses. One of the good hits for cooking is that after garlic is cut or chopped it should be left to stand for about 10 minutes before cooking, such that all alliin is allowed to react and turn into allicin.

**IV. Medical Applications**

Many of the garlic’s medical properties have been already mentioned in above discussion, but the closer look at some most important applications follows:

**Natural Antibiotic**

One of the basic and well-known properties of garlic is its antibiotic ability. Allicin is the main compound responsible those anti-bacterial and anti-fungal properties. "Several researches have shown that garlic inhibits a variety of microbes, including bacteria, fungi, viruses and parasites."(1) All the way back in the middle of nineteenth
century, Louis Pasteur, reported garlic bactericidal properties. Later on the other characteristics are also determined. Researches find out that aqueous extract of garlic, reduce the growth of several zoo pathogenic fungi. Some of the fungi that causes athlete’s foot and ringworms, might be easily healed with garlic.(1) One of the practical applications is that during the wars allicin has been used to fight infections when no other drugs were available.(7) Close attention is also focused on common yeast from our bodies, Candida Albicans, which can weakened the immune system and has high resistance toward many drugs, but it is indeed sensitive to garlic. Garlic even in its highly diluted juice form decreases the growth of Straphylococcus, Streptococcus, Bacillus and Vidro.(1) In Weizmann Institute of science, team of researchers reported that allicin blocks two groups of enzymes that are very common in different forms of microbes. Therefore garlic makes a good anti-microbial drug in broad mining. “Microbes are unlikely to develop resistance to allicin, because to do so they would have to alter their own major enzymes, which would lead to their destruction.”(10)

The problem that might arise is that allicin is short lived and after just few hours since its formation it loses its biological power.

Cancer Prevention

Since ancient time garlic has been known for its anti-cancerous abilities. Garlic allyl sulfur compounds has been proven as effective inhibitors of the cancer process. They are not just limited to specific carcinogens, but rather to a wide range of different tumors, although the most effective results came from prevention of stomach and prostate cancer. Compounds of allyl sulfur make cells weak to the stress created by cell division, so as cancer cells are dividing the fastest they become damaged by high concentration of allyl sulfur compounds.(11) Cancer Weekly Plus reported that research results of garlic supplementation point out that garlic may inhibit the occurrence of mammary tumors. In their studies they also found that aged garlic might repress breast cancer. “We are seeing that garlic has a direct anti-carcinogenic effect. It seems to work as a detoxin and also performs somewhat of a repair process on damaged DNA.”(9) Department of nutrition in the Pennsylvania State University performed a study with colon tumor cell line HCT-15 and find out that addition of Dialyl Disulfide (DADS) into a culture medium decreases the growth of several established human tumor cell lines, including colon tumor. In one of their study they compared effects of presence or absence of DADS in the known chemotherapeutic agent 5-FU. “Treatment with DADS alone depressed mean tumor growth by 33%, whereas the depression caused by 5-FU treatment was 37%. Combining DADS and 5-FU was no more effective in reducing the growth of HCT-15 xenografts than the use of either agent alone.” In their results they also state that, “providing DADS with 5-FU at least partially prevented the depression in spleen weight and protein caused by 5-FU treatment alone.”(2)

In a few last years garlic gain a lot of attention with its anti-cancerous properties, because it has a least number of side effects compared to most other cancer medications. Beside that, there are also evidence that, “garlic might reduce the side effects of chemotherapy.”(9)
Heart Disease Prevention

Garlic has especially high significance on cardiovascular system and in prevention of heart diseases. Majority of heart diseases are directly related to life stile and proper diet. Garlic has properties to lower blood pressure, lipid and cholesterol level, as well as to prevent blood clotting and reduce artherosclerotic buildup (plaques) in arteries. All of these properties would support prevention of heart diseases.

The lipid lowering effect has been demonstrated by the two “meta-analysis more reliable than the analysis of any single study’s data could be.”(6) In a first trial 410 patients have been put on examination and the results suggested that one half to one clove of garlic per day, decreased total cholesterol level by 9%. One year later, in a second trial 952 patients were involved an it shows that the dosage of 600-900 mg of garlic powder per day, lowered cholesterol level by 12%.(5) However, not all clinical studies showed very successful: “The failure of five recent clinical trails to show significant reduction in serum cholesterol by nonenteric-coated garlic powder tablets, four of which used an allicin-standardised product (brand 1), contrasts to many prior positive trails with the same brand. Some evidences indicates that allicin, formed enzymatically from alliiin, is favorable for the hypolipidaemic effects of garlic, although this issue is htly debated between companies.”(5)

In Japan and China garlic have been used for centuries as a treatment for hypertension. Hypertension makes one of the major risk factors of atherosclerosis.(1) Another meta-analysis clinical trial with 415 patients, showed that: “garliccaused a modest but significant reduction in both systolic and diastolic blood pressures.”(5)

As already indicated ajoenic compounds have great influence on reducing the stickiness of the blood cells. “In a double-blind, placebo-controlled study on 60 volunteers with elevated cerebrovascular risk factors and increased spontaneous platelet aggregation, it was demonstrated that 800 mg of garlic powder per day over 4 weeks led to a significant reduction in platelet aggregation and circulating platelet aggregates.”(5) In a studies on animals, garlic showed increase in plasma fibrinogen, reduced blood clotting time and fibrinolytic activity. This leads to the suggestion that the garlic might help in preventing thrombotic disorders.(1)

V. Side-Effects (7&11)

Although garlic has such variety of health benefits and its supplements are generally safe, it is important to indicate some of its side effects or possible complications:

- Large doses of raw garlic might upset the stomach and digestive truck. Cause diarrhea, dizziness, facial flushing, rapid pulse, occasional allergies and isomnia.
- Garlic could thin the blood, which might caused bleeding problems. It is recommended not to consume a garlic prior to surgery or along woth other blood thinning medications and supplements such as ginkgo, policosanol and vitamin E.
AIDS patient should not use garlic supplements because they might reduce the effectiveness and blood levels of some AIDS drugs.

VI. Conclusion

Garlic has been known and used in medical purposes since inmemorial time, although a huge progress was made since then, still there are much to be learned about their compounds and their benefits. "The health benefits of garlic likely arise from a wide variety of components, possibly working synergistically."(4) Understanding the molecular mechanism and pharmacology of bioactive compounds of garlic is important in facilitating scientific evaluation of new therapeutic approaches of this traditional plant.
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