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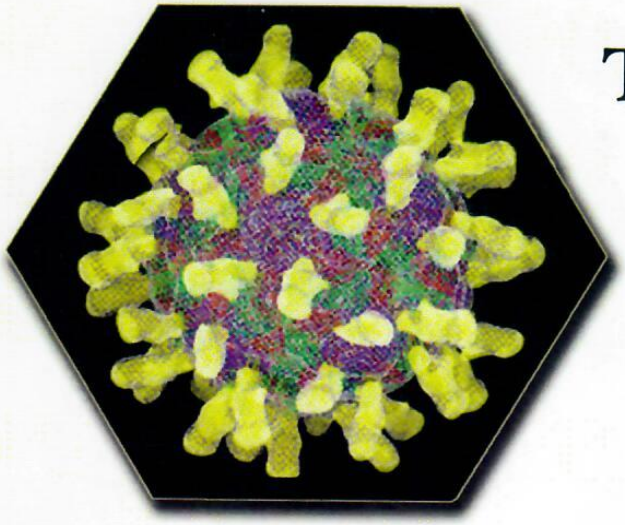

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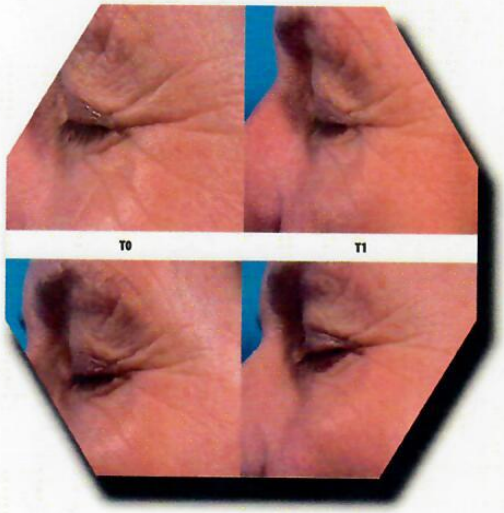

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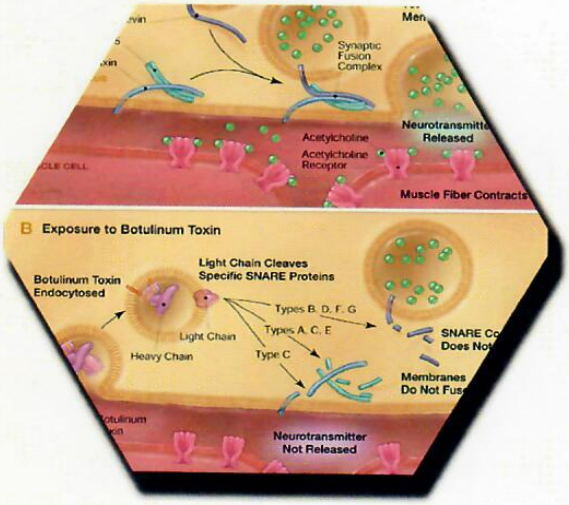

Spring 2009

The Lander College of Arts and Sciences
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Rhinoviruses: The Quest for a Cure

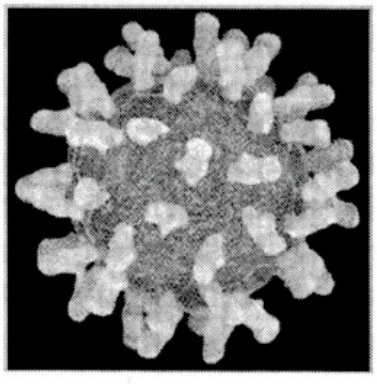
Michelle Gordon- Grunin

INTRODUCTION

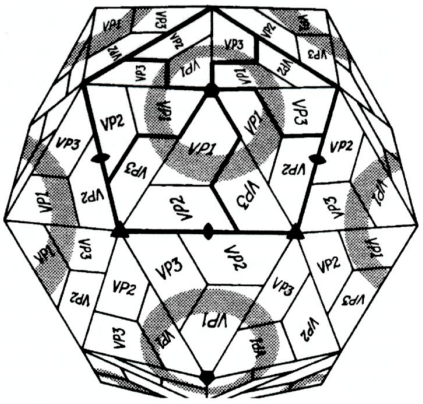
Rhinoviruses, also known as Human Rhinovirus, abbreviated HRV, are one of the many causes of the common cold. In fact, around 50 percent of all cold are caused by rhinoviruses, with the other major candidates being coronaviruses, influenza A or B virus, and minor causative agents like parainfluenza virus, respiratory syncytial virus, adenovirus, and enterovirus (Makela and Puhakka, 1997). However, due to the complex molecular structure of rhinoviruses, a cure for the common cold caused by HRV is still in the making. Several new treatments have been discovered, impacting the virus as different stages of its life, hopefully to prevent those colds that are cause by HRV. Most are still in the process of development, and some have adverse effects. Hopefully, in the near future, a cure will be developed, saving millions of people per year from that annual plague. (Greenberg, 2003).

INTRPDUCTION TO RHINOVIRUS

Rhinoviruses, or Human Rhinovirus are one of the most commonly studies viruses today. (Bella and Rossmann, 1999). The rhinovirus is a fairly small virus, only 30 nanometers, and it belongs to the Picornaviridae (pico means small, virdae, meaning virus) family of viruses. There are approximately 110-115 serotypes of rhinovirus, serotype being the testing of microorganisms for recognizable antigens on its surface.



HRV 16, computer simulated model developed by Purdue researchers
http://findit22.chipublib.org-Cold virus



Organization of the external viral proteins VP1, VP2, and VP3 in the icosahedral shell of HRV's and other picornaviruses. Each protein is repeated 60 times. The canyon is shown shaded. Taken directly from (Rossmann et al., 1999)

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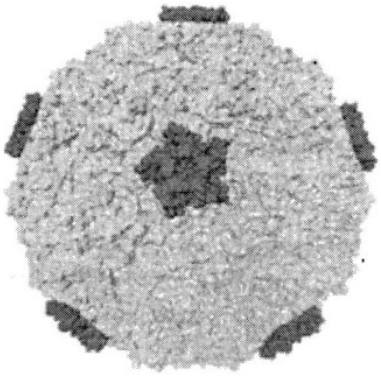
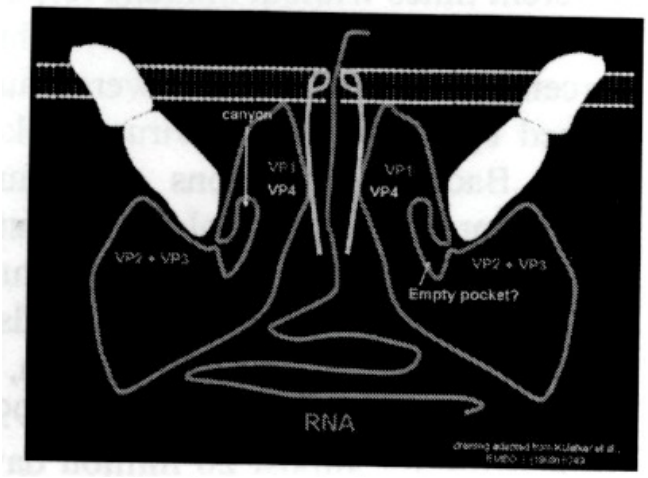
These numerous serotypes are what are responsible for the reinfection process, since different types could affect a person at different times without immune response. (Tolan, et al., 2007) according to a study done in 1997, when two hundred young adults were tested, 50 percent of the colds found were cause by rhinovirus, and the rest were caused by varying other viruses, like coronavirus, or influenza A and B virus. Bacterial infections that cause cold-like symptoms were rare, leaving proof that the cold is almost exclusively a viral disease. (Makela and Puhakka, 1997) According to studies, almost 30-50 percent of all adult colds, and 10-25 percent of colds in children are cause by rhinoviruses. (Bella and Rossmann, 1999) According to Stephen Greenberg, M.D., (2003), in 1996, the common cold was responsible for almost 20 million days of missed work, 22 million days of missed school, and 27 million physician visits in the U.S. In 1998, 76 million visits physicians were tracked up to the common cold, 50 percent of which is caused by HRV. In the United States each year, consumers seeking relief from cold symptoms spend $2 billiom-3 billion on over the counter products to prevent the common cold (Anzueto and Neiderman, 2003).

Rhinovirus is a non-enveloped virus that has only a single stranded, positive sense RNA molecule as its genome. (McCoy, 2004) The capsid is almost spherical, icosahedral in shape, and symmetrical. It is about 300 Angstroms in diameter, (about 30 nm) and is composed of around sixty copies of viral proteins. Theses viral proteins, specifically, VP1, VP2, VP3, and VP4, make up the surface of virus to create an exterior shell. VP4 specifically forms the interior or the capsid, which is in direct contact with the viral RNA (Bella and Rossmann, 1999). In fact, when the rhinovirus structure (specifically HRV 14) was mapped, all of the hypotheses about the structure were proven right (Rossmann, et al., 1985).



The 'starfish like' protuberance. Referenced from http://www.wikipedia.org



Rhinovirus complexing with the ICAM receptor allows entry into a host cell. http://pathmicro.med.sc.edu/virol/pol24.gif

VPI, VP2, and VP3 each take the shape of an eight-stranded molecule, each with a beta-pleated sheet structure, known as the beta-barrel, and the run anti-parallel to each other (Badger, et al., 1988). These fit together in a description like a 'jelly roll', and form the outer surface of the capsid. The capsid is around 5 nanometers thick. (Smyth and Martin, 2001) Unique to the

rhinovirus, at each five-fold vertex, the corners of the polygonal sphere, there is a 'star-fish like' protrusion or protuberance, made up of five copies of VPI, along with a 25 angstrom deep depression or canyon encircling it. This cavity is what makes the canyons. (Bella and Rossman, 1999)

VP4 is also shorter in the HRV, only around 70 residues, or portions of a larger molecule, added on, instead of around 240- 290 residues, like VP1-3, and it is lacking in any special structure. It is on the internal surface of the capsid, near the RNA, and has its N-terminus near the five-fold vertex, and the C-terminus near the three-fold axis of the capsid. VP4 is also covalently bonded to a myristic acid group, giving five symmetrically relates myristic acid groups near the five-fold vertex, and a channel running through the inner and outer surfaces of the capsid (Smyth and Martin, 2001). The protuberances are antigenically diverse among the different serotypes of the rhinovirus, making the canyons different as well (Talaro and Talaro, 2002). VP1-3 contain the antigenic sites that are important for the host immune response, so if they are diverse, that allows for reinfections (Greenberg, 2003).

As for the receptors on the VP molecules, the rhinovirus can be grouped into two-three different types based upon receptors. The first major group, compromising around 91 serotypes have a cell surface glycoprotein known as intracellular-adhesion molecule-1, known as ICAM-1. The minor group, around 10 serotypes, binds to molecules of LDLR, low density lipoprotein receptors. The last group has a receptor that is yet to be discovered (Bella and Rossman, 1999). The LDL receptor that HRV serotype 2 bound to is also known as alpha-2-macroglobulin receptor, or a low density lipoprotein receptor related protein (Hofer, et al., 1993) Dr. Michael Rossmann, with Roland Ruecker at the University of Purdue in 1985 actually discovered the 3-D structure of the rhinovirus, and came up with the canyon hypothesis (Radetsky, 1991).

MODE OF TRANSMISSION AMONG HUMANS

The rhinovirus usually attacks the upper repertory tract in humans. It can be transferred either through aerosol, which is the inhalation of small particles of virus, or through direct contact, by touching the nose with a contaminated hand. However, once it is inside the nose, it moves to the nasopharynx, lodging in the nasal mucosa epithelia cells. It binds to ICAM-1, or an LDL receptor, and infection begins among the host, or human cells (McCoy, 2004). The conjunctiva of the eye may be involved, but because HRV attaches to epithelial receptors, it is less so (Tolan, et al., 2007) Contagious behavior would be construed as nose blowing, sneezing, physically touching environmental surfaces or tissues with the nasal secretions. Within a household, 50 percent of the time infection spreads, but within a school, it ranges from 0 to 50 percent, leading scientists to believe that it requires long-term contact to take effect (Tolan, et al., 2007). The virus replicates in the ciliated cells in the nasal epithelium, although it has been found in research that the non-ciliated cells of the adenoid also may support HRV infection (Arruda, et al., 1991).

Viral shedding occurs in large amounts, specifically, as Robert Tolan, M.D. states, 'as many as 1 million infectious virions present per mL of nasal washings'. This shedding can occur before the host realized that he or she has a cold, and can last as long as 3-4 weeks after the HRV cold dissipates (Tolan, et al., 2007). According to studies, around 95 percent of people exposed to a

HRV strain that they have not previously encountered will develop an infection, and 75 percent of those who are infected display symptoms (McCoy, 2004).

SENSITIVITY

Uniquely among the common viruses, rhinovirus has a sensitivity to temperature. It can only thrive in a temperature between 33-35 degrees Celsius, unable to survive in a normal body temperature of 37 degrees Celsius. This also limits its choice of receptor binding, because it needs the receptors in the upper respiratory tract, and not in the lower respiratory tract, which has a higher temperature (Tolan, et al., 2007).

Rhinovirus also has a sensitivity to pH, because if the virus is swallowed, the decreased pH in the stomach will prevent infection. The rhinovirus capsid dissolves in low pH, which effectively destroys the virus (McCoy, 2004). It is stated that a pH of 3-5 renders that virus unstable (Fiala and Guze, 1970).

SYPMTOMS

Although rhinovirus infections affect people around the world at all seasons, it seems to be epidemic in fall and spring times, causing doctors to prescribe unnecessary antibiotics, which contribute to antibiotic resistance in bacteria that were present at the time (Rotbart and Hayden, 2000). Despite what a parent may tell a child, getting wet, chilled, or exposure to cold weather are not clinically proven to increase the likelihood of contracting HRV (Tolan, et al., 2007). However, according, to a study done in the fall months on 346 adults, 224 were diagnosed as having a cold due to HRV, showing that rhinovirus is the largest contributing virus causing colds during the fall months (Arruda and Pitkaranta, 1997).

Symptoms may begin within twelve hours after infection, and start with the release of cytokines to initiate an inflammatory response. This causes airway hyper-reactivity, and an influx of neutrophils in the nasal mucosa and secretions (Rotbart and Hayden, 2000).

The incubation period normally is 1-3 days, and the most common symptoms are rhinorrhea (runny nose), nasal stuffiness, and sneezing. Other symptoms could be a sore or scratchy throat, facial pressure, headache, cough, hoarseness, malaise, chills, or feverishness. Significant fever is uncommon in adults, but in infants and young children it is present more often (Rotbart, H., Hayden, F., 2000).

HRV can also cause upper and lower respiratory tract complications. Acute otitis media (AOM), could be caused by HRV induced infections, and most cases of acute sinusitis are thought to be a secondary bacterial infection from a primary case of HRV. In children with AOM, viruses have been detected in 11-41 percent of middle ear fluids, and rhinovirus constitutes 8 percent of that. In acute sinusitis, HRV has been detected in 40 percent of sinus brushings (Rotbart and Hayden, 2000). According to Robert Tolan, M.D., (2007) 24 percent of patients with AOM have rhinovirus in their nasopharyngeal secretions.

HRV can also exacerbate asthma in adults and children. In a two-year study done in adults with asthma from 19-46 years of age, colds were associated with 71 percent of exacerbations, and rhinoviruses are the most commonly identified pathogens, HRV also is associated with lower respiratory tract infections. Up to 40 percent of exacerbations in patients with chronic bronchitis are associated with HRV, and in infants younger than 12 months, HRV is

associated with lower respiratory tract illnesses that required hospitalization, including bronchiolitis, and bronchopulmonary dysplasia (Rotbart and Hayden, 2000). HRV may also cause laryngotracheobronchitis in infants, and in cystic fibrosis patients, rhinovirus is the culprit for 57 percent of respiratory exacerbations (Tolan, et al., 2007).

VIRUS ACTION AND INFECTION ATTACHMENT

The rhinovirus attacks a host using the established mechanisms of viral infection. Firstly, the viral capsid interacts with specific receptors on the cell membrane. The receptor binding sires on rhinoviruses are inside the canyon made by VP1-4, which surrounds each 'star-fish', five-fold axis (Smyth and Martin, 2002). When neutralizing antibodies were tested against rhinovirus' surfaces, it was discovered that the virus continued to evade antibody neutralization actions. The mutations in shape that caused this were located at four distinct antigenic surfaces, at the most exposed regions on the virus- on the rim of the canyon depressions. However, molecular residues at the bottom of the canyon are conserved, and immunologically secluded, and the canyon is too narrow to allow antibodies access to the receptor sites. This led to the discovery that the canyon is the receptor binding site, and that the rhinoviruses can hide their binding sites there, protecting them from antibody attack, while they created external residues to confuse the host's immune surface. This is known as the canyon hypotheses (Bella and Rossmann, 1999 and 2000). This protects the virus from any immune response that counteracts its cell receptor binding.

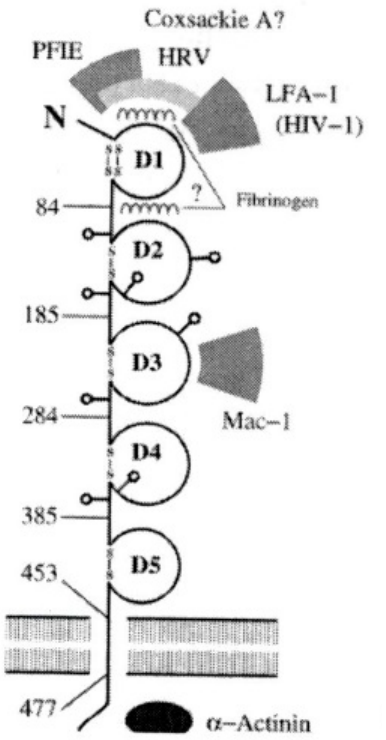
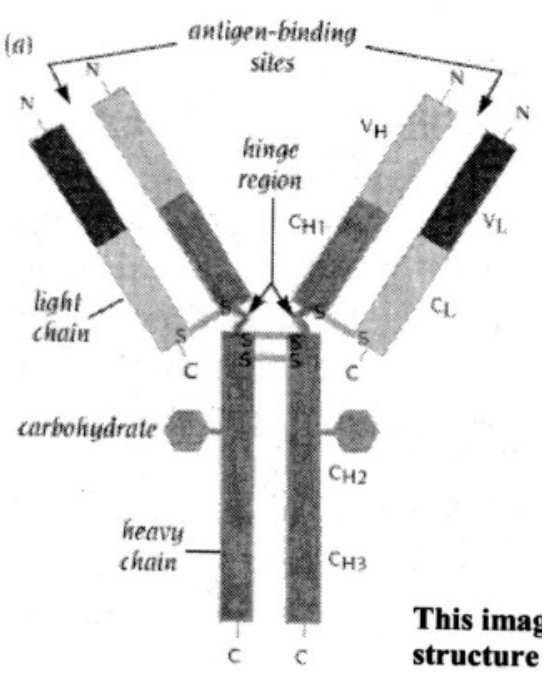


Fig 1. Domain structure if ICAM-1. Each Ig domain is represented schematically by a circle closed by one or two disulfide bonds. Amino acid numbers indicate the beginning and end of each domain. Approximate locations of relevant binding sites are shown. Lollipop-shaped structures indicate N-linked glycosylation sites. (Bella and Rossmann, 2000)



This image from Branden & Tooze shows the structure of an IgG immunoglobulin molecule.
http://images.google.imgres?imgurl=http://pps00.cryst.bbk.ac.uk/course/section11/IgG.gif&imgrefurl=http://pps00.cryst.bbk.ac.uk/course/section11/immunog.html&h=349&w=285&sz=19&hl=en&start=3&tbnid=SsURfzzb9uyQjM:&tbnh=120&tbnw=98&prev=images%3Fq%3Dimmunoglobulin%2B%26gbv%3D2%26vnum%3D10%26h1%3Den

The ICAM-1 receptor is a glycoprotein cell adhesion molecule (hence, CAM), that has an extracellular component made up of immunoglobulin chains. Immunoglobulin (Ig) chains are the building blocks of antibodies, molecularly described as two sets of beta-pleated sheets running antiparallel to each other and linked by disulfide bonds. ICAM-1 has five Ig chains, also known as domains, along with a transmembrane region into the cell, and a short cytoplasmic domain inside the host cell. It has been described as a lollipop structure, due to the several domains connected (Bella and Rossmann, 1999). The ICAM molecule has five immunoglobulin like domains, known as D1-D5 respectively. D2-D4 are glycosylated, and D1 is the primary binding site for rhinoviruses, and for the ligand that ICAM-1 binds naturally, known as lymphocyte function-associated antigen 1 (Olsen, et al., 1993). Some rhinoviruses have been known to stimulate the ICAM-1 expression on host cells to increase the chance of infection (Tolan, et al., 2007).

UNCOATING

When HRV attaches to the ICAM-1 receptor, this initiates entry into the host cell. During the un-coating stage of viral infection, the RNA across the host cell membrane into the cell, but the actual mechanism is still not known for HRV (Bella and Rossman, 2000). ICAM-1 is a transmembrane protein, and its two terminal ends show an Ig like-fold, solidifying the structure hypothesis. On the tip of domain D1, there are three loops important for HRV binding, known as DE, BC, and FG. HRV is usually specific by species, and does not recognize ICAM-1 in any other species other than human, and also ignores similar CAM receptors, like ICAM-2 or ICAM-3 (Bella and Rossmann, 1999).

Richard Colonno, a scientist from Merck Laboratories, actually was the first one to discover the I-CAM receptor, along with the receptor antibody to prevent HRV from bonding to the host cell. In a method known as “Colonno’s brute force method”, he performed 8,000 different tests, and discovered that the I-CAM antibody prevented mice nasal cells from becoming infected (Radetsky, 1991). Colonno discovered, using receptor antibody that he developed, that HRV used the canyon to bind to its ICAM receptor, and discovered the location of the plaques and residues inside the canyon (Colonno, et al., 1985). 20 out of 24 HRV serotypes tested proved positive for the ICAM receptor, and the I-CAM antibody that Colonno developed seemed to prevent receptor binding for 78 out of 88 serotypes tested (Colonno, et al., 1985).

Uncoating is the second step on rhinovirus infection of a host cell. The HRV must have a stable enough capsid to be able to transport itself to the host cell and bind with a cell receptor, but it also must be able to disassociate when needed to allow the RNA genome to enter the host cell. However, research has shown that the crucial step for rhinoviruses is the loss of the VP4 protein. It seems that the myristic acid that is covalently bonded to the VP4 terminal end can interact with the host cell membrane, causing the release of VP4, and the genome, allowing the RNA to enter the cell. The acid lies near the inner edge of the five-fold channel, allowing the genome to leave the capsid through the channel. However, the capsid must disassemble enough to allow this to happen. Uncoating mechanisms have been tested and observed with HRV14, in

which acid conditions were introduced, or as with HRV16, a preparation of its receptor was exposed to it, initiating uncoating (Smyth and Martin, 2002).

In addition, there is a canyon contained within VP1, known as a pocket, which is hydrophobic. Structural analysis has shown that there is a fatty acid in the pocket, known as a pocket factor. This pocket factor has an 'inhibitory but reversible' effect on uncoating. This allows the pocket to stabilize the capsid until it is time for it to disassemble when faced with a host cell. Residues inside the pocket become more stable, along with the N-terminal end of VP3, which allows the protein to stabilize (Smyth and Martin, 2002). This pocket factor fatty acid also protects the virus in between its cell to cell transit, between neighboring cells (Xing, et al., 2003). So, when the rhinovirus binds to the cellular receptor, it causes the virion particle to disintegrate, and the RNA genome is released directly into the host cell (Sompayrac, 2002).

REPLICATION

Replication is the next step in the viral infection process. The RNA moves through a membrane pore that is generated by the N-terminals of VP1 and VP4, during the uncoating process. It has been suspected, but never proven, that the RNA leaves through one of the 12 vertexes at the starfish shaped protuberances. It has been theorized that the virion undergoes conformational changes after the RNA leaves, which leads to capsid disassembly, but it has never been shown. ICAM-1 also locks the virus in an 'open state' so that the RNA can be released without the virion folding in on itself. When the virus expands, by locking the ICAM receptor in place, which allows for movement of the RNA out of the virion. Once the RNA exits, this leads causes rearrangements in the virion cell, namely that the VPI molecule twists, allowing for a larger molecule that eventually disintegrates (Xin, et al., 2003). The RNA genome is then injected into the cell from the acidic endosomes that internalize the virus, like the myristic acid groups of the VP4 (Smyth and Martin, 2002).

Rhinoviruses trigger a chemokine and cytokine response once they have entered and started infecting the cell. This response exacerbates the symptoms of the common cold, and some asthma patients, due to the inflammation that results (Virus Weekly, 2007). The cytokines, specifically are interferon-gamma and interleurkin-6 and interleurkin-8, along with interleukin-1 alpha. (Anzueto and Niederman, 2003). This leads to nasal discharge, nasal congestions, sneezing, and throat irritation (Tolan, et al., 2007). The immune response attracts immune cells, and sends chemical messages to neighboring blood vessels, causing leaking of capillaries, glandular secretion, and causes stimulation of nerve fibers. This causes the sneezing and coughing reflexes, in addition to a pain sense (McCoy, 2004).

The RNA rhinoviruses is a positive sense RNA, meaning that it serves as the viral mRNA, and can be immediately translated by the host cell without involving DNA. The RNA contains between 7500 to 8300 nucleotides, and encodes a single large polyprotein (Belsham and Sonenberg, 1996). The complete HRV genome has been mapped for rhinovirus-14, which has 634 non-coding nucleotides, 6537 coding nucleotides, and again a 47 nucleotide region of non-coding nucleotides. The molecular structure of HRV is most closely associated with enteroviruses, another of the family picornavirdae (Stanway, et al., 1984). It is translated one time by the ribosomes of the host cell, and then the polyprotein created divides itself up into several viral proteins. One viral protein created is the viral RNA polymerase, which then makes complementary copies of the original viral RNA, which are negative strands, in the sense that

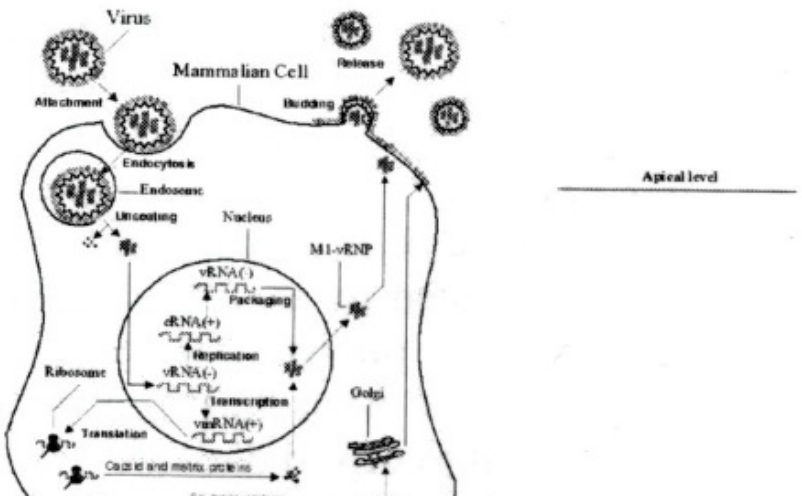
they are the exact opposite of the original strand. These copies of the viral RNA are then copied again, so complementary, positive sense strands are then created, and translated to make new viral proteins (Sompayrac, 2002).

These new viral proteins then undergo processing by virus-encoded proteases, and finally produce the mature virus proteins. This mature viral protein contains about 11 different polypeptides plus some partially processed products. Four of these proteins, with around 60 copies of each one, make up the virus capsid, and other proteins are involved in replication (Belsham and Sonenberg, 1996). The virion capsid protects the many copies of the virion, until the rhinovirus leaves the cell (Sompayrac, 2002). The virus inhibits the host cell's own transcription and translation, modifies or destroys the intracellular membranes of the cell (e.g., the organelles), destroys the cell itself through lysis, and finally releases the mature viruses, ready to infect the next cell (Belsham and Sonenberg, 1996).

The RNA genome is replicated though a RDRP, a RNA-dependent RNA polymerase which is a double stranded RNA intermediate to help the replication process. The host cell ribosomes are taken over, and initiated by an IRES, an internal ribosome entry site (Belsham and Sonenberg, 1996). The IRES allows the ribosomes to begin translating the original RNA genome without the "cap" structure normally present in a genome (Sopayrac, 2002).

The 5' terminal end, or UTR, untranslated coding region, of the RNA is uncapped, which is unusual. The rhinovirus 5' UTR is able to direct protein synthesis without mRNA, and is now referred to as an IRES, or a ribosome landing pad. The IRES is located about 150 bases from the initiation codon on the 3' UTR, but the distance can be greatly modified with little or no effect on rhinoviruses. Rhinoviruses do translate poorly, due to the fact that they, unlike DNA, do not have a checking factor built in to check the nucleotides before translation (Belsham and Sonenberg, 1996). The process by which the cell recognizes the IRES sequence is not known, but many initiation factors as well as other specific cellular proteins help. Three factors have been identified so far, polypyrimidien tract binding protein, La auto antigen, and PCBP (poly(rc) binding protein). The binding of PCBP to the 'clover lead' RNA at the 5' end enhances viral translation (Gamarnik and Adino, 1998).

To prevent interference from the host cell, the HRV encodes a protein that disrupts the normal cap-dependent initiation of the host cell. This shuts down all protein synthesis from capped, cellular mRNAs, except from its own uncapped RNA genome. It only takes about eight hours for a rhinovirus to reproduce, and to make thousands of new viruses (Sompayrac, 2002).



IMMUNE RESPONSE

The body's natural immune response does try to prevent the inhalation of these virions. HRV enters into the lowest part of the nasal cavity, and starts its replication there, without moving deeply into the lower respiratory and digestive systems to be destroyed by the acidic contents of the stomach. This internal defense prevents rhinoviruses from causing intestinal or gut infections. However, there is another defensive immune response. When cells are under attack by a virion, they produce 'warning-proteins' known as Interferon-alpha and interferon-beta. Interferon binds to receptors on uninfected cells, alerting those cells to virion invasion. These cells then spontaneously destroy themselves, to limit the spread of the virus. It is the presence of a large quantity of double stranded RNA in the cell that causes it to produce interferon (Sompayrac, 2002).

HRV however, has a way to deal with the interferon signaling problem. It interferes with the production of interferon by shutting down the host cell's system for transporting interferon out of the cells. So, HRV infected cells produce very little interferon. This also means that HRV has not built up any resistance to interferon as an anti-viral mechanism. HRV is mainly destroyed in the body by the innate immune system, by the phagocytes and NK cells, so that a rhinovirus infection is usually over in a few days. This causes a problem, because the infection is over so quickly that the adaptive immune system, like the B and T lymphocytes, are not activated, and neutralizing antibodies are not created for that infection. So, there is no way to prevent a second rhinovirus attack, even from the same strain of HRV, because there were no antibodies created from the first attack. And, HRV uses antigenic drift, so there are over one hundred different strains of HRV all around in the public. This is when mutations that are introduced during viral replication are used to produce different strains of the same virus. A person can continue being re-infected several times over a matter of weeks (Sompayrac, 2002).

Macrophages do provide one helpful immune response to rhinoviruses, in that they produce interleukin-1, a cytokine that triggers a low fever. HRV can't tolerate higher temperatures, so it can help control the spread of the virus (Sompayrac, 2002). Interleukin-1 alpha, interleukin-6, and interleukin-8 have been found in nasal secretions, and are responsible for most of the symptoms (Anzueto and Niederman, 2003).

TREATMENT FOR HRV AND NEW DEVELOPMENTS

Because of the canyon hypothesis, along with the 'attack and surrender' mode of infection of the rhinovirus, a HRV vaccine is impossible, and even if it were possible, it would not be cost effective, due to the necessity of vaccination for over 100 different types of HRV, due to antigenic drift. However, anti-viral therapy has been developed for HRV.

The WIN-family of compounds are common anti-viral agents being tested as a defense against HRV. They bind to the pocket in VPI, which is hydrophobic, preventing the virus from binding to the host cell. Usually this pocket is filled with the 'pocket factor', a lipid compound, and when this pocket is filled with an anti-viral compound, it stretches the pocket, expanding the

beta-barrel, producing an open conformation, and preventing capsid uncoating (Hadfield, et al., 1999). There are currently nine WIN compounds being tested, yet only a few of them show efficacious results (Pevear, et al., 1989).

The WIN compounds generally contain three aromatic rings, known as A, B, and C. some rhinoviruses' pockets only interact with ring C, while others interact with A and B. Ring A is usually a methylisoxazole ring, ring B, a substituted phenoxy group, and ring C, a five-member heteroatom ring (Zhang, et al., 2004). Variations in the pocket if the rhinovirus, like a more hydrophobic 'toe end' of the pocket, and a more hydrophilic 'heel end', lead to the different bindings of the WIN compounds, and lead to different efficacies (Hadfield, et al., 1999).

WIN 54954

The WIN 54594 molecule is an oral anti-viral compound that is active against rhinoviruses and enteroviruses. It works by binding to the hydrophobic pocket inside the VP4 protein on the capsid surface, preventing the replication of the virus by interfering with the virus uncaoting process, and by changing the cell receptor site to not allow HRV to attach to the host cell. When WIN 54594 was tested in cell experiments, it inhibited 80 percent of the rhinovirus serotypes that were presented to it. As such, the developing scientists started to perform clinical trials on WIN 549594. However, when WIN 54594 was tested by volunteers in two different trials who were infected with HRV type 39 or type 23, it had minimal effect. The scientists that developed it feel that the reason was that the human nasal epithelial cells did not take up sufficient amounts of the drug to make a real difference in the sire of the viral infection. If the scientists refine the drug, it may have potent anti-viral effects (Turner, et al., 1992). It did reach phase II clinical trials, but due to the low efficacy in *vivo*, and some side effects, it was stopped (Hadfield, et al., 1999). The side effects included adverse effects of flushing and a rash (McKinlay, 2001).

WIN 52084

WIN 52084 is also another anti-viral molecule that attaches to the hydrophobic pocket in VPI on a rhinovirus capsid, preventing its attachment to the host cell, and limiting its uncoating mechanism. It also stabilizes the capsid, allowing it to be inactivated by temperature or acid related influences (Lewis, et al., 1998).

WIN 51711

WIN 51711 was a compound that bound to the viral capsid, specifically to the interior of VPU, preventing the virion from binding to the host cell receptor (Sperber and Hayden, 1988).

This WIN compound, known as disoxaril, had broad implications in inhibiting picornavirus activity, specifically HRV, and it interacts in a similar way to the other WIN compounds. However, when it entered phase I clinical trials, it failed toxicity tests, leading it to be stopped (Hadfield, et al., 1999).

PLECONARIL

According to the research in the United States, in 1994, there were 66 million cases of the common cold, caused by HRV. Pleconaril, a new oral drug developed, is a small molecule inhibition of rhinovirus that is developed for the entire picornavirus family (Pevear, et al., 1999). Pleconaril is {3- [3, 5 dimethyl-4-[(3-methyl-5-isoxazoly)-propyl]-phenyl]-

5(trifluoromethyl)-, 2, 4-oxadiazol}. It integrated into the pockets of the capsid at VP4, and inhibits the viral capsid uncoating. Pleconaril blocks the attachment to the host cell receptors, which in turn, inhibits viral replication. It was the first anti-picornavirus compound to be submitted to the FDA. In the clinical trials, it did reduce symptoms and duration of the colds, and with animals, it has penetrated the cells involved, and protected them. In phase II studies, it showed reduction of symptoms significantly, as compared with the placebo (Anzueto and Niederman, 2003). It is a WIN compound, WIN 63843, and is a third generation of the original two WIN compounds, WIN 51711 and WIN 54954, and is currently in phase III trials (Hadfield, Diana, and Rossmann, 1999). According to a 2004 report, it has been in the phase III trials, and is performing efficaciously (Zhang, et al., 2004).

Pleconaril has been tested on 1024 individuals who received it three times daily, and reduced the time to heal 3.5 days, instead of seven days. Individual symptoms also resolved themselves sooner in time to heal 3.5 days, instead of seven days. Individual symptoms also resolved themselves sooner in the pleconaril patients. The side effects seemed to be similar for both those on pleconaril and those with the placebo, and as such, it really has no adverse effects. It has a clinical (due to the reduction of symptoms) and antiviral effect (Rotbart and Hayden, 2000). However, because of safety concerns based upon potential drug-drug interactions, the FDA did not approve pleconaril, but new formulations are being considered (Greenberg, 2003).

INTRANASAL INTERFERON

Interferon molecules have antiviral, anti- proliferative, and immunological effects, mostly associated with the 'suicide' response of infected cells. Scientists have developed a synthetic copy, intranasal interferon-alpha2, (or intranasal interferon alfa-2 beta) which has activity against natural rhinovirus infections. However it has not been beneficial in treatment, due to the severe side effects of nasal irritation and bleeding (Anzueto and Niederman, 2003). In fact, after double-blind trials had been performed with interferon alfa-2 beta, there were no differences in the respiratory symptoms scores, and although there were less active viral particles in the nasal washings from those receiving interferon, there were instances of nasal bleeding, and was associated with toxicity to the volunteers (Hayden, et al., 1987). It still remains to be seen if other interferons or other methods can prevent these effects (Sperber and Hayden, 1988).

Interferon alpha administered intranasally through the major study worked very well in preventing HRV colds. Out of the 14 volunteers who received the placebo, 6 had definite rhinovirus infections, while 0 out of 10 with the interferon-alpha2 had been infected. During the third week of testing, interferon-dosed patients complained of nasal discomfort, nasal obstruction, and/or blood tinged mucus. The results tend to indicate the prevention of infection entirely, but due to the long term side effects, it cannot be prescribed long term. It has been administered through a spray and through drops, and has had a huge anti-viral effect. With regard to the drops, and a lower dosage of interferon, there was no intolerance, and it did prevent colds, but there were long term side effects. Possibly, for short term, this drug could be used (Farr, et al., 1984). However, due to the severe symptoms, it is not useful for treating the (generally mild) cold symptoms (Mossad, 1998).

SOLUBLE ICAM-1

Richard Colonno had discovered the difference in receptors among the different strains of HRV, determining what we know commonly now, that there are two major receptors, and most use ICAM-1. As many as 115 serotypes were discovered and out of the 24 tested, they all shared the same ICAM-1 receptor on the host cells (Abraham and Colonno, 1984). After Colonno used his "Brute Force Method' to discover an antibody, but as the synthetic antibody was not eventually cost effective, more scientists took on the race to find a different cure (Taubes, 1999).

Greve and McClelland discovered in 1989 the major HRV receptor on human nasal epithelial cells, known as the intercellular adhesion molecule-1, abbreviated ICAM-1. They discovered it by sending monoclonal antibodies to the host cells, and they recognized the ICAM-1 protein on the cell surface and bound to it (Greve, et al., 1989). They made the antibodies by injecting mouse cells with human HRV-infected cells, and so the mouse would then make antibodies for all the proteins on the host-cells surface. When they finally discovered that one of these monoclonal antibodies worked, it was against ICAM-1, already discovered as a cell receptor molecule by Springer, another scientist working at his own laboratory (Taubes, 1999).

Greves and McClelland, along with a different scientist, Hayden developed a synthetic of what is commonly known as soluble ICAM-1, or sICAM-1, a form of the receptor that was not bound to the cell wall but free to float in solution. This would act by competitive inhibition, attaching to the HRV before it would attach to the host cell's ICAM-1 (Taubes, 1999). Human cells naturally make a form of sICAM-1, but HRV acts on the cell to upregulate the membrane bound ICAM-1, and down regulates the sICAM-1 in the extracellular space, so that it can develop on the host cells and not be impeded by sICAM-1. So, a synthetic of sICAM-1, if developed into a usable drug, could have a large effect. sICAM-1 has been proven to have antiviral properties both *in vitro in* and *in vivo* (Whiteman, et al., 2002). The down regulation of ICAM is so strong that although the ICAM right after ICAM infection is upgraded to prevent the infection within the first 24 hours, it is almost immediately downgraded to the baseline level by day 9. The up regulation of ICAM is done in response to various stimuli, including ozone exposure, interleukin-5, TNF alpha (tumor necrosis factor-alpha), interleukin-1, and CD8 T lymphocytes (Winther, et al., 2002). Hayden performed four clinical trials on 196 student volunteers, with his version of soluble ICAM-1, known as Tremacamra. Virus shedding was detected from the experimentally induced HRV colds. Of the 177 subjects used to determine efficacy, 81 received Tremacamra, and 96 received placebo. There was a 45 percent drop in symptoms, a 23 percent drop in the clinical colds, and a 56 percent drop in mucus, and above all, even if the drug was administered after infection, it still reduced symptoms, and it has preventative measures (Turner, et al., 1999). A test in chimpanzees with rhinovirus-16 infection showed that the s-ICAM molecule was successful in preventing infection, through checking nasal washing and shedding (Huguenel, et al., 1997). Unfortunately, the development of Tremacamra has been halted. The reason given has been the only marginal clinical benefit observed in a highly controlled setting, with the drug being administered five to six times a day (McKinlay, 2001).

SOLUBLE LDL

Soluble forms of the LDL receptor have also been tested, but not extensively, due to the fact that only a few serotypes of HRV actually use the LDL receptor. It seems to inhibit infection by causing aggregation of the virus, unlike the sICAM molecule (Turner, 2000).

The LDL receptor was analyzed and then a synthetic was developed, containing the seven low density-lipid receptor ligand binding repeats that were found on original LDL in cells. The soluble LDL was tested against cells *in vitro* and found to bond to them, proving that the synthetic was identical to the biological LDL. When the group of HRV that uses an LDL receptor, HRV2, was tested against a synthetic soluble LDL receptor, it inhibited the HRV infection *in vitro*. The virion particles formed large aggregates, preventing binding to the LDL receptor in the host cell. In addition, some HRV particles were also prevented from binding to the host cell receptor due to competitive inhibition between the soluble LDL and the host cell LDL. More investigation is probably necessary on this compound (Marlovits, et al., 1998).

AG7088-RUPRINTRIVIR

AG7088 has been synthesized at Agouron Pharmaceuticals in San Diego, and is an antiviral compound, which inhibits the 3C protease in HRV. It was originally tested with cells in the laboratory, and delivered statistically significant results in inhibiting infection by HRV (Zalman, et al., 2000). And enzyme that is encoded by the virus, 3C protease, is the enzyme that cleaves the viral proteins from the polyprotein molecule created in the first step of translation. This allows the virus to replicate and assemble itself. So, some low-molecular weight drugs have been developed to inhibit the 3C protease and prevent HRV from translating. AG7088, one of these drugs, shows good in vitro activity against HRV, and is now being reformulated to maximize delivery to the nasal cavity (Anzueto and Niederman, 2003). In fact, when tested, AG7088 showed that it irreversibly inhibits the 3C protease, preventing translation (Binford, et al., 2004). It had been tested in 1999 on 868 subjects in a phase II study of naturally acquired picornavirus colds, and a trend was observed towards reduction of the total respiratory symptoms (McKinlay, 2001). Ag7088 was found to be active against 48 HRV numbered serotypes as well as 46 unnumbered types and 4 other picornaviruses (Binford, et al., 2004).

This AG7088, or known as Ruprintrivir, has reduced the number of cold victims in its study, from around 44 percent with HRV in the group to 70 percent on a placebo. It had no major side effects, just nasal irritation or blood-tinged mucus (Hayden, et al., 2003). Studies have shown that AG7088 did not prevent experimental rhinovirus infection, but it reduced illness severity. The side effects, nausea and taste disturbance, were mild (Greenberg, 2003). It was given as a nasal spray, and positively reduced the proportion of those subjects with a positive viral culture out of 202 subjects. The overall infection rate was reduced by 28 percent, but although it has antiviral effects, it didn't diminish the frequency of catching a cold (Hayden, et al., 2003). As such, AG7088 is being reformulated to maximize its effects, and to allow better delivery to the nasal cavity (Greenberg, 2003), with further phase II trials in play (McKinlay, 2001).

VIRAL CAPSID BINDING COMPOUNDS

There are many viral capsid binding compounds in existence, which have been used for picornavirus infections. These include amantadine, rimantadine, and zanamivir. However, with rhinoviruses, these drugs give no major clinical benefit. These drugs bind to the hydrophobic pockets in HRVs capsid, and then inhibit the uncoating of the virus and attachment via ICAM-1. In addition, the side effects of most of these drugs outweigh the slight benefits they give against HRV (Mossad, 1998). These compounds have been tried in influenza viruses, and had excellent

antiviral activity, but most have not demonstrated the same in human clinical trials of HRV. Many of these compounds have limitations in dosing, delivery, tolerance, bioavailability, solubility, and safety, and as such, only preclinical trials re performed with these agents (Anzueto and Niederman, 2003).

A specific capsid-binding compound, BTA188, created in Australia, has been shown to inhibit antiviral activity. It had been tested, and inhibited 87 out of 100 specific serotypes of HRV, although it has yet to be tested on humans. BTA188 has been tested on dogs and rodents, with antiHRV activity, and a good uptake of the compound by the cells in question. This compound should undergo further testing and refining in the future (McKinlay, 2001).

ENVIROXIME

Enviroxime is an antiviral agent made from a benzimidazole derivative. It is believed that enviroxime works by inhibiting the viral RNA polymerase replication complex, (Sperber and Hayden, 1988) thus targeting viral replication inhibition of the virus. It inhibited the 3A coding region of the viral RNA, not letting it be translated, and effectively shutting down the process. However, its development has been halted, because it cannot be administered orally, and in clinical studies when it was administered nasally, it had limited antiviral activity. However, other similar compounds are under investigation (Anzueto and Niederman, 2003). Side effects were observed, like nausea and vomiting. There was limited antiviral activity in several trials. In a study with our sprays per day, there was no benefit shown, with no reduction in viral shedding or symptoms, and a study with six sprays per day showed the same results. It was also tested on naturally occurring HRV colds, with no specific advantages or antiviral effects.

However, although enviroxime had minimal antiviral effects, other similar compounds, like enviradene, are still under consideration, as are other methods of administering these compounds, like a topical delivery (Sperber and Hayden, 1988).

PIRODAVIR

Pirodavir, a substituted phenoxy-pyridanzinamine, is a compound that possesses anti-picornavirus activity (Tolan, et alk., 2007). Several pyridazinamines, like R61837, have already been tested to have clinical activity against many serotypes. It binds to the HRV capsid, and prevents capsid uncoating, cumulating in no infection. Four double-blind trials were performed on volunteer subjects experimentally induced with HRV colds, and pirodavir was administered intranasally. When sprays were given six times a day, colds developed in 100 percent of the placebo subjects, while only 58 percent of the pirodavir treated subjects became infected. Pirodavir also was associated with an unpleasant taste, but that was the only serious complaint. However, it would have to be administered with frequent nasal sprays daily during the duration of a cold (Hayden, et al., 1992).

However, clinical trials have not shown any decrease in rhinovirus viral shedding or symptoms, and as such, it has not been used for HRV (Tolan, et al., 2007). It was effective when given prophylactically, but had no effect on established infections (Turner, et al., 1999).

ZINC

There have been many studies done on the anti-viral effects of zinc on HRV. Some show that zinc beneficial, and some don't. Zinc's mode of anti-viral action is still subject to much discussion, although several theories include competitive inhibition, which prevents the HRV from binding to ICAM-1 blocking viral entry in to the cells, inhibiting viral capsid protein synthesis, stabilizing the membrane of the host cell, inhibiting prostaglandin metabolites, and increasing interferon production in the host cell (Mossad, 1998).

According to one study done on acute power upper respiratory infections, primarily caused by rhinoviruses, zinc gluconate lozenges were given to volunteers, and the duration of illness was not significantly reduced. The severity of the illness was reduced, but the adverse side effects, like nausea and altered taste, were reported by fifty percent of the volunteers. Therefore, according to that study, zinc lozenges were ineffective. The authors concluded that zinc had prevented rhinovirus replication by complexing with the capsid proteins, and preventing the proteases from binding to them (Smith, et al., 1989).

A study was performed by one group who found 40 percent reduction in symptoms, but they used unflavored zinc gluconate tablets and unflavored calcium tablets, leading to a difference in taste (Eby, et al., 1984). Another study performed also found significant reductions in symptoms by using zinc lozenges, but found no antiviral effects. This may have been due to the nasal washings used to dilute a sample for testing may have removed from the zinc, and the effect of zinc may have been only due to the actual presence of zinc in the sample at the time, instead of having an effect once it had just touched the viral sample, and didn't need to be actually present (Al Nakib, et al., 1987). It could be that the efficacy of the lozenge is related to the saliva concentration of zinc, and as such, the saliva can't impact the nasal mucosa, leaving most HRVs untouched, making all the lozenge studies invalid (Eby, 1988).

According to another study done by Gwaltney, Farr and colleagues, who tested zinc lozenges as well, zinc therapy did not reduce the viral symptoms, or alleviate the cold manifest symptoms. This study concluded that participants in other studies may have tasted the bad-tasting zinc lozenges and ascribed healing benefits towards them unduly, and as such, they developed a taste-matched placebo that also tasted bitter to prevent the volunteers from realizing which tablet was the active medication. They found that zinc truly had no noticeable effect (Farr, et al., 1987).

MAST CELL STABILIZERS

Mast cell stabilizers are those drugs like Nedocromil and sodium cromoglycate. They are administered intranasally or inhaled, and have reduced the severity of natural and experimental HRV colds. However, they prevent the chemokines and cytokines from being released, reducing symptoms, but they also do a small part in down regulating the ICAM-1 receptor, so the virus can't bond to the host cell. They have been shown to have no effect on viral shedding or viral response to the infection, so although they have some anti-viral effects, it is minimal (Mossad, 1998).

AQUEOUS IODINE

There have been many studies and arguments, some of which that are still going on regarding the method of transmission of HRV. In the University of Virginia, Jack Gwaltney wanted to prove that HRV colds spread through direct contact, like touch. He, and a colleague,

Owen Hendley, developed an iodine solution that killed the virus through hand contact. However, the solution smelled bad, and turned skin brown, but those who used the solution had 40 percent fewer colds. Elliot Dick of the University of Wisconsin, developed what was known as virucidal facial tissues, known as Dr. Dick's Killer Kleenexes, D2K2 for short. In a test, 60 percent of those who used cloth handkerchiefs developed a cold. Elliot Dick also tested for the mode of transmission. He discovered that HRV developed through aerosol contact, not through touch, through an experiment with human volunteers. The D2K2 tissues were marketed under 'Avert', from the Kleenex Company, but didn't sell so well. In addition, Gwaltney, the chief proponent of the touch transmission hypothesis found that they only worked 10 percent of the time in his study (Radetsky, 1991).

ACID SOAKED TISSUES

Jack Gwaltney, along with his colleagues, developed the virucidal nasal tissues. These were nasal tissues, like Kleenex, that had been impregnated with malic and critic acids, along with sodium lauryl sulphate. The idea was to reduce the pH in the nasal cavity, causing spontaneous capsid disassembly to prevent HRV infection. Working against a placebo of saccharin acid, they caused a 14 percent drop in cold infection rate. So, when used, these virucidal tissues may have a small effect, but not a major one (Farr, et al., 1988).

Gwaltney continued to submit his theory developed in 1978 that HRV was transmitted by hand to hand contact. Although iodine is probably the most effective in preventing the spread by touch, it comes with side effects listed earlier…Under Patent 6034133, Jack Gwaltney, Owen Hendley, and Deborah Thacker registered their idea for a virucidal hand lotion, which contained the same ethyl alcohol, citric acid, and malic acid of the tissues, and was not dangerous to the skin. According to a study done against iodine, this lotion was just as effective at halting the spread of the virus, due to lowering the pH to around 3 (Hendley, et al.-Patent, 2000).

ANTIBIOTICS

According to studies, although antibiotics are commonly prescribed for HRV, there is no clinical benefit or antiviral activity. A study of 1,500 children found that antibiotics did not affect the HRV caused colds. Some cold sufferers, around 20 percent, according to a Swiss study, have pathogenic respirator bacteria, like *streptococcus pneumonia*, *Haemophilus influenzae* and *Moraxella catarrhalis*, for which the antibiotics may be helpful, especially if treated with amoxicillin clavulanate. However, for those patients without bacteria, the antibiotics do not help, and in fact, cause five times more gastrointestinal intolerance and reactions. So, antibiotics should not be prescribed for HRV (Rotbart and Hayden, 2000). However, doctors continue to prescribe unnecessary antibiotics, leading to $37.5 million in 1994 spent for HRV related prescription of antibiotics, contributing to drug-resistant bacteria (Mossad, 1998).

Several studies continue to have been performed, using demethylchlortetracycline, amoxicillin and cotrimoxazole, and cephalexin. The effects for all these studies were mostly gastrointestinal, but most had positive effects. According to a review of all the studies, it states that antibiotics are probably beneficial for acute purulent rhinitis, but they support the 'no

antibiotics' as the first line of defense due to the unclear studies and the side effects (Arroll and Kenealy, 2006).

A study was done on 109 patients with COPD (chronic obstructive pulmonary disease), and were observed for twelve months while receiving erythromycin therapy. The results showed that 76 percent of those who did not receive the therapy caught a cold while 13 percent who received the erythromycin therapy caught a cold and since HRV is the major cause of the common cold, antibiotics may be beneficial (Suzuki, et al., 2001).

In a recent study, the antibiotic erythromycin did inhibit HRV infection in tracheal epithelial cells. It reduced the susceptibility of reinfection, the nuclear factor-kB activation, the number of acidic endosomes, and the cytokine production. The study suggested that erythromycin reduces the ICAM-1 receptor and blocks the rhinovirus' entry into the cell by way of the endosomes. This is the first time that macrolide antibiotics have actually helped in an experimental way. However, when clinical trials were performed, the macrolide antibiotic did no better than a regular antibiotic, trimethoprim-sulfamethoxazole (Suziki, et al., 2002).

Corticosteroids have been shown to inhibit the rhinovirus action through inhibiting interleukin activity, specifically NF-kappa B activity, but these steroids actually increase virus replication, having no anti-viral activity as was once thought (Turner, 2000).

NF-Kappa B is a substance used to indicate to cells to produce tumor necrosis factor-alpha. This factor, TNF-alpha, is used to exacerbate virus infections by starting the body's immune responses. When HRV infects the cells, stimulating macrophages, these factors are released, leading to the inflammatory responses (Laza-Stanca, et al., 2006).

NITRIC OXIDE

When HRV attacks a human system, it caused interleukin production, specifically IL-8 and IL-6, causing inflammatory measure. However, when a nitric oxide donor, specifically 3-(2-hydroxy-2-nitroso-1-proplyhydrazino)-1 propanamine, also known as NONOate, was applied, it inhibited the rhinovirus replication and the cytokine production from the body by releasing nitric oxide. This nitric-oxide releasing effect may have both an anti-inflammatory and an antiviral effect (Sanders, et al., 1997). It seems likely that the release of NO may inhibit early events in the viral infection process. NO has been tested in other picornaviruses, and had an effect against the replication of these viruses, and as such, further testing will be done to see it is has the same effect on HRV (Sanders and Proud-patent, 1998).

R61837

R61837 is another compound that inhibits the replication of rhinoviruses. When tested in vitro, it inhibited 74 percent of the HRV serotypes. When administered intranasally in frequent dosages, starting 1 hour before an HRV cold infected a subject and continuing for six days afterwards, it reduced the symptoms and mucus production, along with inhibiting the replication process. Further studies on this compound need to be done (Sperber and Hayden, 1988).

PDTC

PDTC, or pyrrolidine dithiocarbamate, is another antiviral compound, which works against all tested HRV serotypes yet. However, the studies are not conclusive as to how exactly it prevents HRV infection. The studies suspect that metal irons are involved in some way, since

adding metal ions to PDTC blocks its antiviral effects. PDTC actually inhibits NF-kappa, which had been adding metal ions to PDTC actually inhibits NF-kappa B, which had been mentioned earlier, and PDTC inhibits the polyprotein processing of HRV. However, how it accomplishes this is still not understood. More research on this antiviral drug is needed to come to more conclusive results (Krenn, et al., 2005).

NATURAL REMEDIES

There have been some natural anti-viral remedies as well for rhinovirus. A Chinese herb Agastache folium, had been used for the common cold, and a company named Roche extracted a chemical from the herb that stopped HRV from multiplying within cells. It binds to the capsid surface of rhinoviruses to prevent them from infecting a host cell by binding to the host cell receptor. However, its effect and anti-viral activity is not well known yet, and the chemical, Ro-09-0415 is undergoing testing (Scott, 1987).

Ro-09-0415 is actually a phosphorylated ester attached to the original antiviral flavone from the Chinese herb. It seemed to absorb well, but ineffective eventually, in large dosages, like 1200 mg attached to cells (Sperber and Hayden, 1988).

A different capsid binding agent, Ro-09-0410, was developed, also from the same compound. It also seemed to have adequate levels in blood, but the drug was undetectable in nasal washings, and seemed to have no anti-viral effect, and actually increased mucous production, which didn't alleviate symptoms (Sperber and Hayden, 1988).

DICHLOROFLAVAN

Dichlorofalvan is another capsid-binding agent, preventing viral uncoating and attachment to the host cell receptor. It inhibited viral activity best when it was added together with the virus, but it did show antiviral activity even when it was added replication of a single cycle of HRV (Tisdale and Selway, 1983). When it was administered orally three times daily, it was ineffective in inhibiting HRV infection. When tested in nasal washings, it was not detected, despite adequate levels of the drug administered. When it was administered intranasally, a high level of the drug was detected, proving that intranasally was the correct application. However, when the nasal drops were administered five times daily, they failed to reduce HRV infections, showing that adequate levels of the drug were not taken up by nasal cells (Sperber and Hayden, 1988).

CONCLUSION AND SUMMARY

Rhinoviruses are one of the most common and well known pathogens to date. They were the first virus crystal structure mapped, and the quest for the cure for the common cold is well known and documented, including its mode of attack, and how it affects a host cell. There are even many old proverbs regarding rhinoviruses that have sprung up since ancient times. From England, "stuff a cold starve a fever", from Germany, "sauerkraut is good", and from India, "one cold in the head is as bad as ten diseases'. Many doctors go by the proverb, "untreated colds last a week; medical attention can end them after seven days" (Biddle, 2002). William Osler, a John Hopkins doctor, stated "there is just one way to treat the common cold-with contempt". For colds are the cause of more sickness in the world than all other disease combined. (Radetsky, 1991).

Unfortunately, the cure for this pathogen has eluded scientists for decades. New treatment have sprung up, like soluble ICAM-1 or pleconaril, and natural antiviral compounds like zinc or dichloroflavan. Each has a different antiviral effect, from inhibiting the replication of the virus to preventing binding to the host cell receptor. Hopefully, the scientists and 'cold-warriors' battling this insignificant virus will eventually find a cure for the common cold, caused by the most perfect pathogen, human rhinovirus.

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Botulinum Toxin

David Moshayev

INTRODUCTION

Botulinum toxin is a neurotoxic protein produced by the bacterium Clostridium botulinum. Although botulinum toxin is the cause of the disease botulism and can be used in a terrorist attack, there are also many other uses for botulinum toxin. Botox, a derivative of botulinum toxin, is used for cosmetic purposes. Botulinum toxin is also used in medicines to control certain conditions marked by involuntary muscle contractions. The objective of this paper is to present a strong review of botulinum toxin so that one can see all the good and bad that is botulinum.

Clostridium botulinum is an anaerobic, Gran positive spore forming, rod shaped organism. The spores are heat-resistant and are widely distributed in nature. They occur in both cultivated and forest viscera of crabs and other shellfish (FDA, 1992). *Clostridium botulinum* was named, in the late 1700s, after a sausage (botulus being the Latin word for sausage), when 13 people ate from the same sausage and got the disease. In 1949 botulinum toxin was discovered to be a blocker of neuromuscular transmissions (Caya et al, 2004).

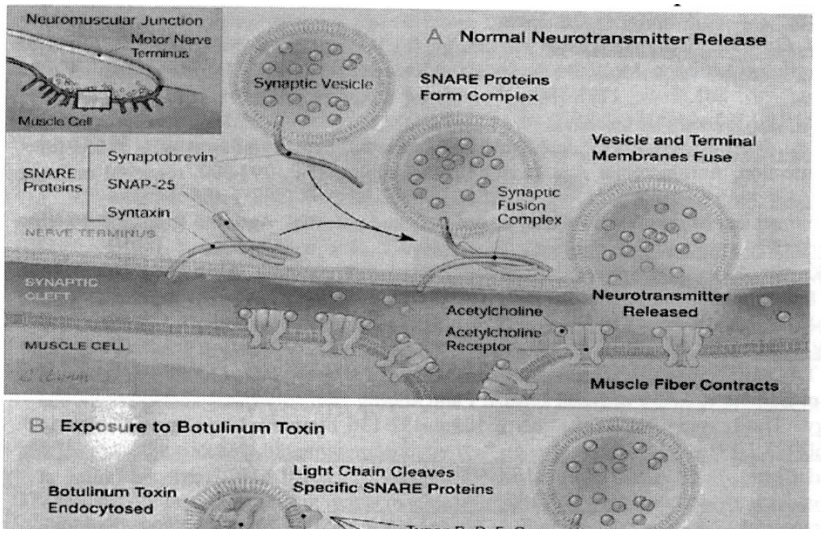

Figure 1. (Arnon S.)

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Botulinum toxin is "the most poisonous substance known," says Stephen S. Arnon, M.D., head of the Infant Botulism Prevention Program as the California Department of Health Services (Vanhelova, 1995), with a lethal dose of about 200-300 pg/kg, meaning that one hundred grams could kill every human on earth.

There are seven antigenically distinct serotypes associated with C. botulinum; A, B, C, D, E, F, and G. Most cases however are associated with types A, B, E, and F. The toxin works by entering the release of the neurotransmitter substance, acetylcholine, (see illustration) which initiates the signal for muscle contraction. In short it results in a progressive flaccid paralysis.

The classic symptoms of botulism include blurred vision, double vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. Infants with botulism appear lethargic, feed poorly, are constipated, and have a weak cry and poor muscle tone. These are all symptoms of the muscle paralysis caused by the bacterial toxin. If untreated, these symptoms may progress to cause paralysis of the arm, legs, trunk and respiratory muscles (Josko, 2004). In food-borne botulism, symptoms generally begin 18 to 36 hours after eating a contaminated food, but they can occur as early as 6 hours or as late as 10 days. There are three clinical forms of botulism poisoning, distinguished by the manner in which they are obtained: food-borne botulism, wound botulism and infant botulism. Less than 200 cases of botulism are reported each year in America. Of these cases approximately 25% are food-borne, 72% are infant botulism, and the rest are wound botulism.

Food botulism is the classic case of this disease. It occurs after the ingestion of preformed neurotoxin in inadequately processed food. This form of botulism is still the most widespread form worldwide. The most common culprits in food-botulism are improperly prepared home-canned foods, especially low acid vegetables such as corn, carrots, asparagus, and beans. The reason home-canned foods are susceptible to botulinum is because the spores are very durable and can withstand temperatures as high as 120 °C. This is especially problematic in high altitudes where the boiling temperatures is <100 °C (Talaro, 2002).

In infant botulism the toxin is produced when C. botulinum spores germinate in the intestines. Although we ingest these spores all the time, because they are nearly everywhere, infants are more susceptible due to the lack of a protective gastrointestinal bacterial flora and in part due to the relatively reduced levels of clostridial-inhibiting bile acid as compared to adults (Cox and Hinkle, 2002).

Wound botulism is simply the entry of the bacterium through a break in the skin. A significant number of new cases involve intravenous drug use; needle puncture sites may become infected with organisms, including C. botulinum. Under conditions of tissue necrosis and anaerobiosis, such as those seen in a subcutaneous abscess, C. botulinum spores can germinate and produce the neurotoxin, which will make its way to a neuromuscular junction of the skeletal muscles (Caya et al, 2004).

The respiratory failure and paralysis that occur with severe botulism may require a patient to be on a breathing machine (ventilator) for weeks, plus intensive medical and nursing care. After several weeks, the paralysis slowly improves. If diagnosed early, foodborne and wound botulism can be treated with an antitoxin which blocks the toxin from circulating in the blood. This can prevent patients from worsening, but recovery still takes many weeks. Physicians may try to remove contaminated food still in the gut by inducing vomiting or by using enemas. Wounds should be treated, usually surgically, to remove the source of the toxin-producing bacteria. Good supportive care in a hospital is the mainstay of therapy for all forms of botulism. Currently, antitoxin is not routinely given for treatment of infant botulism (Sobel, 2005).

Therapy for botulism consists of supportive care and passive immunization with equine antitoxin. Optimal use of botulism antitoxin requires early suspicion of botulism. Timely administration of passive neutralizing antibody will minimize subsequent nerve damage and severity of disease but will not reverse existent paralysis (Arnon, et al 2001).

BIOTERRORISM

The potency of botulinum has caused it to become a very important subject I bioterrorism. The United States has been investigating the possibility of the weaponization of botulinum toxin during the early years of World War II. Botulinum toxin has been developed as an aerosol weapon by several countries. It is estimated that one gram of aerosolized toxin can kill 1.5 billion people. Later on, after the 1991 Persian Gulf War, Iraq admitted to the United Nations inspection team to having produced 19,000 L of concentrated botulinum toxin, of which approximately 10,000 L were loaded into military weapons. These 19,000 L of concentrated toxin are not fully accounted for and constitute approximately 3 times the amount needed to kill the entire current human population by inhalation. It is noteworthy that Iraq chose to weaponize more botulinum toxin than any other of its known biological agents (Shulka and Sharma, 2005).

In the event of an attack, early recognition of outbreaks of botulinum depends on heightened clinical suspicion. Aerosol dissemination may not be difficult to recognize because a large number of cases share a common temporal and geographical exposure and will lack a common dietary exposure. However, identification of the common exposure site initially may be difficult because of the mobility of persons exposed during the incubation period. Though botulism and botulinum toxin are not contagious a microbe intentionally modified to produce botulinum toxin might be contagious.

Extremes of temperature and humidity will degrade the toxin, while fine aerosols will eventually dissipate into the atmosphere. Depending on the weather, aerosolized toxin has been estimated to decay from between less than 1% to 4% per minute. At a decay rate of 1% per minute substantial inactivation of toxin occurs by 2 days after aerosolization.

The potency of botulinum toxin has led to speculation that it might be used to contaminate a municipal water supply. This scenario is unlikely for two reasons. First, botulinum toxin is rapidly inactivated by standard potable water treatments (e.g., chlorination, aeration). Second, because of the slow turnover time of large-capacity reservoirs, a comparably large inoculum of botulinum toxin would be needed. In contrast with treated water, botulinum toxin may be stable for several days in untreated water or beverages. Any outbreak of botulism, should bring to mind the possibility of bioterrorism, but certain features would be particularly suggestive.

The following are features of an outbreak that would suggest a deliberate release of botulinum toxin:

- Outbreak of a large number of cases of acute flaccid paralysis with prominent botulinum palsies
- Outbreak with an unusual botulinum toxin type (i.e., type C, D, F, or G, or type E toxin not acquired from an aquatic food)
- Outbreak with a common geographical factor among cases (e.g., airport, work location) but without a common dietary exposure (i.e., features suggestive of an aerosol attack)
- Multiple simultaneous outbreaks with no common source

Therapeutic botulinum toxin represents an impractical bioterrorist weapon because a vial of the type-A preparation currently licensed in the United States contains only about 0.3% of the estimated human lethal inhalational dose and 0.005% of the estimated lethal oral dose (Arnon et al, 2001).

Recognition of a covert release of a finely aerosolized botulinum toxin would probably occur too late to prevent additional exposures. When exposure is anticipated, some protection may be conferred by covering the mouth and nose with clothing such as an undershirt, shirt, scarf, or handkerchief. In contrast with mucosal surfaces, intact skin is impermeable to botulinum toxin.

In the United States, an investigational pentavalent (ABCDE) botulinum toxoid is distributed by the CDC for laboratory workers at high risk of exposure to botulinum toxin and by the military for protection of troops against attack. A recombinant vaccine is also in development. The pentavalent toxoid has been used for more than 30 years to immunize more than 3000 laboratory workers in many countries. Immunization of the population with botulinum toxoid could in theory eliminate the hazard posed by botulinum toxins A through E. However, mass immunization is neither feasible nor desirable for reasons that include scarcity of the toxoid, rarity of natural disease, and elimination of the potential therapeutic benefits of medicinal botulinum toxin. Accordingly, pre-exposure immunization currently is neither recommended for nor available to the general population. Botulinum toxoid induces immunity over several months and, so, is ineffective as post exposure prophylaxis (Dressler and Hallet, 2006).

Due to the fact that current antitoxins are ineffective once the botulinum toxin entered the neuronal cells, it is essential that the development of a practical post-exposure prophylaxis takes place. One possibility could be the use of photo-chemically irradiated botulinum toxin in the presence of micronutrient riboflavin (vitamin B2) to result in oxidation of the toxin. Encouragingly, one study has shown significant protection using this method. At this stage more research is being done and the findings will be reported in due time (Eubanks, et al, 2005).

Another possibility for vaccinating against botulinum toxin is the inoculation with a plasmid that produces a fragment C of botulinum toxin inside the body. Once the harmless fragment is transcribed and translated, the body will elicit an immune response against it, just like a standard vaccine. This has only been tried in mine but still has great potential. Because the production of a DNA vaccination does not require botulinum toxin, only the plasmid that produces a response, it would be a great solution for the lab workers that would otherwise prepare the vaccine in unsafe conditions (Shyu, et al 2000).

MEDICAL/COSMETIC USES

Most things with destructive powers, if harnessed, can be used for great advancements in human civilization. Botulinum toxin (BTX) is no exception, with uses that range from treating excessive eye contractions to cosmetically taking away frown lines. The treatments gained FDA approval in 1989 and the use of cosmetic Botox was approved in April 2002.

Strabismus (squint), blepharospam, hemifacial spasm, and glabellar (forehead) lines are all problems that occur from over active muscles. The effect of BTX is to weaken the muscle to the point where the muscle will only contract when the individual wants it to. Although the FDA has approved for these conditions, and the National Institute of Health consensus conference of 1990 also included BTX as safe and effective therapy for the treatment of adducter spasmodic dysphonia, oromandibular dystonia and cervical dystonia, there are many off label uses. Off label uses include the treatments of: facial wrinkles other than glabellar lines, migraine headaches, tremor disorders, sphincter dysfunction and other spasticity disorders (Klein, 2004).

Adductor spasmodic dysphonia (ADSD) is defined as a movement disorder primarily affecting voice production. Voice characteristics of ADSD include a strained-strangled, harsh, low mono-pitch voice quality with stoppages and pitch breaks resulting from hyperadduction of the vocal folds. Botox is the treatment choice for ADSD and the overall efficacy has well been established. However, Botox injections do not result in speech intelligibility similar to that of normal, non-ADSD speakers (Bender et al, 2004).

Most of the time when referring to the uses of botulinum toxin we mean type-A (Botox), but type-B (Myobloc) has also gained approval by the FDA (for cervical dystonia not cosmetic use) and a comparison must be made so that we understand the benefits of both, and use the better of the two for our advantage. There were several studies done to try to see the effects of type-B injections for facial rhytides. Botox was also found to have a longer duration of effect and Myobloc was found to have a quicker onset of action but the duration of effect is dependent on dose. Another important feature that myobloc has is, if a person develops neutralizing antibodies that prevent clinical effects from one type of botulinum they could still have the other type of work. Resistance has been reported in 3% to 5% of patients with cervical dystonia who were treated with large doses of botulinum type-A therefore type-B will be a good option for them. Whatever the differences are, type-B is found to be safe and effective and with further trial and

clinical experience it is conceivable that each type will have its own set of indications (Sadick and Matarasso, 2004).

There are some uses for BTX that are under experiment. One study is proposing promising results for obesity treatment. The pilot study has assessed eight subjects with severe obesity. The subjects were injected with 500 UI of BTX-A in the gastric antral region at ten different points. The treatment works by reducing gastric mobility therefore leading to early satiety. Overall, a single dose of BTX-A was well tolerated by all eight patients and a reduction in weight was shown at one month. By the third month a subgroup of patients showed further weight loss (Albani et al, 2005).

Another investigated use of BTX-A is the treatment for non-neurogenic detrusor over activity (overactive bladder). Twenty patients with this bladder problem were given suburothelial injections of 200 UI of BTX-A. The injection was performed at 40 sites of the posterior and lateral walls of the urinary bladder. Although the bladder capacity was increased, the bladder sensation was greatly impaired and the postvoid residual urine volume increased significantly. These unfavorable results might prevent the wide spread use of this therapy, but is still a good option for people who cannot tolerate the usual treatments of antimuscarinic drugs or intravesical vaniloid therapy. The next step would be to adjust the doses in order to help perfect the therapy (Kuo, 2005).

Botulinum toxin was initially intended to treat disorders characterized by excessive muscle contractions. It was noted to also alleviate pain. After studying the effects on pain, botulinum toxin was found to not only block acetylcholine but also block one or more pain neurotransmitters. This new found use is being researched for therapy of migraine and tension-type headaches. The studies done did not have compelling results and more research needs to be done (Schulte-Mattler and Martinez-Castrillo, 2006).

In order to be effective botulinum toxin injections must be administered into the muscle rather than interstitial tissue. Electromyogrophy (EMG) and ultrasonography are two guidance techniques that can be used to ensure proper chemodenervation. Chemodenervation refers to use of a chemical to prevent a nerve from stimulating its target muscle. The advantages of EMG guidance are precision, safety and less chance of side-effects. EMG guidance is used for cervical muscles and deep or small limb muscles. Ultrasonography is used for injections in the urinary system and salivary glands (Pathank, et al, 2006).

As of 2006, Botox injection is the most common cosmetic operation in the United States. It is used for facial wrinkles by weakening or paralyzing the muscles that cause facial rhytides. Though the effect is temporary, it is extremely popular because it has a low risk of side effects and is an easy technique to acquire.

CONCLUSION

There are some difficulties that prevent or slow advancement in the fields that are part of botulinum toxin. First of all, the danger involved with handling the toxin does not allow rapid changes in medicine. Second, the distribution of mass vaccination is almost impossible because the small market size combined with the high price of development (Casadevall, 2002). Another reason that might be stopping mass vaccination is the inevitability of attack, meaning that even if money is spent on defense against botulinum toxin type-A the terrorists will just attack with

another one of the seven types. Plus they can always use a different agent such as anthrax or any other biological weapon.

The ability of botulinum toxin to be used both in a bioterrorist attack and in numerous medical fields is what makes it so amazing. The fact that the bacterium Clostridium botulinum is so durable and can be found all over the world shows that society cannot just ignore it and hope for the best. Research must continue in the fields of medicine and in the fields of prevention.

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Biofilms in Medicine

Marshall Gelbman

BIOFILMS: AN INTRODUCTORY OVERVIEW

In 1862 Louis Pasteur introduced the "Germ Theory of Disease." Subsequently the study of microbiology has flourished greatly and its medical significance has continuously grown. Many microbial organisms implicated in disease have been identified and studies. A general science of medical bacteriology has been determined and is widely taught. Such study has been largely based upon the activity of individual free-swimming (planktonic) cells and colonies that they form. However, microorganisms often form communities called biofilms which can have properties that very different from their planktonic predecessors.

Biofilms are mucoid aggregates of microorganisms which tend to grow on surfaces exposed to water. Biofilms are not the only form of microbial cell aggregate but are distinguished from other aggregates by specific properties. Another form of microbial aggregate is the familiar bacterial colony. Colonies tend to feed on their undersurface and utilize the gaseous surface above for gas exchange; they are usually clones of a single preceding cell (Wimpenny 2000). Biofilms are characterized by their locations at phase boundaries (ibid.) and their production of Extracellular Polymeric Substance or EPS. The sliminess of biofilms is due to their enveloping EPS matrix. The phase boundary at which biofilms generally form is Solid: Liquid (there are a few examples of biofilms growing at other phase boundaries but they tend to have industrial or environmental applications). An additional characteristic of biofilms which is

not generally cited in their description is the strong alteration of their cell physiology from that of planktonic cells (Donlan and Costerton 2002).

Biofilms grow on many surfaces, in many environments, and with many different effects. The most common example of a biofilm is dental plaque; however biofilms also grow on many other natural and artificial surfaces within the body for which they have been implicated in many diseases. Biofilms are also prevalent on household surfaces such as cutting boards, counters, toilets, sinks, tubs, and the interior of pipes.

Although biofilms appear to serve only adverse functions, they have many positive users in the environment and industry. For example, biofilms are used in water treatment filters to metabolize organic substances that are within the water. This strategy has been shown to decrease microbial proliferation downstream (Cunningham 2007). Biofilms are also used in "Bioremediation" activities in which they are utilized to metabolize toxic materials that contaminate soil or water into safe (possibly beneficial) byproducts (ibid).

Biofilms tend to have different medical implications than do planktonic cells and thus require different treatments. Treating biofilm infections requires an understanding of biofilms. To truly understand the effects of biofilms we must take a deeper look at their structure and

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physiology.

THE SCIENCE OF BIOFILMS

It is initially important to note that although we may investigate a variety of controlled laboratory-grown biofilms of defined compositions, the biofilms found in natural environments have highly heterogeneous compositions comprised of numerous bacterial species as well as fungal organisms. The composition of a biofilm changes dynamically as some organisms and organic material are incorporated from the surroundings and some are emitted to the surroundings (Wimpenny 2000). The composition is regulated by a complex variety of genetic and environmental factors.

FORMATION AND GROWTH

Ironically, biofilms have been shown to form more easily in high shear environments (where high mechanical pressure, such as the flow of fluid, is applied to the biofilm). Additionally, the biofilms that form in high shear environments are much stronger than those in low shear environments. Research also shows that biofilms form as easily on smooth surfaces as they do on rough surfaces (Donlan and Costerton 2002).

Busscher and van der Mei depict biofilm proliferation in eight semi-sequential steps (Busscher and van der Mei 2000).

1) Deposition of a conditioning film on the substratum surface
2) Mass transport of microorganisms to the substratum
3) Initial adhesion of microorganisms to the substratum

4) Co-adhesion to attached microbes
5) Anchoring by appendages and polymers to the substratum
6) Co-aggregation of planktonic microbes
7) Growth of the biofilm
8) Detachment of biofilm material.

Each of these steps is an independent phenomenon worthy of great research and description.

Colonization by microorganisms is always preceded by the development of a conditioning film of macromolecules that absorb to the substratum surface. Conditioning films form due to the diffusive and ubiquitous properties of macromolecules. The composition of a conditioning film is related to the chemical properties of the substratum surface and the macromolecules within its surroundings. Examples can include salivary matter on dental surfaces, tears on contact lenses, and urinary components on a catheter surface (Ibid).

There are a variety of methods by which microorganisms reach a substratum; these include passive forms of transport such as Brownian motion, convective transport (movement by fluid), and sedimentation (due to difference in the specific gravity of the microbes and the mass fluid surrounding them), as well as active (flagellar) transport. Current research indicates that chemotaxis mediated active transport is not a factor, yet there is only limited research on such a premise (Davies 2000).

The initial adhesion of microorganisms is by van-der Waals forces while there is even some repulsion due to the corresponding negative electrostatic charges of the substratum and microbial surfaces. Cellular motility appears to be the force that counteracts such repulsion (Davies 2000). Other early factors can be acid base interaction as well as hydrophobic interaction. The degree of relative hydrophobicity between the cell, the substratum and surrounding liquid has been shown to influence initial attachment (Ibid.). This initial adhesion is quite reversible, however it becomes stronger as water is removed from between the interacting surfaces.

Microbial adhesion becomes irreversible when the cells are anchored by EPS polymers and/or cellular appendages such as pili. These macromolecular structures adhere by dipole, ionic, hydrogen bond, and hydrophobic interactions. Research indicates that phenomena such as the secretion of EPS and the protrusion/extension of cellular appendages are activated in response to surface association (Davies 2000).

Adhesion throughout a biofilm is apparently a common theme in the science of biofilms. Two predominant styles of cell-to-cell adhesion are seen in the development of a biofilm; Coadhesion and Coaggregation. Coadhesion refers to the binding of a planktonic cell to a biofilm cell. Coaggregates are planktonic aggregates of microbial cells which can be incorporated as units into biofilms by binding the substratum or by Coadhesion (Kolenbrander et al. 2000).

Following the “early events” of biofilm formation, the biofilm proliferates primarily by cellular growth of the biofilm cells. Research has shown that substrata-attached *Pseudomonas* reproduce at an extremely slow rate, with a generation time inversely relates to the attachment strength, indicating that growth is not an early event (Busscher and van der Mei 2000).

An essential step in the biofilms development cycle is detachment. Biofilms are subject to numerous physical and chemical forces many which can disrupt cell-to-cell interactions causing

detachment by part of the biofilm. If the interaction between the substratum and biofilm cells is broken, complete detachment occurs (Ibid.).

Microbes throughout nature display defense mechanisms towards their competition. Early colonizers of biofilms have been shown to prevent further colonization by other species through the secretion of specific bio surfactants which alter the chemical or physical properties of the surface in ways which prevent attachment of the undesired species (Ibid.).

STRUCTURE OF BIOFILMS

Prior to the use of Confocal Lens Scanning Microscopy (CLSM) in biofilm study, there were many misconceptions of biofilm structure. Biofilms were thought to be homogeneous unstructured accumulations of bacteria on surfaces. CLSM provided accurate images of unprepared live biofilms which contradicted these early beliefs. Prior study had been with electron microscopy which requires dehydration of samples (Donlan and Costerton 2002).

Dispute had raged over the correct biofilm structure with three common models observed. Some believed biofilms to be irregular branched or simple stalked structures, others believed biofilms to be fairly flat and homogeneous structures while others argued that biofilms are composed of mushroom or tulip shaped structures which are internally accessed by pores. Subsequent study has indicated that all three models are correct and depend on the available nutrient resources. The first model had been found in water distribution systems, which are low nutrient environment. The second model appears where nutrient concentration is very high e.g. the human body. The third model is observed in laboratory growth in which media containing moderate nutrition is utilized. Both presence of specific substrates and concentration of those present relate to a biofilm's structure (Wimpenny 2000).

As previously described, biofilms in nature are composed of multiple species and mutants with structural and physiological properties that can be harnessed for the entire "community". Under certain growth conditions P. *aeruginosa* biofilms have been shown to form mushroom shaped structures. Research has shown that the stalks of the mushroom are formed of a specific population and the mushroom caps are formed by a motile subpopulation which travels up the stalk to reside atop it (Parsek and Fuqua 2004).

Biofilms are composed of approximately 15% cells and 85% matrix. The structural units of a biofilm are microcolonies; these structures feature many of the biofilm implied properties such as quorum sensing, antimicrobial resistance, and detachment. Water channels flow between microcolonies of sessile cells. Microcolonies of biofilms in environments with high shear forces have been shown to assume tadpole shapes which oscillate in the bulk fluid. Interestingly, detachment of microcolonies can have dire medical results as they can travel in the planktonic manner while retaining such properties as antimicrobial resistance (Donlan and Costerton 2002).

Considering that the matrix content of biofilms dwarfs that of cells, understanding the EPS which forms the matrix is of great importance.

The abbreviation of EPS has remained a staple term in biofilm study, however it has multiple long forms associated with it. They include extracellular polysaccharides, exopolysaccharides, exopolymeric substances, exopolymers, and extracellular polymeric substances (Flemming et al. 2000).

Although confusion in determining the correct long form of the term "EPS" may be quite trivial, there is practical confusion in determining the composition of EPS. This is primarily due to the dynamic properties of this substance. The composition of EPS differs based on which organisms are present, and by their surrounding environment (Parsek and Fuqua 2004).

While polysaccharides such as alginate were believed to be the primary components of EPS, many studies have shown proteins and nucleic acids to prevail in quantity. In addition to polysaccharide, protein and nucleic acid components, EPS also contains lipids and phospholipids as well as humic substances (Flemming et al. 2000).

EPS is formed primarily of polymers bearing charged functional groups such as phosphate, carboxylate, and sulfate groups. Alginate which has been found in large concentrations within P. aeruginosa biofilms is formed of mannuronate and guluronate monomers; the carboxyl groups of these moieties are anionic. Other anions prevalent in EPS are proteins and nucleic acids. The charged functional groups of these polymers relate to biofilm structure (Flemming et al. 2000).

UNRAVELING THE "BIOFILM PHENOTYPE"

As described above, the behavior of cells embedded within a biofilm, differs greatly from that of planktonic cells. Julian Wimpenny states in regard to the behavior of biofilms as communities that "the sum of its activities is greater than the sum of all the activities of is constituent members," and that "…a community might have *emergent* properties" (Wimpenny 2000). Theses distinctions are both genetic and environmental in source.

In terms of generic expression, there is a definite deviation in the physiology of the bacterial cells of a biofilm. In regard to the environmental influence of phenotype, there are apparent differences in the collective action of the varied multitude of microbial cells that form a biofilm and their planktonic analogues.

Environmental differences can be attributed to the presence of the surrounding EPS matrix and to the heterogeneity of the biofilm population. The variety of species and mutants within a biofilm can be though to act together as a single multicellular unit which utilizes different cell types for the differing functions for which they are optimally suited. However, the genetic basis for the biofilm phenotype is a much more complex matter requiring a more complex explanation.

Molecular explanation of the biofilm phenotype is related to two cell density-dependent mechanisms; quorum sensing and gene transfer.

As its name implies. Quorum Sensing (QS) is a microbial cell to cell (pheromone) signaling system which is dependent upon cell concentration. Signal molecules are secreted by some cells; if the cell density is low they diffuse providing a minimal effect. If the cell density is high, a sufficient (threshold) quantity of signal molecules is present to activate the receptors of other cells in the vicinity inducing a signal transduction cascade which activates the expression of a number of genes (Stoodley et al. 2000).

Quorum sensing signals induce a multitude of properties including the development of genetic competence (the ability to genetically transform), synthesis of antibiotics, and even virulence (Cvitkovitch et al. 2003).

Such quorum sensing activities occur frequently when there is a high concentration of cells. Biofilms always indicate a high concentration of cells and are thus probable locations for quorum sensing to occur. Additionally, quorum sensing pathways have been shown to induce biofilm, development (Stoodley et al. 2000).

Quorum sensing is also implicated in bacterial dispersions from biofilms. Such dispersion occurs by expression of density- dependent genes which code for enzymes that degrade EPS matrix thus freeing cells from it (Davies 2000).

There are various specific quorum sensing pathways utilized. Gram negative bacteria such as P. aeruginosa primarily on Acyl-Homoserine Lactones (AHLs) as inducers. Gram positive species, such as the various streptococci, have their own variety of inducers molecules. For example, many streptococci utilize molecules classified as Competence Stimulating Peptides (CSPs) which act by QS to activate cascades leading to genetic regulation of numerous properties which are likely to include those which influence the "biofilm phenotype" (Cvitkovitch et al. 2003).

During horizontal gene transfer (transformation and conjugation), fragments of genetic material are transferred among microbial populations conferring a variety of phenotypic properties to non-descending cells.

Cells must be in a state of genetic competence to accept DNA by gene transfer. Competence stimulating peptides are so-names due to their induction of competence in their recipient cells. This implies that high cell density, as is found within biofilms, greatly increases the level of transformation. Gene transfer is also increased within biofilms due to the presence of an "abundant extracellular gene pool" (Cvitkovitch et al. 2003).

Conjugation rates also appear to be higher for surface associated cells than for cells within liquid culture (Ehlers 2000).

It is apparent that the increased level of gene transfer within biofilms provides a source of phenotype distinction from planktonic microbes. As noted other factors are quorum sensing, and environmental distinctions such as the protective Eps matrix and the communal interaction of differing species and mutants.

BIOFILM INFECTIONS

Above, numerous physiological and structural features of laboratory studied biofilms are described. It is now appropriate to discuss the biofilms that grow within the human body. Biofilms have been found to grow extensively on a number of medical and anatomical surfaces within the human body. In fact the NH indicates that more than 60% of microbial infections are of the biofilm type (Hentzer and Givskov 2003).

Is it important to note the many specific biofilms observed within the body, as well as to truly understand their implications and their distinctions from planktonic flora.

BIOFILMS AND CHRONIC INFECTIONS

As previously mentioned biofilms are phenotypically distinct from suspended microbes; yet the primary methods microbial research have been studies of planktonic cell cultures. This is unfortunate as a multitude of human infections are biofilm based.

Previous bacterial epidemics were planktonic cells that could be easily eliminated with antibiotics and by increasing immune function by vaccination. These infections acted in the acute

manner. However, with such conditions quite controlled, a newer breed of infections has appeared. These infections are not as invasive yet persist for prolonged periods of time with sporadic flare-ups. These diseases also appeared to be caused by common organisms for which the victims were perceived to possess immunity. When the organisms were cultured and tested for antibiotic susceptibility they were deemed sensitive to the conventional drugs; yet patient treatment with the antibiotics failed. These chronic infections, resistant to traditional antimicrobial elements were determined to be of the biofilm, type. Biofilms are noted to be the most defensive prokaryotic "life strategy" (Costerton et al. 2003).

Progressing in the field of medical microbiology requires acknowledgment of the distinct biofilm phenotype, research methods altered for biofilm study, and realization that biofilms induce chronic infections requiring altered treatment mechanisms (Ibid).

SURVEY OF HUMAN BIOFILM INFECTIONS

Below are some examples of common biofilm growth on the natural surfaces of the human body, as well as biofilms which colonize implanted medical devices.

1) *Dental Biofilms and Implications*

The most common example of biofilms in the body, and possible the most studied, is that of dental plaque.

The initial event in plaque formation is the development of an acquired pellicle on the enamel surface of teeth. An acquired pellicle is a protein rich conditioning film derived from saliva. Pellicle formation is followed by colonization by normal oral flora. In the days following colonization, a biofilm matrix begins to appear. These events directly follow cleaning of the enamel surface (Donlan and Costerton 2002).

If proliferation of the biofilm is undistributed for a period of 2-3 weeks, a biofilm with a depth of 50-100μm is observed. This biofilm is termed plaque. If the plaque becomes mineralized by calcium and phosphate ions it becomes calculus or tartar. Eventually plaque colonizes the lateral surfaces of teeth as well as the gingival sulcus (between the tooth and gingival surface); such plaque masses induce periodontal disease and dental caries (erosion of the teeth) (Ibid.).

2) *Native Valve Endocarditis*

Vascular injuries commonly induce a form of endocarditis termed Nonbacterial Thrombotic Endocarditis (NTBE).

A potentially fatal biofilm infection of the body that is linked to bacterial Native Valve Endocarditis (NVE). NVE is caused by adhesion of microorganisms of the endothelial surfaces of the cardiac valves which are damaged by NTBE.

In regions of NTBE a high level of fibronectins are secreted by the cells, platelets, and fibroblasts present. Several bacteria feature receptors and bind the fibronectins; formation of microcolonies follows (Ibid.).

Research shows that these bacteria/fibronectin unions develop so that the bacteria are encapsulated within fibronectins which protects them from phagocytosis. Fibronectins also bind leukocytes and can thus hinder their motility (Ibid.).

Biofilms on heart valves can directly damage the underlying tissues. These biofilms can also disseminate fragments by detachment which can form emboli. Fungal

biofilms are found to be the predominant culprit of theses emboli as their biofilms can be thick (Ibid.).

3) *Biofilms and Otitis Media*

Otitis Media (OM) is a bacterial induced inflammation of the middle ear tissues. Common among children is Chronic Otitis Media with Effusion (COME). COME is a chronic condition in which a viscous fluid is found within the middle ear. Biofilms have been found on the mucosal surface of the middle ear of COME patients (Costerton et al. 2003).

One mechanism utilized to treat COME is the insertion of tympanostomy tubes into the ear to alleviate pressure buildup. Unfortunately many of these tubes have been demonstrated to provide a new surface for biofilm colonization. However, silicone tympanostomy tubes bombarded with ions have been shown to remain uncontaminated (Donlan and Costerton 2002).

4) *Biofilms and Cystic Fibrosis*

Cystic fibrosis (CF) is a recessive genetic disorder found predominately among Caucasians of European decent. CF is primarily characterized by respiratory infections as well as other abnormalities throughout the body.

During infancy and early childhood, the lungs of CF patients are infected by organisms such as *Staphylococcus* aureus and *Haemophilus influenzae* which can cause tissue damage. Such damage to the epithelia increases the adhesion of *Pseudomonas aeruginosa* cells. *P. aeruginosa* subsequently become the primary colonizers and induce chronic infection. Chronic *P. aeruginosa* infection is the predominant cause of respiratory dysfunction and subsequent death in CF patients (Lyczak et al. 2002).

An important factor in the *P. aeruginosa* infections of the CF patient's airway is it growth as a biofilm. Electron microscopy has demonstrated the presence of *P. aeruginosa* biofilms in CF lungs. As explained above, growth in the resistant biofilm phenotype is implicated as a cause of chronic disease. Additionally, research has related the quorum sensing regulation of *P. aeruginosa* virulence to that of biofilm growth (Lyczak et al. 2002).

5) *Biofilms and Central Venous Catheters*

Central Venous Catheters (CVCs) are utilized to administer substances into large veins of the neck chest or groin. Many pathogenic micro-flora colonize the lumen and external surfaces of CVCs. Biofilms of multiple species have been found growing on CVC surfaces (Murga et al. 2001).

An early event in biofilm growth on CVC surfaces is the development of a conditioning film of blood on the catheter surface. Although catheters are flushed after blood is drawn through them, it is assumed many serum proteins remain on the surface. The blood proteins fibronectin (described above in regard to NVE pathogenesis) and fibrinogen have been shown to affect surface attachment of microbes, inducing attachment of many organisms (Murga et al. 2001).

6) *Biofilm Growth on Urethral Catheters*

Urinary tract infections are extremely common among patients with long-term urinary catheters installed. Clinical evidence shows that is it quite complicated to eliminate such infections while the catheter is present (Stickler et al. 1998).

Permanent urinary catheters can go unchanged for as long as 3 months allowing infected urine to circulate within them. Biofilms have been shown to grow on the interior of such catheters, often to densities which impede urinary out-flow (Stickler et al. 1998).

Biofilms within urinary catheters have been observed in-vivo and in-vitro by scanning and transmission electron microscopy (Donlan and Costerton 2002).

ANTIBIOTIC RESISTANCE OF BIOFILMS

Numerous clinical scenarios and research studies have been demonstrated the inherent resistance of biofilms to conventional antimicrobial agents. Generally biofilms cannot be eradicated by the same antibiotic regiments that can eliminate their planktonic constituents (Costerton et al. 2003).

It is accepted that the resistance of biofilm enveloped microbes to antimicrobial agents cannot be related to a single factor but is the result of multiple factors which are tied to the biofilm phenotype (Parsek and Fuqua 2004).

RESTRICTION PENETRATION

The initial factor considered for antimicrobial resistance in biofilms is the penetration restriction of their matrix. Restricted penetration is caused by two major mechanisms; the action of the matrix as a diffusion barrier, and the binding of matrix polymers to antimicrobial particles.

Consideration of the restricted penetration model has demonstrated its ability to hinder influx of large molecules such as lysozymes; however it does not eliminate the entry of small antimicrobial molecules. Penetration restriction has been shown to merely slow the penetration of such drugs. Such retarted diffusion can however protect biofilms from degradable antimicrobials as it presents the opportunity for degradation factors such as beta-lactamases to act. the synergistic cooperation of diffusion restriction and antimicrobial destruction/modification is a highly effective resistance mechanism (Lewis 2001).

ALTERED GROWTH RATE

Another factor commonly cited in the discussion of antimicrobial resistance of biofilms is the relationship between growth rate and the killing effect of antimicrobials. Biofilm cells have substantially decreased growth rates; many antimicrobials require cells to be growing for any efficacy. Most other antibiotics (e.g. advanced beta-lactams such as cephalosporins and fluorouqinolones) feature decreased efficacy with decreased growth rates (Lewis 2001).

ALTERED PHENOTYPE

A predominant factor cited in the discussion of biofilm resistance to antimicrobials is the “biofilm phenotype”. It is believed that biofilms feature physiology that is distinct from that of planktonic cells. As described above such distinction are the results of quorum sensing, increased gene transfer and possible surface association. These factors affect the expressed genotype of cells. An altered phenotype can result in alterations to antimicrobial absorption and efficacy (Parsek and Fuqua 2004).

PERSISTER CELLS

Kim Lewis describes the presence of subpopulation of "persister cells" within the biofilm which are not easily eradicated. It is believed that even with the aforementioned mechanisms of antimicrobial resistance most of the biofilm cells can be eliminated with certain antibiotics; however these persister cells are not eliminated.

Such subpopulations of persisters are found within planktonic populations but are believed to be eliminated by the immune system after antibiotics eliminate the vast majority of the cells. Lewis hypothesized that the biofilm matrix protects the persister cells from immunity factors thus maintaining persistence (Lewis 2001).

The hypothesis that biofilms protect persists from immunity factors may require review. Parsek and Fuqua quote Jeff Leid to have reported at the Biofilms 2003 meeting that human leukocytes do penetrate biofilms (Parsek and Fuqua 2004). It is likely that persisters are responsible for increasing the resistance of biofilms by a different mechanism.

TREATMENT AND PREVENTION OF BIOFILM INFECTIONS

Numerous biofilms relevant in medicine have been discussed as well as their highly resistant nature. It is now essential to propose strategies for preventing, eradication, and treating, biofilm infections. There is not a single target for such strategies but a multitude of targets which must be considered.

PREVENTION OF BIOFILM GROWTH

Prior to exploration of strategies for the treatment of existing biofilms, it is wise to consider some methods by which biofilm growth can be initially prevented.

The general steps in biofilm formulation described above indicate mechanisms by which biofilm formation can be inhibited.

Initially, primary colonizers must adhere to the substratum surface. If adherence to the substratum can be decreased, biofilm growth can also be decreased. One approach to prevent biofilm growth on medical devices is to alter the surface properties of the biomaterials. This hypothesis is supported by J. Chandra and associates who demonstrated that chemical modification of biomaterials influenced the ability of *C. albicans* to form biofilms on them (Chandra et al. 2005).

In addition to material alterations, adjustment of clinical procedures and standards can also decrease biofilm formation on medical devices. For this to occur it is important that the medical community recognizes the existence of biofilms as well as their dire implications. Modifications should be made in the scheduling and methods by which medical devices are installed and replaced (Costerton et al. 2003).

There are also occasions when it is appropriate to utilize prophylactic antibiotic therapy to eliminate planktonic populations to prevent colonization of anatomical and medical device surfaces. Acknowledging the patterns of human biofilm infections can help to identify such occasions.

ELIMINATING BIOFILMS

While there is no central solution, many mechanisms have been proposed to eliminate biofilms. Most of the proposed methods will require much more intensive research prior to any

clinical relevance. However, many biofilm infections can be eliminated utilizing current antimicrobial agents by unique regimens which are depicted simple laboratory susceptibility tests. A prevalent theme is the administration of various antibiotic combinations. Saginur and associates as well as Slinger and associates found that specific combinations of antimicrobials are effective in treating biofilms. These same antibiotics are ineffective against biofilms when administered independently (Saginur et al. 2005, Slinger et al. 2006).

Assuming that persister cells are the predominant basis of biofilm resistance, Kim Lewis presents a possible treatment regimen. Lewis suggests administering a bactericidal antibiotic, withdrawing from treatment, and then re-administrating to the agent.

The first administration is to eliminate the majority of cells; the normal cells. The withdrawal period is to allow growth of the remaining (persister) cells. During this time the vast majority if the population will lose their persister phenotype leaving a relatively insignificant population of persisters. These "normal" cells will now be eliminated by the second drug administration. This mechanism is proposed primarily for cases of direct antibiotic application; where drug delivery is controlled. For example: administration of antibiotics via aerosol directly to the Cystic Fibrosis airway (Lewis 2001).

Many new antibiotic targets have been proposed for the treatment of biofilms. One such methods in the inhibition of quorum sensing. Theoretically, quorum sensing can be inhibited in three ways: inhibition of signal generation, inhibition of signal dissemination, and inhibition of signal reception (Hentzer and Giskov 2003).

Interestingly, efficacy of antibiotics against biofilms has been shown to increase in the presence of ultra sound or low-strength electric fields (Donlan and Costerton 2002). These phenomena are not yet clinically significant.

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Bisphosphonates and Osteonecrosis of the Jaw

Chaya Leah Katz

ABSTRACT

Bisphosphonate-related Osteonecrosis of the Jaw (BRONJ) is a condition which, according to the American Association of Oral and Maxillofacial Surgeons (AAOMS), adversely affects between .8-12percent of the population, a small yet significant amount of adults. Osteonecrosis of the Jaw (ONJ) is characterized by the death of bone and suffering patients present with either a non-healing extraction socket or an exposed jawbone. In general, afflicted patients have been treated with a class of drugs known as the Bisphosphonates (BP). Bisphosphonates were originally developed in order to treat and manage many metastatic diseases of the bone and stabilize bone loss caused by osteoporosis. Recently, oral surgeons have seen many patients with necrotic lesions on the jaw. The common theme between these patients was that they had all received chronic bisphosphonate therapy. This paper will attempt to review current medical literature on this most important topic. To facilitate this, the paper will first delve into the histology and physiology of the bone. Once that is understood, the history of the BP's will be traced: 1) Why they were developed, 2) their early chemical structures, 3) how they

evolved, and 4) what are the current recommendations for patients who are suffering. Reduction of bone vascularity and of the normal remodeling of the bone, and the accumulation of micro-damaged bone, both causes of necrotic lesions to the jaw bone, will be explained and various case studies will be discussed with regard to their diagnoses and treatment/management plans of BRONJ.

INTRODUCTION

There are many conditions which can affect the calcification of bones in humans. These include Paget's disease (Osteitis Deformans), osteoporosis, multiple myeloma, metastatic breast and prostate cancer, and solid tumors of the bones. In all of these diseases, both the quality and quantity of the bone is compromised. This can lead to pain, fracture, spinal cord compression, and hypercalcemia, all of which are associated with a high morbidity (Hotobagyi, GN et al, 1998). The Bisphosphonates, as a class of drugs, were developed to manage these complications and they act by strengthening the bone. They accomplish this through a variety of proposed mechanisms. However, in recent years, much controversy has begun to arise regarding a significant side effect of this therapy-namely- the development of osteonecrosis in the jaw which seems to occur more frequently in those patients receiving Bisphosphonate therapy.

DESCRIPTION OF BONE

Human bone is a complex tissue with functions which run the gamut from the obvious job of providing support and protection for the body's organs, to that of being the body's most important reservoir of calcium, thereby enabling it to maintain calcium homeostasis.

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It is composed of two main components: 1) an organic matrix which is strengthened by 2) firmly attached inorganic calcium safe deposits. The organic component is composed of 95% collagen and ground substance. The collagen matrix is what gives bone its great tensile strength. The ground substance is made up of extracellular components and proteoglycans. The inorganic component is composed of calcium and phosphate combined chemically to form a compound known as hydroxyapatite (Berne et al., 2004). These calcium salts, which are intimately bound to the collagen matric, give bone its extremely high compressive strength (Guyton, 2005).

Bone calcifies in stages. The main cell involved in bone production is the osteoblast. Osteoblasts secrete collagen monomers. These monomers polymerize rapidly and form collagen fibers. The combination of the collagen fibers and the ground substance is called osteoid. Within a few days, the polymerized collagen begins to have calcium salts precipitating on it surface and within a few weeks there are fully hydroxyapatite crystals adhering to the collagen (Guyton, 2005).

Bone is laid down in concentric circles surrounding the blood vessels and nerves within it and which supply it with nourishment. The process begins from the periphery and as the bone is formed, the lumen becomes smaller (Berne et al., 2004). Some osteoblasts become

encased between these concentric layers or lamellae of bone and are then known as osteocytes. As the lumen becomes smaller due to bone deposition, there is the beginning of an encroachment upon the blood vessels and nerves supplying the bone and formation ceases via a negative feedback system. The residual canal through which the vessels and nerves run is called a Haversian canal and each new area of bone deposited in this way is called an osteon. In short, bone is composed of multiple osteons or Haversian systems. As bone ages, it slowly becomes brittle due to the increase in inorganic to organic ratio. The body compensates for the increased ratio by constantly remodeling the bone, i.e. that all through life there needs to be a constant resorption of the old brittle bone and a concomitant deposition of new flexible bone with the ideal ration of components to ensure the proper compressive and tensile strengths of the bone that are needed to support body functions. This task is accomplished by a specialized cell called the osteoclast (OSTEO= bone, KLASTOS= broken –in Greek). This is a large, multinucleated, phagocytic cell (Wheater et al., 2006).

The mechanism of the osteoclastic activity is extremely complex. Briefly, what occurs is as follows. When the time comes for the bone to be resorbed, the osteoclast sends out villus like projections which secrete, 1) Proteolytic enzymes released from lysosomes which have the ability to digest organic material, and 2) Citric and Lactic acid which dissolve the mineral component of the old bone. Additionally, whole fragments of bone salts and collagen are phagocytosed by the villi (Guyton, 2005). Normally, there is equilibrium between deposition and resorption. The osteoclasts are active for about 3 weeks, producing tunnels of about 1 mm in diameter and a few mm in length. This area is then invaded by osteoblasts and new bone formation using new matrix is begun (Guyton, 2005).

As mentioned earlier, bone serves as the body's reservoir of calcium to maintain serum levels. Mobilization of calcium into and out of the bones is mainly regulated by two hormones, each of which is antagonistic to the other. **Parathyroid hormone** (PTH) secreted by the Parathyroid gland causes the rapid dissolution of bone by releasing the calcium and phosphate into the bloodstream. This is accomplished rapidly via direct action but there is also a slower, more sustained phase with the PTH acting to stimulate the production of osteoclasts, which in turn dissolve the bone, releasing the calcium (Guyton 2005). **Calcitonin** on the other hand, is a hormone having the antagonistic effect to PTH. Secreted by the thyroid gland, it serves to decrease the formation of osteoclasts (Wheater et al., 2006) (Guyton, 2005).

With this basic understanding of one histology and physiology, the diseases which affect the bones can now be understood. Once the bone diseases have been clearly outlined, the rationale for the development of the BP drugs can be understood.

DISEASE OF THE BONE

Osteoporosis is the most common metabolic bone disorder in adults. What occurs in this disease is a decreased rate of bone matrix formation. However, the rate of bone mineralization remains normal and steady. In essence what is occurring is that whatever matrix is formed is mineralized normally, but, since there is not enough matrix being formed, the amount of mineralized bone being produced is not keeping up with osteoclastic resorption. This results in a net decrease in volume of mineralized bone per unit volume (Rose and Kaye, 1990). When there is a loss of 30% of bone mass, there is the beginning of pain and increased risks of bone fracture to the individual. The cause of this disease is multifactorial including the natural

decrease in estrogen production in post-menopausal women, which in turn causes a decreased stimulation of the osteoblasts. [In osteoporosis, inhibiting bone resorption by osteoclasts preserves bone density] (Marx et al, 2005).

Cushing's syndrome, another bone disorder, is defined by an increase in the production of corticosteroids which again decreases the osteoblastic function. Any syndrome which decreases the production of protein or increases the catabolism of protein (as examples, diabetes or hyperthyroidism) will result in a generalized osteoporosis (Anderson and Scotti, 1980). Multiple myeloma is a malignant disorder of the plasma cells (Rose and Kaye, 1990). The marrow becomes replaced with abnormal immature plasma cells and they in turn secrete abnormal immunoglobulins which are found in the serum and urine (Rose and Kaye, 1990). Radiographically, there is generalized osteoporosis and there are osteolytic lesions most commonly found in the skull and mandible. These are referred to as 'punched out' lesions. Metastatic prostate and breast cancers invade the bones and the tumor cells produce cytokines which stimulate osteoclasts to resorb the bone (Conte P.F. et al., 1994).

HISTORY OF BISPHOSPHONATES

The bisphosphonate drugs were developed in order to combat many of the diseases of the bone. In the early 1960's, Fleisch demonstrated that the inorganic pyrophosphates (PPi) had the ability to bind to hydroxyapatate crystals in bone and prevent their dissolution by osteoclasts (Fleisch H. et al, 1966). He also found that oral forms of PPi were inactivated by the phosphatases in the stomach. As a result, the organic BP's were developed. The organic BP's were more resistant to the gastric insult and were proven to not only inhibit hydocyapatate dissolution directly by making the hydroxyapatate crystal more resistant to dissolution, but they also prevented bone resorption by inhibiting osteoclastic activity (Gutta and Louis, 2007).

The BP's are similar to the PPi's chemically. The major difference between the two is that the BP's have a carbon atom bridging the phosphate atoms which causes the phosphonate atoms to be more resistant to hydrolysis (Gutta and Louis, 2007). The BP structure looks as follo

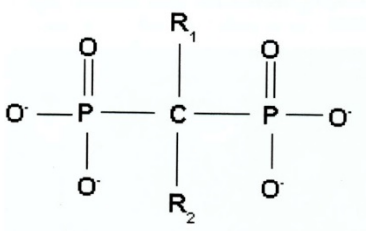

Fig. I. The basic chemical structure for bisphosphonates. (Gutta and Louis, 2007)

The R1 chain always contains a hydroxyl group (-OH) which imparts clinical affinity for bone. The difference in the potency that each BP compound has on bone lies in the alterations of the R2 side chain. The most powerful Bisphosphonates are those with an amino group in the R2 chain of which Zoledronate (Zometa) is the most potent. The following is a list of the currently available Bisphosphonate compounds, their chemical structures, potency and route of administration.

Chemical structure, potency and route of administration of various bisphosphonates

Agent	*R1 side chain*	*R2 side chain*	*Relative potency*	*Route*
Etidronate (Didronel)	OH	$-CH_3$	×1	Oral
Clodronate	Cl	—CL	×10	Oral/IV
Tiludronate (Skelid)	H	-S- ⬡ -Cl	×10	Oral
Pamidronate (Aredia)	OH	$-CH_2-CH_2-NH_2$	×100	IV
Neridronate	OH	$-(CH_2)_6-NH_2$	×100	Oral
Olpadronate	OH	$-(CH_2)_2N(-CH_3)_2$	×1,000	IV
Alendronate (Fosamax)	OH	$-(CH_2)_3-NH_2$	×1,000	Oral
Ibandronate (Boniva)	OH			

MECHANISMS OF ACTION

When the Bisphosphonates are administered, they disappear rapidly from the blood and enter into the bone, remaining there for an extended period of time. Some BP's have half of up to 10 years (Kasting and Francis, 1992)! As mentioned previously, osteoclasts produce acid which dissolves the hydroxyapatite mineral. Since hydroxyapatite absorbs the bisphosphonate and they are incorporated into it structure, as the osteoclast causes the hydroxyapatite dissolution, the bisphosphonates are released and then subsequently absorbed by the osteoclast-thus causing the death of the osteoclast (Marx et al., 2005).

How does this death occur? There are a number of proposed mechanisms of action for the Bisphosphonates. The first generation of Bisphosphonates, such as Clodronate and Etidronate, do not contain any amino groups. After being absorbed by the osteoclast, these Bisphosphonates operate by being metabolized into cytotoxic forms of ATP (Adenosine Triphosphate) which are then incorporated into the osteoclast, causing its apoptosis (Reszka and Rodan, 2003). The more potent Bisphosphonates, i.e. those with amino groups, act by interfering with the production of

FIGURE 6. Photomicrograph of necrotic bone shows empty lacunae. Sequestrum is surrounded by neutrophils and bacterial debris (hema-toxylin and eosin, original magnification x 100). (Ruggiero et al., 2004).

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Additionally, there have been research papers sho to produce a strong osteoclast inhibiting factor (Vi ɔit osteoclast recruitment (Ruggiero et a., 2004).

CASUSES OF OSTEONECROSIS OF THE JAW

The inhibition of the osteoclasts bodes well for the prevention of bone breakdown. However, there many studies showing that concomitant to prevention of bone breakdown, is the development of osteonecrosis. There are many explanations given for this. The first and primary theory is that as the osteoclast function is inhibited, there is a reduction in the normal remodeling of bone. The old bone is not resorbed properly, resulting in an accumulation of micro damaged bone which has less vascularity than healthy bone. Moreover, in a normal, healthy individual, when bone is resorbed by the osteoclasts, the resorption itself stimulates the release of many cytokines and growth factors (Bone Morphogenic proteins- BMP's) which stimulate the osteogenic precursors to differentiate into mature osteoblasts and produce new bone (Storm et al., 1993). When the osteoclasts are inhibited, the release of the cytokines and BMP's is diminished, resulting in a decreased production of new bone (Ruggiero et al., 2004). A second theory given for the reduced vascularity of the bone is that the BP's have been shown to inhibit capillary neoangiogenesis, and to inhibit vascular endothelial growth factor (Marx et al., 2005). Aredia, one of the Bisphosphonate drugs, actually has been shown to decrease bone blood flow in rats (Ruggeiro et al., 2004). Although this second theory sounds attractive, studies have shown that there are many other drugs, such as Thalidomide and alpha 2a- Interferon, which are more potent anti-angiogenic drugs that the Bisphosphonate drugs which do not cause osteonecrosis of the jaw (Marx et al., 2005). Further supports for the osteoclast inhibition theory as being the primary cause of the necrosis, comes from understanding the disease called Osteoporosis, an inherited autosomal dominant trait characterized by the loss of osteoclastic function. These patients present with an identical clinical picture of bisphosphonate- induced exposed bone osteonecrosis in the jaw although angiogenesis is normal (Ibid).

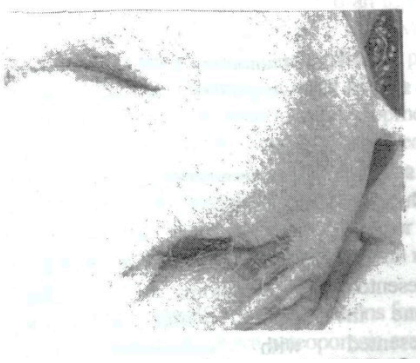


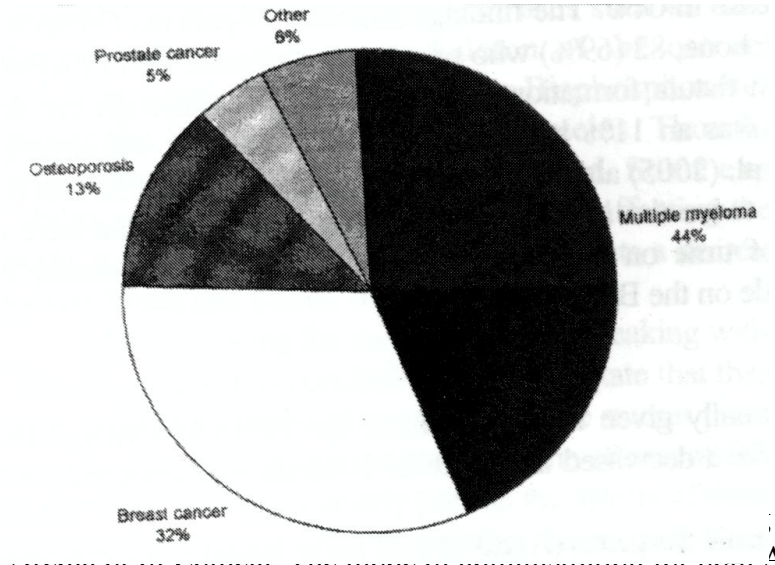
Fig. 2. Chronic orocutaneous fistula with necrosed bone. (Gutta and Louis, 2007)

The development of the osteonecrosis is interestingly noted to be only in the jaws. This is in line with the fact that there is a much greater turnover of one in the jaws due to the presence of teeth and the remodeling of periodontal ligament space. Given this greater turnover, an increased blood supply is needed in the jaw and the consequent lack thereof will cause the necrosis of the jaw (Marx et al., 2005). Osteoclasts live, on average, for about 150 days. (Berne et al., 2004). If after that time no osteoclastic activity occurs, then no BMP, cytokines and other factors are

produced to stimulate new osteoblastic activity. Eventually, the osteon becomes acellular and necrotic, the capillaries become involute and there is a general necrosis of the bone (Marx et al., 2005).

CASE STUDIES

One major study in the ONJ areas was performed by Ruggiero et al (2004), who noticed that there were a growing number of patients at his Oral Surgery Department in the Long Island Jewish Hospital who were being diagnosed with refractory osteomyelitis in the jaw. The typical presentation was a non-healing extraction site progressing to sequestrum formation, exposed bone, localized swelling, and purulent discharge. Up until February of 2001, there had only been one or two patients presented with this condition and when they did, it was always patients who had been receiving radiation therapy. However, not one of these patients had been receiving radiation therapy! Ruggiero's study spanned from February of 2001 to June 2003 and involved 63 patients. There were 45 females and 18 male patients ranging in age from 43 to 89 years of age. The most common oncologic diagnosis was multiple myeloma. The breakdown of the diseases can be seen from the following graph: (Ruggiero et al., 2004)



; infusions of the bisphosphonate Aredia or of Zometa. The mode of administration for both Aredia and Zometa is via IV. [The oral BP's are primarily used to treat osteoporosis (AAOMS, 2006) (Reszka and Rodan, 2003)]. Aredia, which is a first generation Bisphosphonate, is administered over a 2 to 24 hour period every 3 to 4 weeks at a dose of 90 mg. Zometa is administered as a monthly infusion at a dose of 4 mg over 15 minutes (Ruggiero et al., 2004). The duration of therapy ranged from 6 to 48 months.

The breakdown of the medications is seen in the following chart:

Twenty four of these patients (38%) presented ONJ symptoms with maxillary bone involvement. One patient presented symptoms *of both* maxillary and mandibular lesions. Nine of the 63 patients had *no* history of a recent dentoalveolar procedure and yet still presented with exposed and necrotic bone I Microscopic exams of patients' jaws showed necrotic bone, bacterial debris, and granulation tissue. Six of the patients had the signs of osteolysis prior to extraction suggesting that there were lesions prior to any dental procedures.

Another landmark study documenting the exposure of bone in the jaws of patients taking the BP's was begun by Marx in 2003 and involved 36 patients. The present study in 2007, includes those 36 original patients and additional 83 patients. Of the 119 patients followed, 32 (26%) were receiving Aredia, 48 (40.3%) were receiving Zometa and 3 (2.5%) were receiving oral Fosemax. Those patietns on Aredia received 90 mg every 3 to 4 weeks and those on Zometa received 4 mg in the same time interval. Of the patients taking Fosemax, one was taking 10 mg for 6 years and the other 2 had been taking the drug for about 3 years. The mean exposure time until bone became exposed was 14.3 months for Aredia patients, 9 months for patients on Zometa, and 36 months for Fosemax patients. 62 of the patients (52.1%) suffered from multiple myeloma, and 54 (45%) were taking the drugs for bone metastasis from prostate and breast cancer.

Although multiple myeloma is a much rarer disease than cancer and osteoporosis, it is associated with a greater number of ONJ cases because its presence in bone is from the very outset. We see in both studies that multiple myeloma caused an increase in ONJ. The findings include 37 patients (31%) who presented with exposed asymptomatic necrotic bone, 82 (69%) who presented with pain, 28 (23%) with one or more mobile teeth, and 21 (17%) with fistula formation and/or bone exposed through the skin. Subsequent cases were reported where there was an 11% incidence of ONJ in patients taking Zometa (the most powerful of the Bisphosphonates) had twice the incidence of ONJ than patients who were on Aredia. This risk increased as the amount of time on the BP increased- with an estimated 9% increased risk of ONJ for each additional decade on the BP.

MANAGEMENT

Patients who have osteoporosis are usually given the oral form of the BP's (AAOMS, 2006). These forms of drugs are less potent and have a decreased incidence of osteonecrosis associated with them.

The American Association of Oral and Maxillofacial Surgeons (AAOMS), in 2006, issued a position paper on Bisphosphonate Related Osteonecrosis of the Jaw (BRONJ). In it, they noted that it was the Oral Maxillofacial Surgeons who first noticed the correlation between non-healing exposed bone in the jaws and patients who were being treated with Intravenous BP's. The AAOMS, after reviewing the available literature and case studies on the subject, has

concluded that the incidence of BRONJ in the population ranges from .8% to 12% and that with more time and exposure, the figure is likely to rise. The risk factors include the potency of the drug and the duration of time spent on it. The local factors include any insult to the mucosa, such as extractions, implant placements, periapical surgery or periodontal surgery involving osseous injury, which can lead to an ingress of bacteria. Patients who are receiving intravenous BP's and undergo dentoakveolar surgery are seven times more likely to develop BRONJ than patients not having surgery at all. This also applies to patients with inflammatory dental disease (AAOMS, 2006). Novartis, the manufacturer of both Aredia and Zometa has added labeling to their products providing cautionary language in relation to development of osteonecrosis.

Interestingly, the studies quoted earlier show that the mandible has the greatest incidence of developing the osteonecrosis (2:1 over the maxilla). This is due to the mandible having more varied anatomy (e.g. tori, mylohyoid ridge) than the maxilla and the mucosa of the mandible is thinner over those areas. Age is also an important factor to take into consideration. With each decade of age, there is 9% greater risk for BRONJ in patients with multiple myeloma who are on the IV BP's than for those who are not.

The AAOMS recommendations for prevention of BRONJ are as follows:

Patients who have osteoporosis are usually given the oral form of the BP's. These forms of the drug are less potent and have a decreased incidence of osteonecrosis associated with them. However, the risk increases when the duration of therapy exceeds 3 years. If elective dental surgery is planned, it is best to discontinue the oral BP's for 3 months prior to surgery to reduce the risk of developing BRONJ. Patients who are about to initiate BPIV therapy should have all elective surgery performed prior to initiation of therapy and ideally should wait 14-21 days to allow osseous healing and mucosalization. Patients with dentures should be examined for all areas of possible trauma. Patients on IV therapy should avoid procedures that involve osseous injury.

There are three stages in classifying osteonecrosis, as put out by the AAOMS:

Stage 1- Patient has exposed necrotic bone with no evidence of infection. This early stage of osteonecrosis can be managed with oral rinses such as .12% chlorhexidine.

Stage 2- Patient's symptoms include exposed bone with pain and infection. These severer symptoms can be managed with antibiotics, in addition to the oral rinses.

Stage 3- Patient is suffering from exposed necrotic bone, pain and infection, together with either pathologic fracture, extra oral fistula formation or osteolysis. These conditions need to be managed with surgical debridement and resection (Marx et al., 2007).

The potent Bisphosphonate drugs inhibit the mevalonate pathway which produces isoprenoid proteins such as farsenyldiphosphate and geranylgeranyldiphosphate. These proteins are essential to production of the GTP'ases such as Ras, Rho and Rac. These in turn are essential in the production of the cytoskeleton of the osteoclasts and in the integrity of its ruffled border. The future involves designing Bisphosphonate with selective activity against GTP'ases excluding the Rho and Ras groups of proteins. Thus the mevalonate pathways will be spared and the osteoclastic activity will not be affected. Some experimental compounds like NE10790 have that ability to inhibit bone resorption without affecting the osteoclast. Denosumab is a new generation antibody. It is a human 1gG2 molecule that affects a decrease in bone resorption but does not affect the osteoclast (Gutta and Louis, 2007).

After reviewing the case studies and speaking with both an oral surgeon and a general dentist, I feel that the evidence is conclusive enough to state that there is a large risk involved when using Aredia, Zometa, and other Bisphosphonate drugs. The studies presented in this paper and many other studies in this area to date have primarily examined the effects of BP drugs on humans- and not on animal models. Thus, the study results accurately portray the effects of these drugs on humans who have developed ONJ. Obviously, the importance of elucidating the mechanism of this complication is paramount to devising strategies for preventing BRONJ. If the underlying mechanism primarily involves bone remodeling, then eliminating the diseases and conditions that upregulate bone remodeling before starting bisphosphonate therapy can, in some cases, prevent this complication. Although these studies serve to alert dentists and clinicians about the potential complications of bone necrosis in patients receiving bisphosphonate therapy, many questions remain concerning the exact pathogenesis of the process. Further research is needed to elucidate the exact pathogenesis of the process. Further research is needed to elucidate the exact relationship between osteonecrosis and bisphosphonates. The results gathered from these studies and others are conclusive but the mechanism is not conclusive. Knowledge of the factors that incite this condition offers another means of preventing the exposure of bone once bisphosphonate therapy has begun.

CONCLUSION

In conclusion, BRONJ is a condition which is characterized by the death of the bone, with patients presenting with an exposed jawbone or a non-healing extraction socket. The Bisphosphonate drugs, which are taken by cancer patients, multiple myeloma patients, those suffering from osteoporosis, and others, have scientifically been linked to causing these and other symptoms characterized by bone loss, necrotic lesions, and the reduction of bone vascularity. Ongoing studies are searching for possible explanations of the bisphosphonate mechanism, seeking a more efficient recourse of healing ONJ patients as well as an improved quality of life for those suffering from this condition.

SUMMARY

As this paper has shown, BRONJ is a severe condition which adversely affects a small yet significant number of our population. Oral surgeons have recently seen an ever increasing number of patients who suffer from osteonecrosis of the jaw, as a result of chronic bisphosphonate therapy. It is extremely important that all health professionals, especially dentists, oncologists, and oral surgeons, be aware of the possibility that patients being considered for oral surgery or dental implants may perhaps be undergoing BP therapy. Additionally, patients should be informed of the risk of ONJ, so that they will be able to properly evaluate their dental needs and treatment options before starting therapy. BRONJ is a real and painful condition. However, with the current available information and future research on BP mechanisms still being pursued, there is hope that this condition can be successfully managed and perhaps eliminated.

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Insomnia: Its Causes, Its Consequences, and Its Cures

Shoshana Fried

ABSTRACT

Insomnia is a complaint that affects almost all Americans at some point throughout their lives. However, only chronic insomnia is truly a disorder. This type of insomnia can be caused by various reasons, whether physiological, molecular, or psychological. This paper will elaborate on a number of possible causes for insomnia. Furthermore, this paper will present some of the many adverse effects that are caused by the sleep deprivation resulting from chronic insomnia. There are many possible treatments available to help against insomnia. This paper will explain

some of the behavioral methods. In addition, it will discuss the many different medications that have been used or are still used to treat insomnia, including chloral hydrate, barbiturates, antihistamines, and antidepressants. This paper will show the benefits and risks of benzodiazepines, a class of drugs used to treat insomnia, as well as the benefits and risks or the benzodiazepine-like agents BZRAs. This paper will also mention the new EVT 201drug for insomnia that is currently undergoing testing for its efficacy and safety. In conclusion, this paper will show that each case of insomnia is unique and different, from its cause to its ramifications. Each case must be dealt with and treated individually.

Most people spend about a quarter of their lives sleeping. That's about forty-two hours a week, ninety-one days per year, and, in a lifetime of ninety years, over twenty of them will be spent asleep. Many think of this as wasted time; we cannot accomplish many things on out "To Do" lists while snoozing. However, sleep is an important and essential need of the body, and in fact one could not live long without it. Many restorative and healthy benefits take place during those hours of sleep, making sleep quite necessary for life.

Various parts of the brain are responsible for or involved in sleep, and a few theories exist as to how we fall asleep. The generally accepted view today is that several parts of the brain are involved in sleep regulation. In some areas of the brain, neurotransmitters such as serotonin and norepinephrine are produced by neurons in the brainstem and act on different groups of neurons. Whether we are asleep or awake depends on which neurons receive the signals. In other cases, neurons begin to signal only once we fall asleep. These activated neurons are believed to switch off the signals and keep us awake (NINDS '04). One area of the brain involved in sleep regulation is the raphe' nuclei which are located in a thin strip down the midline of the caudal reticular formation. It is believed that these nuclei promote sleep (Pinel '03). The basal forebrain region and the nearby anterior hypothalamus are also thought to be instrumental in promoting sleep (Pinel '03). Sites throughout the caudal reticular formation are thought to be responsible for controlling the major indices of REM sleep, the stage of sleep most physiologically similar to wakefulness. Different sites control different features of REM sleep, such as core muscle relaxation, cardiorespiratory changes, and rapid eye movements.

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These sites communicate through cholinergic and monoaminergic neurons located in the caudal reticular formation. If all these areas work together, REM sleep is induced (Pinel '03).

Why do we sleep? What do we gain? According to many studies done on both animals and humans, much. Sleep is necessary for survival. According to the recuperation theories of sleep, the body's homeostasis is disrupted while one is awake. Sleep restores the body to homeostasis, for example restoring the body's energy levels. These theories imply that when a great deviation from homeostasis results after a period of wakefulness, sleepiness is triggered and the person will go to sleep. Once homeostasis is reestablished, the person wakes up (Pinel '03).

Another theory proposed is that sleep is necessary for our nervous system to properly function. Sleep gives regularly active neurons a chance to rest and repair themselves while using

other groups of neurons during sleep. Without sleep, daytime neurons would become energy depleted or so clogged up with metabolic byproducts that they would malfunction (NINDS ’04). Sleep also allows for certain neuronal connections that do not regularly occur during wakefulness, which otherwise might degenerate from misuse (NINDS ’04).

Additionally, many cells in the body show higher rates of protein production during sleep. These proteins are necessary for cell growth and repair. In children and young adults, release of growth hormone occurs during deep sleep (NINDS ’04). When a person’s body has an infection, he or she sleeps more due to the increased levels of cytokines, which are powerful sleep-inducing chemicals. This indicates that sleep may help conserve energy and other resources that the immune system can now use to attack the source of infection (Dunn ’04).

The benefits of sleep are many, indicating that sleep is extremely significant. Not only is sleep relaxing and restorative, it is also actively beneficial. Sleep helps the body maintain its normal metabolism and activity, and that is why a deficit of sleep is so detrimental.

Insomnia is defined as “the sensation of daytime fatigue and impaired performance caused by insufficient sleep” (A.D.A.M. ’03). Insomnia is divided into three categories, based on its duration. Transient insomnia, which is experienced by 35% of Americans (Caldwell ’03), is the inability to fall asleep for a few nights. Short-term insomnia lasts for up to three weeks. Chronic insomnia, which is the truly problematic type, is defined when a person finds it difficult to fall asleep or maintain sleep or he feels his sleep is nonrestorative for at least three nights a week for a month or more (A.D.A.M. ’03). The patient also must be in distress and believe that his or her normal daily functioning is suffering from the sleep loss (A.D.A.M. ’03).

Transient and short-term insomnia can have many causes. These types of insomnia can often be a response to a change or a stress, such as travel, an acute illness, a job change, or even an exam. These symptoms are minor and only last for a short while; when the stress is removed or the person adjusts to the change, the insomnia stops and normal sleep returns. Transient or short-term insomnia can often occur in females due to fluctuations in hormones, such as during menstruation or pregnancy. Changes in the amount of light to which a person is exposed can influence his or her ability to fall asleep, along with noise, uncomfortable temperatures, or other environmental disruptions (A.D.AM. ’03). Increases muscle tension, caused by daily stress, will not allow a person to fall asleep easily (Caldwell ’03). Ingesting certain substances, such as caffeine or nicotine, can disrupt sleep and generate insomnia. Many medications include insomnia as a side effect, especially those containing caffeine (A.D.A.M. ’03). However, in all these cases, unless the insomnia persists for more than a few weeks, no treatment is necessary.

Chronic insomnia, however, is more significant. Chronic insomnia is usually caused by a collaboration of different psychological and physical conditions. One important kind of insomnia is psychophysiologic insomnia which is caused by initial transient insomnia. After experiencing difficulty falling asleep for a few nights, the patient begins to associate the bed with a struggle to sleep. This causes even more difficulty in falling asleep. Eventually the patient begins to worry excessively about sleep loss and continually experiences anxiety at bedtime. This cycle continues over a log period and the insomnia becomes chronic (A.D.A.M. ’03).

Chronic insomnia can also be caused by pain or discomfort. Leg disorders especially, such as restless leg syndrome or leg cramps, are often a cause of insomnia. Other medical problems that can cause insomnia include arthritis, heart disease, asthma, Attention Deficit

Hyperactivity Disorder (ADHD), and cancer. Many medications as well induce insomnia. These medications include beta-blockers, certain antidepressants, beta-agonists, and many more. When taken over a long period of time, they can produce chronic insomnia. Substance abuse, especially overuse of alcohol or cocaine, can also play a major role in causing insomnia (A.D.A.M. ’03).

Very often insomnia has an underlying psychological cause. Certain emotional disorders often cause insomnia, especially anxiety, bipolar disorder, and depression. These emotional disorders prevent the relaxation necessary for sleep onset (Caldwell ’03). They also often cause repeated awakenings during the night and the inability to return to sleep after awakening (A.D.A.M. ’03).

Insomnia can be annoying. Lying in bed for hours waiting to fall asleep can be frustrating, and waking up repeatedly throughout the night can be exasperating. However, the effects of sleep deprivation are much more significant and detrimental than that.

Sleep deprivation can cause a decline in certain cognitive abilities. The National Sleep Foundation (NSF) lists a number of consequences of inadequate sleep, including slow thinking or reacting and frequent mistakes. They also include difficulty focusing, a narrowing of attention, difficulty following directions, difficulty remembering information, and poor judgment regarding complex situations (A.D.A.M. ’03). Sleep deprivation can also cause impairment of higher neurological functions. Awareness and perception decline, and vision difficulties often occur. Difficulty with coordination and fine motor skills is also quite common (Caldwell ’03). General performance declines and simple tasks become more difficult or even impossible.

Even more unsettling are the emotional problems caused by inadequate sleep. Sleep deprivation often has an adverse effect on mood or temperament. The NSF includes in its list impatience and quick anger, as well as depression or negative mood. [j] Insomnia can interfere with the activity of hormones and pathways that deal with emotion, so that even minimal sleep deprivation can greatly effect one’s mood (A.D.A.M. ’03).

When one has had inadequate sleep, one is more likely to feel forlorn, depressed, or hopeless. Lack of sleep also causes motivation to diminish. In addition to the actual decline in cognitive abilities due to his sleep deprivation, insomnia also causes a person’s desire and interests to lessen. He would much rather rest than do a math problem or solve a puzzle. Perhaps this is what account for the great disparity in performance when one is sleep deprived (Caldwell ’03). The negative repercussions that result from sleep deprivation can decrease the quality of life as much as living with a chronic condition like heart failure (A.D.A.M. ’03).

During periods of inadequate sleep, many abnormal physiological conditions are observed. A study done in 1991 found that after a single night of three hours of sleep, during submaximal exercise maximum oxygen consumption significantly decreased while heart rate and ventilation significantly increased. A 1993 study investigating the endocrinological effects of sleep deprivation found that TSH, thyroid hormones, estradiol, and LH levels went up, while prolactin levels decreased (Walsh and Lindbolm ’97). Although the reasons for these changes are unclear, their message is not: Sleep deprivation causes many changes in the body’s metabolism, and most of them are evidently not very beneficial.

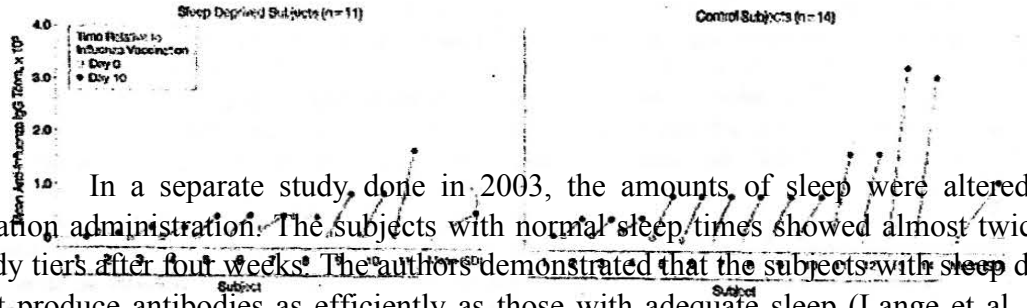
Chronic sleep loss adversely affects metabolic functions, in particular carbohydrate metabolism. A study done at the University of Chicago in 1999 found that after a period of sleep deprivation, glucose conditions were higher than they were after a period of adequate rest (Van

Cauter ’99). These variations in glucose metabolism resembled those in patients with type-2 diabetes (The University of Chicago Medical Center ’99). A subsequent study was conducted in 2006 by the same author. The HbA1c, glycosylated hemoglobin which reflects the average blood glucose blood level over the previous three months, was measured in various patients already diagnosed with diabetes. They found that higher HbA1c results were closely associated with inadequate or poor quality sleep. This study showed that insufficient proper sleep is related to increased blood glucose levels in patients with diabetes (The University of Chicago Medical Center ’06).

The 1999 study concluded that the reduction of metabolic function capacity due to chronic sleep loss is devastating. The metabolic and endocrine changes that result “mimic many of the hallmarks of aging”. Cutting back on sleep “produced striking changes in glucose tolerance and endocrine function-changes that resembled the effects of advanced age or the early stages of diabetes.” These results occurred after less than a week of sleep deficiency! This study showed that chronic sleep loss can “hasten the onset but could also increase the severity of age-related ailments such as diabetes, hypertension, obesity and memory loss” (The University of Chicago Medical Center ’06).

Besides altering endocrine and metabolic functions, sleep deprivation can also have an adverse effect on the immune system. In a study done in 2002, influenza vaccinations were given to two groups of people. One group had been restricted to four hours of sleep for the previous four nights, while the other group had maintained their usual bedtimes before receiving the vaccination. It was found that those in the group of decreased sleep had mean antibody titers less than half of those in the group with normal sleep times. The authors concluded that “the response to influenza vaccination may be impaired in individuals with chronic partial sleep restriction” (Lurie ’02).

Antibody Titers at Baseline and 10 Days Following Influenza Vaccination



Spiegel, K. et al. JAMA 2002;288:1471-a-1472-a.

In a separate study, done in 2003, the amounts of sleep were altered *after* the vaccination administration. The subjects with normal sleep times showed almost twice as great antibody tiers after four weeks. The authors demonstrated that the subjects with sleep deprivation did not produce antibodies as efficiently as those with adequate sleep (Lange et al. ’03). This weakened immune system makes people deprived of sleep more vulnerable to diseases and infections (Dunn ’04). In a study done by the American Cancer Society, they found that men who slept for less than four hours a night had mortality rates ten times greater than those who slept seven to eight hours a night. Perhaps that is why the National Center for Health Service Research in the United States considers proper amount of sleep one of the six most important factors affecting illness and death rates (Caldwell ’03).

Insomnia is not a disease but a symptom (Caldwell ’03). However, insomnia can be cured- not only by taking care of the underlying cause but also by treating the insomnia itself. There are many behavioral therapies and other treatments to stop insomnia that do not involve medications. In addition, hypnotic drugs, which are the class of drugs that can bring on sleep, lengthen sleep time, or improve it in any way (Caldwell ’03).

Behavioral techniques are often favored over medications for many reasons. Using drugs always introduces side effects, interactions, and other restrictions, while making adjustments in behavioral often does not. Behavioral methods can be used by all insomniacs, including those already taking an incompatible medication or elderly patients. Additionally, although medications are equally or even more effective, behavioral techniques often work faster (A.D.A.M. ’03).

There are a number of behavioral approaches for treating insomnia. The first step usually recommended is to check for proper sleep hygiene. This term is used “to describe simple behaviors that may help improve sleep” (Bootzin and Rider ’97). Behaviors that constitute improper sleep hygiene are identified and stopped. These include spending too much time in bed, using substances like caffeine or nicotine that interfere with sleep, attempting to sleep in a poor sleep environment, napping in the late afternoon or evening, and engaging in extremely stressful or exciting activities close to bedtime (Bootzin and Rider ’97). Instead, proper sleep behaviors are set. These behaviors may include establishing a fixed time for going to bed and getting up, taking a hot bath about one and a half to two hours before bedtime, or doing something relaxing such as reading or meditating half an hour before bedtime (A.D.A.M. ’03). Proper sleep hygiene education has proven quite effective, often even after only one or two sessions of evaluation and recommendations (Bootzin and Rider ’97).

One suggestion made to improve sleep hygiene is to exercise in the early evening. A few hours after exercise, a low point in energy is experienced, and this can help the person to fall asleep more easily (A.D.A.M. ’03). Engaging in moderate to heavy exercise causes one to fall asleep more quickly, wake up less often throughout the night, and experience increased amounts of deep sleep (Caldwell ’03).

Another behavioral treatment used for insomnia is stimulus control instructions. These are a set of instructions that focus primarily on helping the patient associate the bed with falling asleep quickly. Some of the instructions include going to bed only when ready for sleep, as well as getting out of bed and leaving the bedroom if unable to fall asleep within ten minutes. Studies have found stimulus control to be one of the most effective therapies for treating insomnia (Bootzin and Rider ’97).

There are other behavioral techniques that can be used in the treatment of insomnia. Some of these techniques are light therapy, progressive muscle relaxation, and cognitive behavior therapy (A.D.A.M. ’03). However, the value of these methods is not so certain and many do not provide conclusive evidence as to their effectiveness. Experts are still studying the benefits and helpfulness of each, and studies are still being conducted to find more ways to help in the treatment of insomnia.

Sometimes, however, behavioral methods are not enough. Each individual’s patient’s case of insomnia is unique, and the treatment provided must match in each case. Sometimes the behavioral techniques do not work well enough, and other proven methods must be used.

Medications can also be helpful in the treatment of insomnia. Some over-the-counter drugs and even drugs regularly given to treat a different symptoms- can be helpful, and sometimes doctors might prescribe a specific medication to treat insomnia.

One drug that was formerly very common as a remedy for insomnia is chloral hydrate. It has been in use for more than one hundred fifty years (A.D.A.M. ’03). Chloral hydrate works quickly, reaching peak blood levels within an hour (Caldwell ’03). It has a fairly short half-life so does not usually produce a hangover after awakening. However, its popularity as a treatment for insomnia has gone down, due to the many adverse side effects and risks it involves. As a direct brain-cell depressant, chloral hydrate can interfere with brain functions and cause confusion and drowsiness (Caldwell ’03). It can also cause inflammation of the skin or irritation of the stomach (A.D.A.M. ’03). Because it is metabolized in the liver and excreted in urine, patients with liver or kidney disorders should not use it (Caldwell ’03). Chloral hydrate also has carcinogenic properties and may damage genetic material. Additionally, the lethal dose of chloral hydrate is very close to the therapeutic dose, and even a slight overdose can be fatal (Pagel and Parnes ’01). Because of all the risks it incurs, chloral hydrate is no longer regularly used in the treatment of insomnia.

Barbiturates is another class of drugs that can be used to treat insomnia. Barbiturates were used as the standard sleeping medication in the years before the 1060’s (Pagel and Parnes ’01). However, addiction and abuse of barbiturates were quite common (A.D.A.M. ’03). They also have a high danger of overdose and have caused the deaths of many, including some famous celebrities like Elvis Presley and Marilyn Monroe (Pagel and Parnes ’01). Presently, due to their potential for abuse and overdoes, these drugs are rarely or never prescribed to treat insomnia (A.D.A.M. ’03).

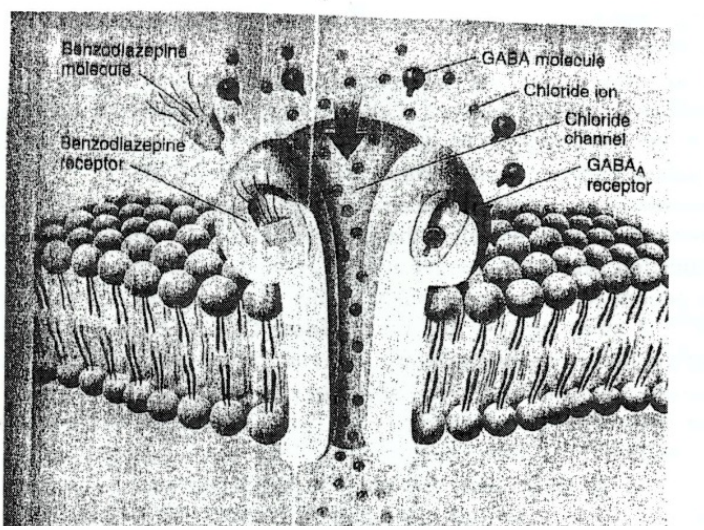
Antihistamines are another type of drug used in the treatment of insomnia. Antihistamines are regularly prescribed to unclog congestion from a common cold or to stop allergic reaction that might become dangerous. A very prominent side effect of antihistamines is sedation. Everyone knows that taking Sudafed or Benadryl will cause one to fall asleep. For certain antihistamines, this side effect is so pronounces that it was decided to market the drugs for insomnia. These antihistamines include diphenhydramine, the one ingredient in the most over-the-counter “sleeping pills,” diphenhydramine, promethazine, and meclizine. However, in most antihistamines leave patients feeling drowsy the next day (A.D.A.M. ’03). They also cause some other adverse side effects, such as dizziness, blurred vision, increased heart rate, and weight gain (Caldwell ’03).

Over-the-counter sleeping pills are used by 3/1% of adults in the United States (Pagel and Parnes ’01). These antihistamines are quick acting and cause quick onset of their hypnotic effects. The effects generally last for six to eight hours (Caldwell ’03). However, many sleeping pills work differently for different people. Sleeping pills often cause daytime sleepiness and cognitive impairment. These side effects often remain until the next day, interfering with daytime performance (Pagel and Parnes ’01). Sleeping pills can also have a diminishing effect on brain function. This dangerous symptom is exacerbated if the antihistamine is reaching with another drug substance that causes sedations (Caldwell ’03). They should be taken with caution and used only as a last resort.

A class of drugs used quite frequently in the treatment of insomnia is sedating antidepressants. Many patients with insomnia also suffer from depressive symptoms, and sometimes the depression is a direct result of the insomnia (Pagel and Parnes ’01). One group of antidepressants used are the tricyclic antidepressants. Tricyclics are absorbed quickly and reach peak blood levels in two to four hours. Tricyclics can increase deep sleep and decrease the number of awakenings during the night (A.D.A.M. ’03). In addition, they cause a gradual improvement in the depressed patient’s mood, although this effect only starts two to three weeks after starting regular dosage (Tyrer ’93).

The required dose to treat insomnia is smaller than the dose used to treat depression, an amount that often causes many negative side effects. However, even the small dose does cause a few minor side effects such as weight gain, increased heart rate, dry mouth, and constipation. It can also can also cause daytime sleepiness and hangover or even weakness and fainting spells (Caldwell ’03). Tricyclics also have a high danger of overdose (Pagel and Parnes ’01). Additionally, if a patient is taking antidepressants, especially a large dose, and abruptly withdraws, he can suffer symptoms of severe anxiety and perhaps even panic. However, withdrawing gradually by slowly reducing the dose over a period of four weeks can usually help circumvent this effect (Tyrer ’93).

The main class of drugs used today in the treatment of insomnia is benzodiazepines. Benzodiazepines act as GABA receptor agonists in the nervous system. GABA (gamma-aminobutyric acid) is the chief inhibitory transmitter in the central nervous system (Pinel ’03). There are three types of receptors for GABA in the CNS; one type is $GABA_A$ receptors. The $GABA_A$ receptor controls the direction and magnitude of the ionic currents of the neuronal ion channels. When GABA binds to the $GABA_A$ receptors, it triggers the opening of a chloride ion pore. This inhibits the firing of new action potentials. Benzodiazepines act by binding to a different site on the $GABA_A$ receptor and thereby increasing the binding of GABA molecules to the receptor (Pinel ’03). This increases the inhibitory effects and slows down the flow of chemical information from one neuron to another (Caldwell ’03). This is what causes the relaxation and sedation that aid in reducing insomnia.



Since they were developed in the 1960's, benzodiazepines have proven their effectiveness. Today benzodiazepines are the drugs of choice in the treatment of insomnia, since they have the highest benefit and the lowest risk of other sleep-enhancing medications (NSF '02). As with most sleep-promoting drugs, benzodiazepines have proven effective for decreasing the time it takes to fall asleep, increasing total sleep time, minimizing the number of awakenings during the night, and improving the quality of sleep (A.D.A.M. '03). Benzodiazepines, though, are less likely to lose efficiency after a few months (A.D.A.M. '03), while many other drugs require higher and higher doses to continue to work against the insomnia. In addition, benzodiazepines have a much smaller abuse potential and overdose danger (Pagel and Parnes '01). The therapeutic index, or the relationship between the effective dose and the lethal dose, is about one hundred for benzodiazepines, while it is a mere two to four for drugs such as barbiturates (Roehrs and Roth '97). For this reason, final overdose of a benzodiazepine is extremely rare (Caldwell '03).

However, benzodiazepines do have certain adverse side effects. As do most hypnotics, benzodiazepines often cause residual daytime sleepiness. Benzodiazepines inhibit the charging of neurons in the central nervous system, and if this effect lingers, drowsiness as well as a slowing of thought processes occurs (Caldwell '03). Coordination and judgement are impaired (Committee on the Review of Medicines 80'), and a general deterioration of neurological functioning is observed (Caldwell '03). Most often these effects are quite subtle, but occasionally they may be severe, resulting in confusion and disorientation and memory loss of preceding events (Caldwell '03). This happens especially with elderly patients who are taking benzodiazepines, as the aging brain seems to be more susceptible to this type of side effect (Caldwell '03). This residual drowsiness has led benzodiazepines to be associated with an increased risk for falls and car accidents, especially within the first week of taking them (A.D.A.M. '03). This is why users of benzodiazepines are advised not to drive or operate heavy machinery while on the medication.

The inhibitive properties of benzodiazepines also cause relaxation of muscles. If this effect persists, patients will experience poor fine motor coordination and generalized weakness. A mild spinning sensation or blurred vision may also occur (Caldwell '03).

Benzodiazepines can also have an effect on one's mood and may increase depression (A.D.A.M. '03). In addition, respiratory depression may occur as a result of taking benzodiazepines. This happens especially in patients with preexisting respiratory disorders, for whom the benzodiazepines exacerbate the respiratory malfunctions (Pagel and Parnes '01).

Some experts do not like to promote the use of benzodiazepines as they distort the normal patterns of sleep. While benzodiazepines increase the time spent in Stage Two sleep, they decrease the time spent in Stage Four sleep and in the restorative REM sleep (Pinel ’03). This causes another negative side effect of benzodiazepines: the patients experiences disturbing dreams (Caldwell ’03).

Benzodiazepines are also problematic in that tolerant to the medication and it no longer works well as the treatment continues (Caldwell ’03). This requires the patient to take continuously higher doses of the benzodiazepine to maintain the same results (Pinel ’01). A study done by the White House Office of Drug Policy and the National Institute on Drug Abuse found that many benzodiazepines tend to lose their efficacy after three to fourteen days of continuous use (Committee on the Review of Medicines ’80).

In addition to tolerance, the body develops dependence on benzodiazepines. This is a state in which unless drug use continues, the body will go into a withdrawal or drug-absence stage (Caldwell ’03). If a patient taking benzodiazepines stops the medication abruptly, he will experience many withdrawal symptoms. One symptom almost always experienced, especially by those who have been taking benzodiazepines over a long period of time, is rebound insomnia. This entails one to two nights of poor sleep and daytime tiredness. Patients may also suffer from increased anxiety and apprehension and perceptual disruption of noise, touch, and movement (Tyrer ’93). Gastrointestinal irritation and disturbed heart rhythm are common withdrawal symptoms as well. Sometimes, patients may suffer the same severe insomnia that caused them to start the drug in the first place (A.D.A.M. ’03). This severe rebound insomnia lasts about one to three weeks until it returns to the level it was at before the drug was stopped (Tyrer ’93). This can be dangerous because doctors observing the return of the original insomnia may conclude that the treatment was ineffective and then prescribe a higher dose of the medication (Committee on the Review of Medicines ’80).

To circumvent these withdrawal symptoms, it is advised to withdraw from the benzodiazepine drugs gradually (Committee on the Review of Medicine ’80). This can be over a period from two weeks to many months (Tyrer ’93). This allows enough time for the central nervous system to adapt to the withdrawal of the inhibitory drug (Tyrer ’93).

Another suggestion to decrease the severity of withdrawal symptoms is to withdraw from the benzodiazepines along with cognitive behavior therapy. A study done in Canada in 2003 studies the differences between gradual tapering of the benzodiazepines and gradual tapering combined with cognitive behavior therapy. The researchers randomly assigned these two withdrawal methods to the patients in the study who had been using benzodiazepines at bedtime for the previous three months or longer. Out of the sixty-five patients, thirty received a gradual tapering schedule of 25% reduction in dosage at one to two week intervals. The other thirty-five received the same withdrawal schedule as well as a ninety-minute weekly session of therapy with a psychologist who gave sleep hygiene education and instructions for stimulus control. Each patient met weekly with a doctor who looked for withdrawal symptoms. Based on the patient’s symptoms the doctor prescribed either the same or a lower dosage of the benzodiazepines. After eight weeks of treatment an evaluation was done. The evaluation found that 38% of those undergoing gradual tapering only had completely withdrawn from the benzodiazepines, while in the combined group the percentage of patients off the benzodiazepines was 77%. Additionally, a

reduction in the dosage of benzodiazepine greater than 50% occurred in 69% of the first group and 97% of the combined patients. After a twelve month follow-up, some patients had reverted back to taking the benzodiazepine or had increased the dosage; however, the percentages were once again much higher for those in the combined group. This study clearly showed that the combination of cognitive behavior therapy with gradual tapering is superior to gradual tapering alone (Baillargeon et al. '03).

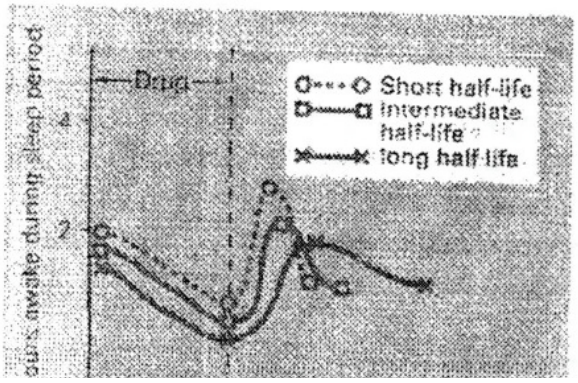
Changes in benzodiazepine use

Patient status and time of measurement	Group; no. (and %) of patients		OR (and 95% CI)	
	Combined treatment	Tapering alone	Crude	Adjusted*
Benzodiazepine-free				
Immediately after treatment	26/34 (77)	11/29 (38)	5.3 (1.8–16.2)	7.9 (2.4–30.9)
At 3 mo	22/33 (67)	10/29 (34)	3.8 (1.4–11.3)	3.9 (1.4–12.1)
At 12 mo†	23/33 (70)	7/29 (24)	7.2 (2.4–23.7)	7.6 (2.5–26.6)
Dosage reduction ≥ 50%				
Immediately after treatment	33/34 (97)	20/29 (69)	14.8 (2.5–284.3)	16.5 (2.7–321.2)
At 3 mo	25/33 (76)	19/29 (66)	1.6 (0.5–5.0)	1.6 (0.5–5.0)
At 12 mo†	26/32 (81)	15/29 (52)	4.0 (1.3–13.6)	4.1 (1.3–13.8)

Note: OR = odds ratio, CI = confidence interval.
*Adjusted for initial daily dose of benzodiazepine.
†For one patient in the combined treatment group, information about benzodiazepine withdrawal was obtained at 12-month follow-up, but data for the dosage reduction variable was not available at that time; hence the difference in denominators.

Due to the many adverse side effects the use of benzodiazepines entails, doctors recommend that the regular use of benzodiazepines should be limited (Roehrs and Roth '97). The Committee on Safety of Medicines advised that benzodiazepines should not be used for more than four weeks (Bashir et al. '94). Benzodiazepines should be prescribed at the lowest possible effective dose and prescriptions should be within the therapeutic range (Committee on the Review of Medicines '80)

However, there is another type of benzodiazepine that produces fewer negative side effects. This type of benzodiazepine may be excluded from the limitation of regular benzodiazepines due to the decreased risks it involves. These are the short-acting benzodiazepines that have much shorter half-lives than the regular benzodiazepines. These come very close to the "ideal sleeping pill", as they have a quick onset, last for a typical night's sleep of six to eight hours, and then are quickly metabolized or eliminated from the body and the hypotonic effects stop. (Caldwell '03).



There is quite a range of short half-life benzodiazepines. Some, like triazolam, have a very rapid onset but last for only a short while. This medication can be helpful for a patient who has difficulty falling asleep but not for a patient who tends to awaken throughout the night, as the drug is cleared from the body before the night is over (Caldwell ’03). Other short half-life benzodiazepines wear off by the next morning. This decreases the “hangover” side effects of drowsiness and impaired neurological functioning (Committee on the Review of Medicines ’80). For this reason, short-acting benzodiazepines are usually prescribed over long-acting ones for the elderly (Hallfors and Saxe ’93).

The withdrawal symptoms from short-acting benzodiazepines differ from the symptoms of withdrawal from long half-life benzodiazepines. In 1991 a meta-analysis was conducted of seven studies done to determine the differences. Each of the studies had a few more dropouts of the withdrawal from the short half-life benzodiazepines than the long half-life ones. When all the studies were combined, the meta-analysis found the difference to be significant. Additionally, the meta-analysis found that the patients on a short half-life benzodiazepine had a 25% greater chance of experiencing anxiety after withdrawal than those on a long half-life benzodiazepine. The authors concluded that patients withdrawing from short half-life benzodiazepines “may be exceedingly uncomfortable and less likely to successfully complete withdrawal” (Hallfors and Saxe ’93).

The claims that short half-life benzodiazepines cause excessive withdrawal symptoms and rebound insomnia led to the banning of one such drug, triazolam, by several countries including Britain (O’Donovan and McGuffin ’93). A review of the topic written in 1993 gave many examples of studies that showed memory impairment and anxiety associated more with short half-life than long half-life benzodiazepines. The United States Food and Drug Administration received reports on adverse side effects on hundred times more commonly for triazolam than temazepam, a different short-acting benzodiazepine with a slightly longer half-life. The review concluded that amnesia and rebound insomnia, “although not unique to triazolam, are more often associated with its use than with other benzodiazepines.” The authors pointed out that there are numerous treatment methods for insomnia, both pharmacological and behavioral, and “triazolam has no compelling singular benefits that outbalance the risks” (O’Donovan and McGuffin ’93).

However, the review warned that “obviously case reports and drug monitoring data are open to many sources of bias, and a drug that has been the subject of media attention may attract

closer scrutiny of its apparent adverse effects by both doctors and their patients" (O'Donovan and McGuffin '93). Furthermore, a letter to the editor written by two doctors in response to the 1993 review pointed out that the only countries in which triazolam is suspended are the United Kingdom, Norway, and Brazil. The authors of the letter claimed that the evidence brought in the review does not support the position that triazolam is more likely to produce withdrawal symptoms than other benzodiazepines. They explained that the authors of the review gave "enormous weight to some studies with exceptionally small sample sizes" and they showed a "tendency to magnify some studies while minimizing large scale work." This resulted in a "conclusion that we believe is not supported by objective fact." Their idea is the same conclusion that the Collegium International Neuropsychopharmacologium and the World Psychiatric Association reached in their reports on the subjects. They conclude that patients who use short half-life benzodiazepines "derive remarkable benefit" (Dinan and Leonard '93).

This conclusion is supported by other studies which claim that although the withdrawal symptoms from a short half-life benzodiazepine occur sooner and are more severe, they last for a shorter time than the withdrawal symptoms from long half-life benzodiazepines (Tyrer '93). A study was done in 1994 over a six-month period to test the effects of physician intervention during benzodiazepine withdrawal. Incidentally, this study found that those patients on a short half-life benzodiazepine were more likely to reduce their dosage than those on a long half-life benzodiazepine. The authors pointed out that the current trend was to switch short half-life benzodiazepine users to a long half-life benzodiazepine when considering withdrawal, as the shorter half-life benzodiazepines were thought to have increased risks of withdrawal symptoms. However, the authors suggested based on their results that "it may be better to leave patients on their short-acting preparations when a dose reduction is being attempted" (Bashir et al. '94).

Some Long- and Short-Acting Benzodiazepines and Their Commercial Names

Long-Acting	**Short-Acting**
chlorazepate (Genene, Tranxene)	alprazolam (Apro-Alprax, Xanax)
chlordiazepoxide (Librax, Librium)	bromazepam (Lectopam)
clonazepam (Klonopin, Rivotril)	estazolam (ProSom)
diazepam (Valium, Rivotril)	fluntitrazepam (Rohypnol)
flurazepam (Dalmane, Somnol)	halazepam (Paxipan)
nitrazepam (Inomax, Mogadon)	ketazolam (Loftan)
quazepam (Doral)	lorazepam (Ativan, Nu-Luraz)
	midazolam (Versed)
	oxazepam (Serax)
	prazepam (Centrax)
	temazepam (Restoril)
	triazolam (Halcion, Nutriazo)

Although benzodiazepines are currently most often prescribed hypnotic, a new group of drugs called benzodiazepines receptor agonists (BZRAs), is increasingly being prescribed and is becoming the new hypnotic drug of choice (A.D.A.M. '03). These non- benzodiazepine BZRAs are even shorter acting than short half-life benzodiazepines and include "Z drugs" zolpidem, zaleplon, and zopiclone, known commercially as Ambien, Sonata, and Imovane.

The National Institute for Clinical Excellence (NICE) in Britain recommends that hypnotic drugs be used only for short periods. In their guidance, they do not distinguish between short-acting benzodiazepines and the BZRAs, explaining that they have found "no compelling evidence of a clinically useful difference" between them. Instead, they recommend using the drug with the "lowest purchase cost" (Gibson '04). However, many researchers and practitioners disagree with NICE's conclusion. The British Sleep Society, a professional organization of medical and scientific staff who focus on sleep disorders, does not support NICE's decision. Professor David Nutt, the society's representative, expressed that "it seems perverse that patients will be forced to run the risk of significant daytime hangover to save a few pence on drug costs" (Gibson '04).

Just like the British Sleep Society, many physicians believe that BZRAs have fewer side effects than even short half-life benzodiazepines (Pagel and Parnes '01). Although they might produce minimal early-morning confusion and nausea (Caldwell '03), BZRAs have much lower risk of lingering sedative effects that will interfere with daytime functioning (Zammitt and Kramer '01). This is especially helpful in the treatment of elderly patients, when this results in fewer risks for falls or memory loss (A.D.A.M. '03). BZRAs only slightly alter sleep patterns, and their potential for abuse is minimal (Pagel and Parnes '01). In patients with respiratory disorders, BZRAs have fewer respiratory suppressant effects (Pagel and Parnes '01). They have a lower risk than benzodiazepines for tolerance and dependence (Lieberman '07). Studies done to evaluate the long-term effectiveness and safety of BZRAs showed that discontinuation did not produce withdrawal symptoms (Zammitt and Kramer '01). The drawback to BZRAs, however, is that these drugs are expensive (A.D.A.M/ '03).

Most recently, Evotec, a research company that "is a leader in the discovery and development of novel small molecule drugs" (WebWire '06), has been working on a new drug to treat insomnia. This drug, labelled EVT 201, is a partial agonist of the $GABA_A$ receptor. As a partial agonist, it employs a difference mechanism than benzodiazepines and BZRAs which are full agonists of the $GABA_A$ receptor.

EVT 201 is currently in the middle of extensive testing process conducted on all new drugs. It recently completed a Phase II clinical trial conducted on sixty-seven patients in sleep labs in the United States. This trial showed that the drug caused significant improvements in the quality of sleep, measured both by subjective assessments and polysomnography. The subjects in the trial did not report any residual sedation. In this trial, EVT 201 was shown to be safe and well-accepted without any significant adverse effects (WebWire '06).

In further trials to be conducted on the drug, additional measures will be studies and researchers will check for any adverse effects. Dr. John Kemp, Chief Research and Development Officer in the Evotec company, pronounced, "If we can confirm this profile of efficacy together with freedom from significant adverse effects in patients with primary insomnia, this would represent a very attractive product in a market in which we believe there are significant opportunities based on a high unmet medical need" (WebWire '06). Perhaps in a few years from now, EVT 201 will be the only medication that can be found on the shelf in the insomnia section of the pharmacy.

The way people think about insomnia has changed drastically over the past fifty years. In the past, if someone complained of having sleep difficulties, "medications that were often

dangerous and addictive were prescribed to induce sleep, while the basis of the patient's complaint was not addressed" (Pagel and Parnes '01). Today, however, the safety of treatments has improved (Lieberman '07), and researchers are still working on finding drugs with better results and fewer adverse side effects. Additionally, our understanding of sleep has grown tremendously, and we are better able to pinpoint the source of the insomnia and treat accordingly. Doctors now understand that not all cases of insomnia are alike. From the psychological, biological, or physiological factors that caused the insomnia, to the endocrinological, behavioral, and emotional outcomes that result, no two cases of insomnia are perfectly alike. Consequently, each case must be treated differently. A method that produced wonderful results when used for Patient X might backfire when tried for Patient Y. Each case of insomnia is unique, and the plethora of treatments available today allows the practitioner to find and prescribe the ones that will work best for each patient.

CONCLUSION

Insomnia is a condition that can happen to anyone. Whether due to improper sleep hygiene, consumption of beverages such as caffeine or nicotine, as a side effect from a particular medication, or during a period of depression, many people are susceptible and may become unable to sleep. This can be dangerous, because as this paper has shown the resulting sleep deprivation has many significant negative consequences. Not only do these effects persist as long as the insomnia lasts, we have seen that some results may have lifelong repercussions. Fortunately, though, there are many treatments available to help in the battle against insomnia. This paper has discussed a few behavioral techniques that are helpful. Additionally, this paper has presented many medications that are used to treat insomnia and has explained the benefits and risks of each type. This paper has shown that although drugs might be more effective than behavioral techniques for many people, no medication is perfectly effective or completely free from negative side effects. Thus, any patient suffering from insomnia must discuss with his doctor the various remedies, weighing the risks and benefits of each. They will then be able to choose the treatment or treatments that will help with his individual case and will best cure him of his insomnia.

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BOTOX and Its Effect on Wrinkles

Rochelle Eckstein

ABSTRACT

Clostridia Botulinum (C. Botulinum) is a bacterium that produces a unique exotoxin, botulinum toxin (BTX). BTX induces flaccid muscle paralysis by inhibiting the release of acetylcholine at the cholinergic nerve endings. Recently, Botox, developed from BTX, has become a popular solution to reduce the appearance of hyper-functional facial rhytids. Facial wrinkles are caused by repetitive muscle contraction, and a treatment that directly addresses this will be effective. Injections of Botox temporarily relax or paralyze these muscles. This paper will briefly discuss the bacterial basis of Botox and its development. It will explain how the mechanism of action of BTX can be used therapeutically and cosmetically as well. The formula and development of Botox and similar drugs will be explained as well as the relevant facial anatomy. A detailed analysis of case studies and comparisons of different formulations are required before determining if Botox is an effective treatment. The side effects and risk factors involved will be assessed, concluding if Botox is a safe as well as effective treatment.

INTRODUCTION

CLOSTRIDIUM BOTULINUM

Clostridium botulinum, a gram-positive, spore forming, anaerobic bacteria, is found in the soil but easily isolated (Arnon et al, 2001). C. botulinum is best known for its neurotoxin produced by growing cells, botulinum toxin, BTX. BTX is the most poisonous of all known poisons and causes the disease botulinum which is characterized by flaccid muscle paralysis (Sharma and Shukla, 2005).

C. Botulinum was first discovered in the 1820s by Justinus Kertner, a Bavarian. Kertner collected data and published two monographs on 230 cases of botulism, giving a complete and accurate description of botulism including symptoms, duration and physical findings. Symptoms include: tear fluid disappears, the pupils dilate, eye muscles are paralyzed, mucous and saliva secretion is suppressed, skin is dry and the skeletal and gut muscles are paralyzed. Cognition is retained throughout all this (Scott, 2004).

Seventy-five years later, Van Ermengem, a professor of bacteriology correctly described the bacterial basis of botulism after an outbreak among 34 individuals who had attended a funeral and eaten raw partially salted ham. Of these, 34, 23 became paralyzed and 3 died. Van Ermengem found the ham was toxic to lab animals, producing a paralytic disease. He isolated the anaerobic bacteria from the ham and the spleen of one man who died. He grew it, named it, characterized its culture requirements and described its toxin (Scott, 2004). Botulinum is from the Latin *botulis*, meaning sausage, from the first incidences of botulism caused by sausages (Boni, Burg, and Kreydan, 2000).

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BOTULISM

Botulism is a rare disease with four naturally occurring syndromes: foodborne, airborne, infant and wound. The bacteria cannot penetrate unbroken skin, so it must be ingested, inhaled, or colonized in broken skin (Arnon et al, 2001). The most common form is foodborne botulism from improperly stored food containing the bacteria. The spores are resistant to heat and survive when foods are not processed correctly. Once ingested, the bacteria colonize in the GI tract and secrete BTX. Inhalation botulism is caught via airborne BTX that is inhaled and absorbed through the lungs. Wound botulism generally occurs in deep wounds associated with IV drug use. Infant botulism is caused by the ingestion on the bacteria spores that colonize in the GI tract and secrete toxin that is absorbed from the lumen. Infants have an immature gut physiology and an inadequate development of gut flora so they are particularly susceptible to infection (Sharma and Shukla, 2005).

Because of its toxicity, the use of botulism as a biological weapon presents a dangerous threat. A single gram of crystalized toxin, evenly dispersed and inhaled, could kill more than one million people (Arnon et al, 2001). However, because of its potency and unique method of action, BTX also has many therapeutic uses as will be discussed later (Sharma and Shukla, 2005).

BOTULINUM TOXIN (BTX) AND MECHANISM OF ACTION

There are seven distinct serotypes of C. botulism, A-G, that are characterized by immunological differences in their toxin (Sharma and Shukla, 2005). All toxins have a similar structure of a large single polypeptide and block the release of acetylcholine at the neuromuscular junction, causing acute flaccid muscle paralysis (Sobel, 2005).

BTX is synthesized as a single-chain polypeptide with a molecular weight of approximately 150kDa. The bacteria possess a protease that nicks the molecule to create a dichain structure, consisting of a heavy chain (100kDa) linked to a light chain (50kDa) by a disulfide bond. The dichain molecule is the active form of the toxin that blocks cholinergic transmission (Simpson, 2004). BTX is extremely potent because it is enzymatic (Arnon et al, 2001). The potency is remarkable because the toxin goes through a lengthy and complex chain of events until it reaches the action site, the peripheral cholinergic nerve endings (Simpson, 2004).

The toxin is released from the bacteria as part of a non-covalent complex together with auxiliary proteins. These auxiliary proteins have no meaningful role at the site of toxin action. Their function is to make the toxin resistant to the harsh conditions in the gut like low pH and proteolytic enzymes. Once the toxin enters the lumen of the gut or airway, it must cross membrane barriers to reach the general circulation. The toxin does not cause cell death; therefore, it does not kill the cells in its path. Rather, it uses transmembrane and transcellular processes to reach its target. A likely mechanism of action has been proposed: the toxin binds to the apical surface of the epithelial cells, undergoes receptor-mediated endocytosis and transcytosis and is delivered to the basolateral surface of the cells. The toxin can therefore reach blood and lymph (Simpson, 2004).

Once the toxin enters the general circulation, it needs to exit the vasculature to reach the extracellular space near the cholinergic nerve cells. There is no single study that describes this exit from the vasculature, but it could be an active transcellular process of para-cellular movement. Since BTX does not penetrate the blood-barrier, it has little ability to impair central cholinergic transmission of intact organisms (Simpson, 2004).

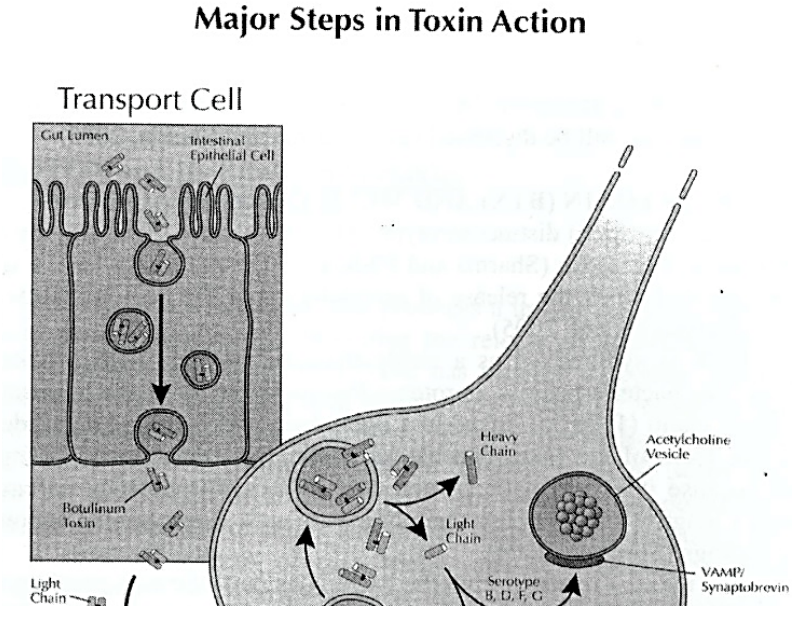
When the toxin reaches the peripheral cholinergic nerve endings, there is a sequence of membrane penetrating events. [Figure 1]

1. The toxin binds to the surface of the plasma membrane
2. This is followed by receptor mediated endocytosis and pH induced translocation across the endosome membrane
3. When the toxin reaches the cytosol, it acts as a zinc-dependent endoprotease to cleave polypeptides that are essential for exocytosis
4. This blockade of the transmitter release accounts for the flaccid paralysis that is characteristic of botulism. Without release of acetylcholine, the muscle cannot contract.

BTX acts preferentially on cholinergic nerve endings, but can block exocytosis from other nerve endings, such as norepinephrine and serotonin, when the concentration of the toxin is increased. While BTX can block exocytosis at all peripheral cholinergic sites, the neuromuscular junction has received the greatest amount of research and clinical attention (Simpson, 2004). No one has yet determined how the toxin is eliminated from the nerve endings. However, the nerve terminals regenerate slowly (Sobel, 2005), and new motor axon twigs sprout to reinervate the paralyzed muscle fibers (Arnon et al, 2001). This process takes several weeks or months, and in the United Staes, 96% of those affected eventually fully recover (Sobel, 2005).

Figure 1

Most of the steps in BTX action occur at two sites. Epithelial cells are transport cells; they bind the toxin and carry it from the lumen of the gut to interstitial fluid and general circulation. Peripheral cholinergic nerve endings like those at the neuromuscular junction are target cells for toxin action. BTX binds to these cells and once internalized into the cytosol attacks polypeptides that are essential for transmitter release (Simpson, 2004).



When BTX is exposed to striated skeletal muscle, it prevents the release of the acetylcholine (ACh) at the neuromuscular junction (Matarasso, 1998). ACh is the major neurotransmitter involved in parasympathetic nerve transmission at the post ganglionic synapse. In skeletal muscles, calcium activates the release of ACh from the presynaptic membrane into the synapse. ACh then binds to the nicotine receptors on the post synaptic membrane. When the nicotine receptors are activated by ACh, they allow transport of sodium and potassium ions across the post synaptic cell membrane. The entry of sodium into the cell causes depolarization of the cell membrane and generation of an endplate potential. The endplate potential initiates propagation of an action potential along the cell membrane of the skeletal muscle, ultimately causing skeletal muscle contraction (Boni, Burg, and Kreydan, 2000). [Figure 2]

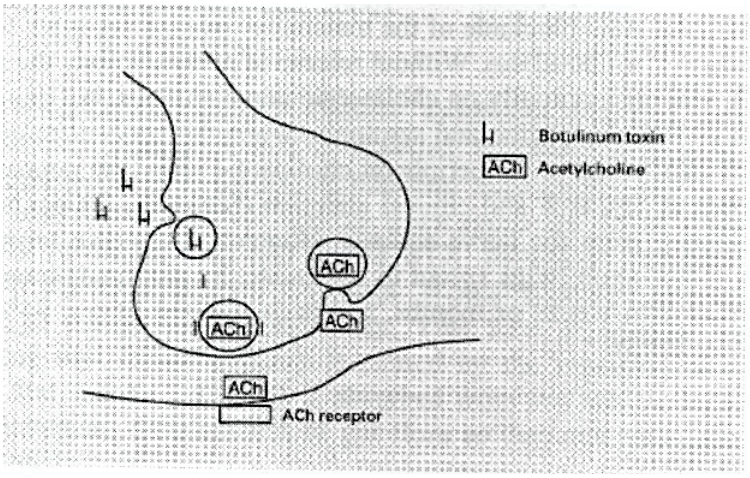


Figure 2

BTX is composed of a heavy chain and a light chain linked by disulfide bonds. Once internalized into the synapse, the light chain dissociates and cleaves the target protein, thus blocking ACh release (Boni et al, 2000).

The receptor at the neuromuscular junction has not been unequivocally identified. The major distinction between serotype A and serotype B are that they attack different substrate. Each serotype cleaves only one peptide bond in its substrate even though the sequence may be repeated in the substrate. Serotypes C and E are similar to serotype A and types D and F are similar to type B. (Simpson, 2004). BTX-A cleaves to a 25-kd synaptosome associated protein (SNAP-25) and BTX-B cleaves to a vesicle-associated membrane protein (VAMP or synaptobrevin) (Carruthers and Carruthers, 2005). Although they both block ACh release, BTX-A blocks the frequency of the spontaneous release by greater magnitude than BTX-B (Simpson,

2004). This difference may be responsible for differences in clinical effects of the two toxins, as discussed later (Carruthers and Carruthers, 2005).

HISTORY OF THE THERAPUETIC AND COSMETIC USES OF BTX

BTX is also used therapeutically and cosmetically. When injected, it produces a temporary chemo-denervation of muscle, resulting in a localized reduction of muscle activity (Carruthers and Carruthers, 2005). When used to treat conditions where there is excessive and uncontrolled cholinergic nerve activity, BTX causes temporary relaxation of muscle where normal function is attained (Simpson, 2004). These potential uses for BTX were not realized until the 1970s, long after it was considered a biological terror threat. In the 1940s, Drachman of Johns Hopkins used small doses of toxin to paralyze the hind limbs of chicks. At that time, alternative therapies were being sought to treat strabismus, such as injections of different anesthetics, alcohols and snake neurotoxins. Techniques to accurately inject extraocular muscle with local anesthetics to determine their function in eye movement had already been developed (Scott, 2004).

The first experimentation on humans began in the 1960s and gained acceptance by the 1990s. Scott investigated therapeutic uses of the toxin first in monkeys and then on humans with strabismus and blepharospasm (Cote et al, 2005). A few picograms of the toxin injected into the target muscle induced a paralysis confined to that muscle with long duration and no side effects. By 1982, the eye muscles were injected for retraction, hemi-facial spasm, blepharospasms and the limbs and neck for dystonia. At first there was a strong aversion to using BTX for these indications, but as there was no adequate alternatives to treat many motility disorders, treatment became more popular (Scott, 2004). In 1989, the FDA approved using BTX-A for treating strabismus and blepharospasm and in 2000, expanded approval to include cervical dystonia (Cote et al, 2005). Other conditions were also treated with BTX in the mid-1990s, such as hyperhidrosis and Frey's syndrome (Scott, 2004).

Cosmetic use of BTX was pioneered by Alistair and Jean Carruthers. Many patients treated for blepharospasm would joke at their 3 and 4 month follow up visits that they were there to "get the wrinkles back out" (Scott, 2004). Carruthers first tested BTX to treat glabellar lines in 1992 and later tested it on selected facial muscles to lift the brow and flatten folds (Carruthers, 2005). In 2002, the FDA approved BTX-A for cosmetic use for temporary improvement to the appearance of glabellar lines. These lines are the only FDA approved rhtyids for BTX treatment, but many physicians use it for off-label purposes as well. The most common injection sites are the glabellar frown lines, crow's feet, wrinkles, the forehead and neck bands (Cote et al, 2005).

BOTOX

BTX-A is available in two formulas: from Allergan in the United States and Canada as Botox, and as Dysport in Britain, France, and Germany. BTX-A is sold in a lyophilized form and needs to be reconstituted with saline before use. BTX-B is available as Myobloc only in the Unites States and is sold in an aqueous solution. The dosages of all three vary greatly and one needs to make sure that the correct dosage for the specific product is used as well as adhering to the manufacturing guidelines (Mantell, 2004). All treatments are temporary and serial doses are needed to maintain the desired results (Mendez-Eastmen, 2000).

Botox is available through Allergen and the toxin is supplied in a 100-unit multi-dosed crystallized complex. The crystalline form must be stored in the freezer at temperatures of -5°C or lower and should be reconstituted immediately before injection (Mendez-Eastmen, 2000). Both Dysport and Botox are reconstituted either with preservative free solutions or with normal saline. When reconstituted with preservative free solutions, the formula should be used within 4 hours, but normal saline retains its potency when refrigerated for 4 weeks. The preservative free saline is refrigerated at 2°-8°C for a maximum of 4 hours to ensure it remains sterile. Many physicians use the reconstituted form 4 hours after reconstitution, but the toxin could denature and jeopardize its sterility (Mendez-Eastmen, 2000). Many patients reported less pain with the injection after using a saline containing preservative. Higher dosages in smaller volume keeps the toxin and its effects more localized and allows for more precise placement and little spread (Mantell, 2004). The mode of measuring toxin strength is paralytic activity in the mouse. One unit is the amount of toxin that kills 50% of a standardized mouse model when injected intraperitoneally (Klein, 2004). Patients can be treated in a sitting or lying position, but sitting is recommended to avoid the toxin spreading to underlying muscles (Boni, Burg, and Kreydan, 2000). The administering physician needs to understand the relevant facial anatomy and only a physician should administer the injection. Local anesthesia is unnecessary. Patients can resume normal activity post injection and may take Tylenol if they experience pain. Nonsteroidal anti-inflammatory agents should be avoided for 7 days before injection as it could exacerbate the symptoms of side effects (Klein, 2004). The FDA approved treatment only for glabellar lines, but Botox is often used for off-label uses as well. The decision to inject Botox in an off-label site should be the physician's and he should follow the recommended dosage and be careful not to give too large of a dose (Mendez-Eastmen, 2000).

Dysport, also from BTX-A, is produced via fermentation and then recovered and dissolved in aqueous solution. It is provided as an air dried powder and is reconstituted and diluted with normal saline before use, similar to Botox. It is available in the UK and used therapeutically for blepharospams, torticollis, pediatric cerebral palsy spacity as well as for cosmetic purposes to treat facial lines. It is not directly comparable to Botox, but there is a ratio of about 1:3 or 1:4 units of Botox to Dysport (Markey, 2004). Myobloc, BTX-B, has the FDA approval to treat cervical dystonia. It is sold in a highly purified liquid formulation from fermented BTX-B. It contains very little protein or inactive toxin because this could increase the risk of antibody formation. It is slightly acidic with a pH of 5.6, so a painful, burning sensation is experienced upon injection. There is no dose determined for cosmetic uses, but a ratio of about 1:125 units of Botox to Myobloc is used (Flynn, 2004).

There are several differences between BTX-A and BTX-B. BTX-B has an increased radius of diffusion than Botox, creating a more uniform effect. The rate on onset of Myobloc is also slightly faster, by about a day. Duration of effect is does dependent, but studies comparing the two show that Myobloc has a shorter effect than Botox. Additionally, Myobloc is very stable even at room temperature because it is not reconstituted (Flynn, 2004).

Injection into the correct part of the muscle is important to achieve maximum benefits. The toxin works best when injected into the muscle belly and are usually not superficial because it easily penetrates muscle (Klein, 2004). Injection by EMG (electromyogrpahic) guidance helps achieve accurate placement by locating the most active part of the muscle responsible for a

particular facial line. It determines which muscles are contracting and contributing to the frown. A combines EMG injection needle is used which requires a larger needle. However, some physicians feel there is no benefit because the anatomy of the glabella is so reliable. Some use the EMG needle only for reinjection, but perhaps reinjection would not be necessary if one was used initially. Additionally, the pattern of muscle activity varies greatly from patient to patient (Mantell, 2004). When EMG injection is used, the area is cleaned with alcohol and allowed to evaporate completely. This is because the alcohol can denature the toxin. The patient then frowns, squints and raises the eyebrows to activate the targeted muscles. A needle is connected to the EMG unit is inserted. After placement, when the muscle is activated, a sound should be heard from the EMG machine. The needle is reinserted if no sound is heard (Mantell, 2004).

FACIAL RHYTIDS

Lines and wrinkles in the face are caused by muscle action and contraction. Treatments such as surgery and implants are only partially effective as they do not address the underlying cause of excessive lines, glabellar lines and crow's feet (Boni, Burg, and Kreydan, 2000). [Figure 3]

Frown lines are caused by the contraction of the frontalis muscle (Boni, Burg, and Kreydan, 2000). The frontalis is the elevator of the brow. This thin muscle covers a large portion of the forehead and is not attached to bone. The action of this muscle is to elevate the brow and wrinkle the forehead. The horizontal creases on the forehead are a direct result of the frontalis action (Vigliante, 2005). 50-60 units of Botox are used to erase these creases (Matarasso, 1998).

Glabellar frown lines are caused by the contraction of the musculus corrugator superciliaris at the medial end of the eyebrow (Boni, Burg, and Kreydan, 2000). This is a deep muscle located against the bone beneath the frontalis and orbicularis oculi muscles. The action draws the eyebrows medially downward, producing vertical glabellar wrinkles. These lines give the characteristic appearance of anger, frustration and negative emotions. Known as the "frowning muscle," it is the principle muscle used in the expression of suffering (Vigliante, 2005). 30-40 units are used to treat the lines (Matarasso, 1998). This muscle is usually stronger in men, so a slightly higher dose is necessary (Boni, Burg, and Kreydan, 2000).

The procerus muscle marks a horizontal groove at the base of the nose (Boni, Burg, and Kreydan, 2000). The primary action of this muscle is to draw the medial angle of the eyebrows which creates the transverse wrinkles at the bridge of the nose (Vigliante, 2005). Generally 30-40 units are needed to improve this furrow (Matarasso, 1998).

The squeezing action of the musculus orbicular oculi causes crow's feet, deep horizontal and oblique furrows at the temporal aspect of each eye (Boni, Burg, and Kreydan, 2000). This muscle a broad, flat muscle, and is the chief muscle surrounding the orbit. Is it the depressor of the brow and eyelid and forceful contraction induces concentric folds. In childhood, these lines are seen only in dynamic situations, such as laughter or squinting in the sunlight. However, in adulthood they are visible even in facial repose. These lines increase with years of sun exposure and dynamic expression (Vigliante, 2005). 20-30 units are needed to treat them (Matarasso, 1998).

The platysma, a broad sheet of muscle, is located in the neck, but is primary function is on the face

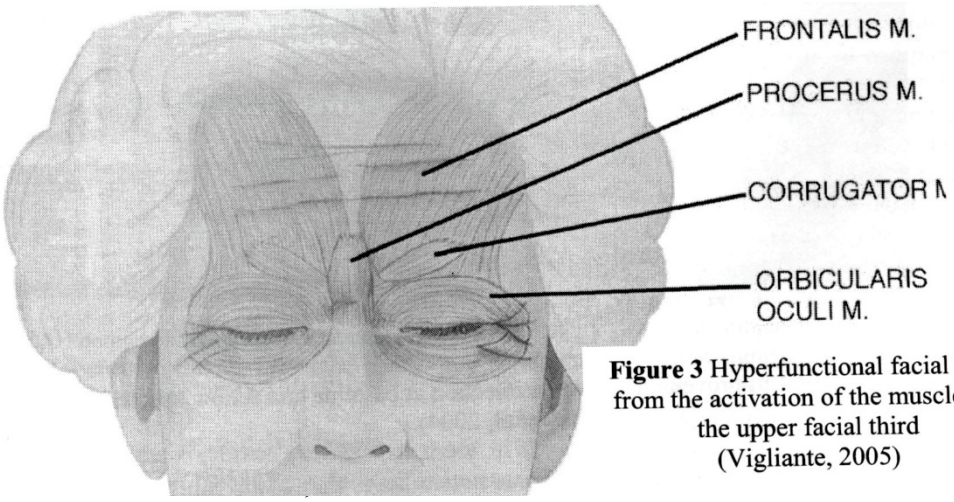


Figure 3 Hyperfunctional facial lines from the activation of the muscles of the upper facial third (Vigliante, 2005)

and mand the mandible, increasing the diameter of the neck and causing hyper-functional bands in the neck. The muscle also acts to draw the lower lip and corner of the mouth laterally and inferiorly, partially opening the mouth as in an expression of surprise or horror (Vigliante, 2005). These bands can be treated with multiple injections (Matarasso, 1998).

CASE STUDIES

THE EFFECTS OF BOTOX ON CROW'S FEET

As mentioned above, repetitive contraction of underlying muscles and their action on the skin produce wrinkles on non-fatty facial areas. Jean Luc Levy, Jean-Jacques Servant and Elisabeth Jouve of France conducted and experiment to determine the duration of a defined dose of BTX-A on crow's feet wrinkles. An objective evaluation of duration of the results of a single injection was provided. The treatment area was the crow feet wrinkles and adults with bilateral symmetric crow's feet were eligible, but those patients who had undergone prior cosmetic surgery, had health conditions, were breastfeeding or were potentially pregnant were excluded. The study included 25 female patients from age 31-65, with a mean age of 48. They were injected at the baseline with 12 units of BTX-A and evaluated at 3, 6 and 9 month intervals. Clinical photographs were taken at the baseline and during the follow up visits with the eyes closed at rest. Patients were positioned in front of the VisioFace special measurement bench with a 3D sensor. 3D microtopography of the skin was recorded with a DermaTop optical 3D in vivo scanner (Levy et al, 2004).

Patients were asked to evaluate their photographs at baseline (T0) and 9 months (T3) to score wrinkle reduction. Observers assessed post treatment and pretreatment photographs. They compared the photographs from the baseline with those from 3 (T1), 6 (T2) and 9 (T3) months after the injection. The result from these assessments by the patients, the 3D profilometry and the independent observers all were similar and showed improvement. Only one patients had a side effect of edema of the lower lid for a month following treatment. The conclusion from this study was that BTX-A is a safe and effective method for treating crow's feet with clear improvement shown at 6 months (Levy et al, 2004). [Figure 4]

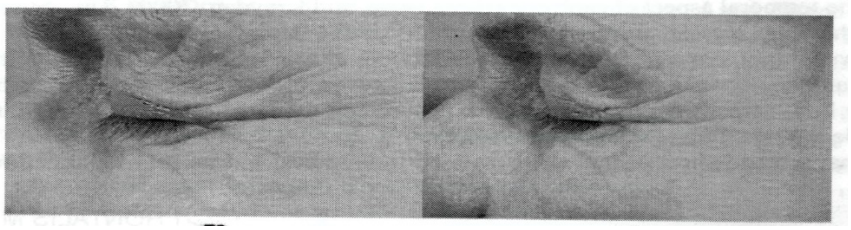


Figure 4: Improvement after one injection at baseline and follow up visits (Levy et al, 2004).

Although the study group for this trial was not so large, the results can be considered objective because of the impartial observers and 3D photography. While the results were positive with few reports of side effects, one should remember that the Crow's feet wrinkles are not a FDA approved injection site.

COMPARING THE EFFECT OF BOTOX AND OTC CREAMS ON GLABELLAR RHYTIDS

Another experiment was conducted by Kenneth Beer to compare the safety and efficacy of BTX-A with topical creams for treating moderate to severe glabellar rhytids. These lines are the only conditions approved by the FDA for Botox treatment. These over the counter (OTC) topical products advertise that they are more effective at treating wrinkles than Botox. This study, a single-center, randomized, investigator blinded parallel study consisted of five treatment groups: Botox, a placebo saline injection, StriVectin-SD, Wrinkle Relax and HydroDerm. Females with clinically diagnosed moderate to severe glabellar lines at maximum frown from the ages of 18-65 were eligible for participation. Criteria for exclusion were pregnancy, previous eyebrow surgery, use of retinoids, hydroxyl acids or products containing vitamins A, C and E, 77 participants were randomly placed into one of the treatment groups. Frowns were assessed both by the patients and a principle investigator, and photographs were taken of the glabellar region of each subject at maximum frown and rest. The first phase was a masked phase with follow up visits at 4, 8 and 12 week intervals. This was followed by an open-label phase where all subjects received open-label Botox injections with a 4 week post injection follow up (Beer, 2006).

All participants were instructed to maintain their standard facial care for the duration of the study. Those subjects in the placebo and Botox groups received injections by a staff member rather than the investigator to maintain the blind. Those assigned to use the topical creams were told to apply it in the morning and evening, massaging the cream gently over the bridge of the nose and eyebrows until absorbed completely. At baseline and each follow up visit, subjects assessed the change in appearance of the wrinkles, the investigator assessed them and photographs were taken. Patients also completed a facial line outcomes (FLO) questionnaire related to self-perception. The primary efficacy measure was the investigator's assessment of glabellar lines at maximum contraction at the follow up visits. Secondary was the subjects'

assessment. Botox treatment resulted in significantly reduced wrinkle severity over the topical creams and placebo. Subjects who received Botox treatment were also more satisfied with the treatment results than those in the other groups and 90% were satisfied with treatment during the open-label phase. Side effects were only reported by 3 participants in the StriVectin-SD group (Beer, 2006). [Figure 5]

This trial was the first that compared the efficacy of Botox to OTC topical creams. The results indicated do not support the advertising claims made by manufacturers of these creams. While they may be a more economic choice, their treatments of hyperfunctional lines were not very different than the placebo. However, the study was not long enough to see the long term effects and duration of treatment. Additionally, the investigator is associated with Allergen, the company that manufactures Botox.

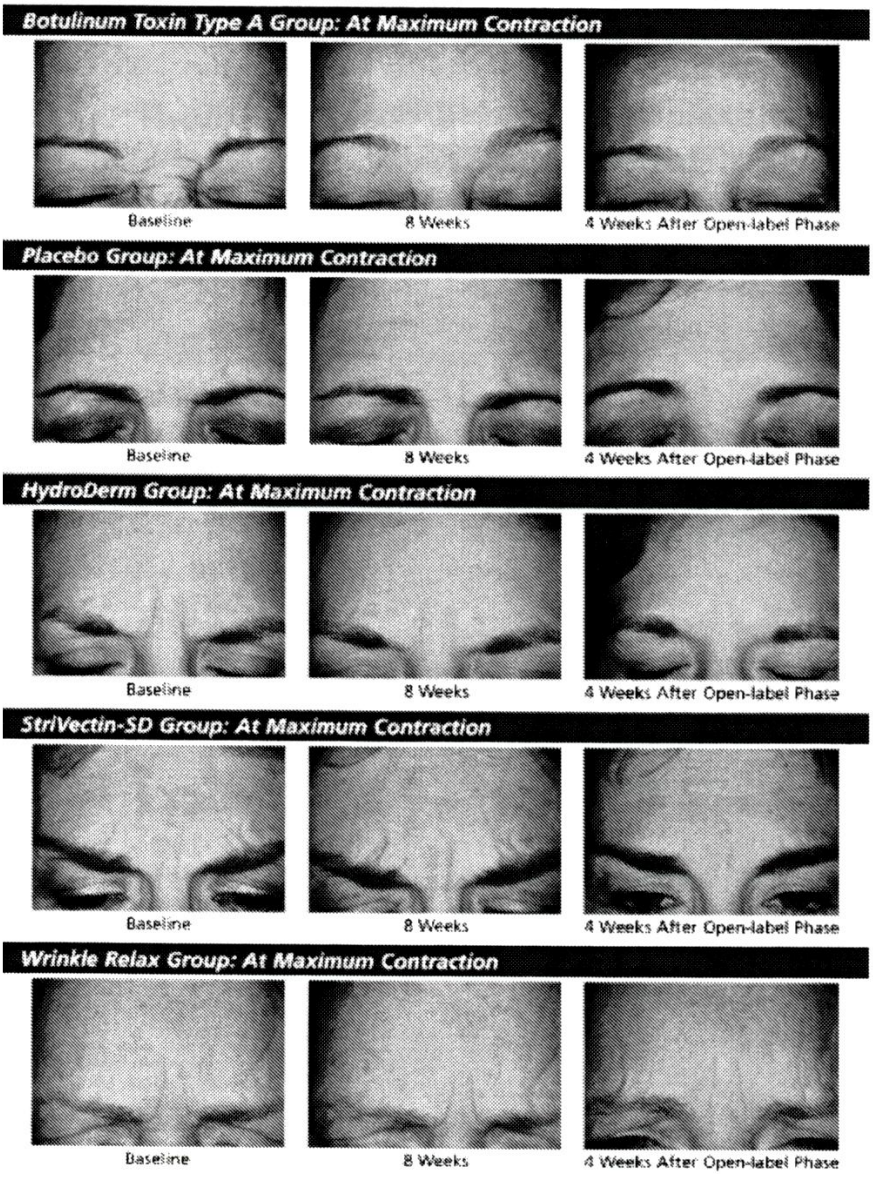


Figure 5: Photographs comparing treatment at baseline, 8 weeks and after the open-label phase. (Beer, 2006)

COMPARISON OF BOTOX AND DYSPORT ON GLABELLAR LINES

There has been little research directly comparing the two formulations of BTX-A which may behave differently, Botox (BTX-A1) and Dysport (BTX-A2). The results and dosages of both formulations are different and cannot be compared. Phillipa Lowe, Rickie Patniak and Nicholas Lowe designed a study to compare the results of both formulations. Patients between 18-55 years of age with moderate to severe glabellar lines at maximum contraction as determined by an investigator were eligible to enroll. Women of childbearing age were required to have a negative urine pregnancy test result. Criteria for exclusion included: facial cosmetic surgery planned during the study, visible scars, prior cosmetic surgery that could interfere with evaluation, history of facial nerve palsy, alcohol abuse, infection at the injection site. 62 patients were randomly assigned to the two groups (59 completed the study). The mean age was 41 and they were predominately Caucasian and female. The mean age of the Botox group was higher, but there were no other major demographic differences (Lowe et al, 2006).

The BTX-A1 group received a dose of 20 units (the FDA approved dose) and the BTX-A2 received a dose of 50 units (reported to be the optimal dose). The dose was divided between 5 injection points. Patients could continue their normal face care regimen, but should not apply creams for 4 hours before the follow up visits. Patients were photographed using the modified

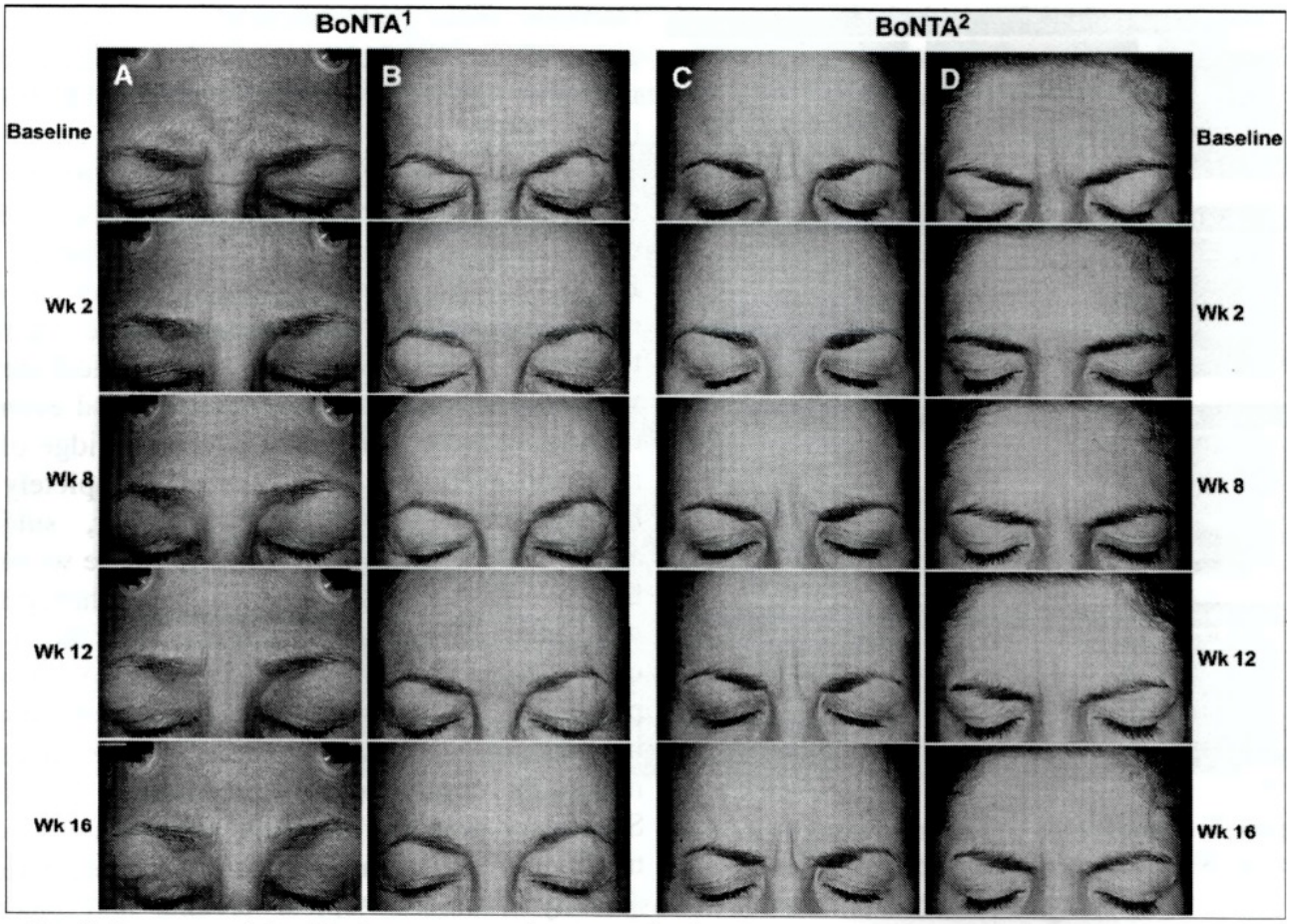


Figure 6: More prolonged duration of effect at week 16 with BTX-A1 (BoNTA1) than with BTX-A2 (BoNTA2) (Lowe et al, 2006).

Canfield system during maximum contraction at each visit. Severity of the lines was graded as none, mild, moderate or severe. The primary outcome measure was improvement of at least 1-grade at week 16. Other outcome measures included patients whose lines were graded as none or mild and incidence of relapse. Using masked assessment of the standard photographs demonstrated that BTX-A1 offers a more prolonged efficacy than BTX- A2. Both groups peaked at week 8 for at least a 1-grade improvement. However, the duration of improvement was generally more prolonged with Botox than with Dysport at week 16. Patient satisfaction was also higher with Botox than with Dysport. Both products were well tolerated and there was no difference in treatment related adverse effects between the two groups (Lowe et al, 2006). [Figure 6]

This study was supported by an unrestricted research grant from Allergen. Furthermore, the age difference between the two groups is considerably significant as younger patients have stronger muscles and may require a higher dosage. While Botox may have a longer duration than Dyspoty, both formulas appear to be effective. Costs cannot be compared because Dysport is not available in the US and Botox is not in the UK.

SIDE EFFECTS AND COMPLICATIONS WITH BTX-A TREATMENT

Botox is a safe, effective treatment without serious side effects. When it is used properly, the complications are a few with mild severity. No irreversible clinical effects have been reported from cosmetic uses. Different treatment areas are associated with different side effects. The most common complication is ptosis of the upper eyelid from injection into the glabellar area, but this could be resolved with eye drops. Eyelid ptosis can be avoided with more accurate placement and lower volume. The most significant complication from treating the frontalis is brow ptosis. The brow shape may also be changed because the muscle responsible for brow elevation is relaxed. To avoid this, the forehead and glabella should not be treated in the same session. Furthermore, the boundaries of the forehead should be defined and injections should not be above the middle brow. This treatment also works best on younger patients (20-45 years of age) and older ones should be injected more cautiously. Reported complications from treatment of Crow's feet include diplopia, drooping lateral lower eyelid and an asymmetric smile from injections placed too low, and this takes longer to resolve than lid ptosis. Strabismus is a very rare side effect, and the patient should be referred to an ophthalmologist for appropriate care. High doses injected into the platysma can produce weakness and dysphagia. Patients with severe over treatment of the neck can have trouble holding their necks erect because the muscles are so weakened. Therefore, injection in any area should be administered carefully in the correct dosage, in the appropriate location and by an experiences physician. (Klein, 2004).

As part of its mission to improve patient and consumer safety, in 2005, the FDA funded Cote et al (2005) to conduct a study reviewing the adverse effects (AE) reported to the FDA after BTX-A treatment. The FDA received reports of AEs through the MedWatch system. Although clinicians are encouraged to report incidences of AEs, it is voluntary, so the number reported is actually only a subset if the incidences that occur. It is very difficult to accurately classify the

AEs into serious and non-serious reports as MedWatch often lacks complete information. AEs that met the US Code of Federal Regulations 600.80 such as: "death, a life threatening adverse drug experience, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapability, or a congenital anomaly/birth defect" were categorized as serious. All other were referred to as non-serious (Cote et al, 2005).

Cote et al (2005) attempted to review all reported cases, both from therapeutic and cosmetic use. Serious AEs were reviewed from December 1989 and May 2003. Due to the large number of non-serious reports, the review was limited from December 2001 to November 2002. 1437 AEs were reviewed, 406 from therapeutic cases and 1031 in cosmetic instances. The proportion of reports classified as serious was higher for therapeutic cases than those cosmetic uses. 217 serious AEs were reported for therapeutic cases that included 28 deaths and 17 seizures. For cosmetic cases, there were 36 serious reports and no deaths. The AEs reported were both known and unknown and for both on-label and off-label uses. The most common reactions were: dysphagia, muscle weakness, allergic reactions, flu-like syndromes, injection site trauma, arrhythmia and myocardial infarction. Non-serious reactions included ptosis and headaches for cosmetic uses. The AEs reported for therapeutic uses were different than those for cosmetic uses since doses are higher for therapeutic purposes and patients have serious underlying diseases that increase the risk of an adverse reaction. Many of the AEs in cosmetic cases were related to improper technique, storage or dilution, higher doses than recommended and injection in sites that are not FDA approved (Cote et al, 2005).

As in any treatment, there are always side effects involved. However, when used properly as advised in the approved labeling, the AEs experienced are generally non-serious and plausible as BTX blocks muscle contraction, so ptosis and dysphagia are likely. Incidences of AEs also decrease when the optimal dose of 20 units is used (Cote et al, 2005).

There is the possibility of developing resistance to BTX because of antibodies produced against the toxin. The likelihood of developing immunoresistance is associated with high doses and greater frequency of treatment (Klein, 2004). All BTX preparations (Botox, Dysport, Myobloc) have complex mixtures of various proteins and excipients such as human serum albumin, lactose, NaCI or buffers. Theoretically, all non-human proteins in the mixture can act as antigens to stimulate formation of antibodies. The antibodies can be formed against the toxin or non-toxin. Only the antibodies against the toxin interfere with the activity of the toxin and there may be partial or complete therapy failure (Dressler and Hallett, 2006). The newer batches of Botox have a decreased amount of protein so chances of antibody production are reduced. The only effect of these antibodies is that BTX-A is no longer effective, but no hypersensitive reaction develop (Klein, 2004). Patients who develop antibodies against BTX-A can be treated with BTX-B as an alternative since the antibodies are antigen specific (Scott, 2004).

CONCLUSION

Botox is a safe, effective and minimally invasive procedure used to reduce the appearance of hyper-functional facial lines. Although it successfully treats a large part of facial lines, the only FDA approved injection site is the glabellar frown lines. There are still ongoing trials to determine the safest and most optimal dosage for the longest duration of effect, but until then, serial injections are needed to maintain the results. Caution should be used with repetitive injections as this increases the risk of side effects and developing immunoresistance. Individuals

should understand the risks involved with Botox injection and consult a physician to determine if this procedure is right for them.

SUMMARY

As this paper has shown, Botox is an effective treatment to temporarily reduce the appearance of facial wrinkles. It is processed from diluted bacterial exotoxin, BTX. BTX acts on the peripheral cholinergic nerve endings to inhibit acetylcholine release and induce flaccid muscle paralysis. Botox uses this same method to temporarily relax targeted muscles in the face responsible for wrinkles. Numerous case studies done over the past several years have proven that Botox is an effective, albeit temporary, alternative to reconstructive surgery to treat and reduce the appearance of these lines. As with any treatment, there are always risk factors involved. By adhering to the recommended FDA guidelines of storage, dosage and procedure, many of these risks are avoided. Patients who opt to receive Botox injections should make sure to choose an experienced physician to ensure a safe and quality result.

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The Effect of Exercise on Alzheimer's Disease

Benjamin Korman

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease, from which there is no recovery. It begins with impaired memory and judgement and progresses to the point where those affected can no longer care themselves. Although the cause of AD is unknown, two significant abnormalities occur in the brain of its victims: neurofibrillary tangles and amyloid plaques. It has been well established that exercise improves mood and general well-being, however this paper will focus on the effect of exercise on AD. It will show that exercise can improve physical functioning of an individual with AD, however more importantly it will focus on how exercise can prevent and/or delay the onset and progression of AD. In addition we will discuss how much exercise is necessary to reduce the risk of AD, despite there being no established exercise prescription at this time.

INTRODUCTION

Alzheimer's disease (AD), the 4th leading cause of death, affects 4.5 million people in the United States and is expected to increase to 13.2 million by the year 2050. AD is a progressive neurodegenerative disease, from which there is no recovery. It begins with impaired memory and judgment and progresses to the point where those affected can no longer care for themselves. (Desai, 2005).

Although the cause of AD is unknown, research has found that two significant abnormalities occur in the brain of its victims: neurofibrillary tangles and amyloid plaques. Neurofibrillary tangles are found in the cytoplasm of abnormal neurons. They consist of twisted nerve cell fibers, which result from the alteration of Tau, a protein which helps support nerve cell structure. These tangles are resistant to chemical or enzymatic breakdown and remain in the brain tissue even after the neuron it began in has died or disappeared (Porth, 2004).

Amyloid plaques are sticky patches formed by insoluble proteins-Beta amyloid-surrounded by the debris of dying nerve cells. These plaques are found in areas of the cerebral cortex associated with intellectual function. Elevated levels of beta amyloids are also associated with a decrease in the enzyme choline acetyltransferase which is required for the synthesis of acetylcholine, a neurotransmitter associated with memory (Porth, 2004).

Initial clinical manifestations of AD are attributed to forgetfulness, emotional upset, or other illness. The individual becomes progressively more forgetful over time, particularly in relation to recent events. Memory loss increase as the disorder advances and the individual becomes disoriented and confused. The ability to concentrate declines. Abstraction, problem solving, and judgement gradually deteriorate. These mental status changes induce behavioral changes, including: irritability, agitation, and restlessness. The individual may become anxious,

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depressed, hostile, emotionally labile, and prone to mood swings (Porth, 2004).

BENEFITS OF EXERCISE

Research has shown that regular physical activity, regardless of the sex or age of the participant, has multiple beneficial effects, including but not limited to, decreasing: mortality rates, coronary artery disease, risk of colon cancer, diabetes, risk of obesity, risk of hypertension, as well as increasing mood and reducing depression (Centers for Disease Control and Prevention, 2003). Exercise is especially important for those with AD as many studies have associated this disease with physical deterioration such as malnutrition, higher risk for falls and fractures and rapid decline in mobility, compared to aged matched controls (Teri et al, 2003). Studies (Arkin, 2003, Rolland et al., 2007) have found that individuals with AD who participated in exercise sessions of one hour twice a week, showed significant improvement in aerobic fitness, duration and upper and lower body strength, as well as slower physical decline.

One study (Teri et al, 2003) looked at the effects of exercise on physical performance for those with a diagnosis of AD. Patients in this study were either assigned to a combined exercise and caregiver training program or to routine medical care. Three months into the program patients in the exercise group showed improved scores for physical role functioning compared to the routine medical care group whose scores actually declined. At 2 years, the exercise group continued to show better physical role functioning scores compared to the routine medical care patients. These studies prove that although a person has a diagnosis of AD, it is possible to progress on a physical level. This is improvement can be beneficial for those caring for an individual with AD, as it enables the person to remain more physically independent and less dependent on the caregiver.

PSYCHOLOGICAL BENEFITS OF EXERCISE

Most research done on the psychological benefits of exercise conclude that exercise improves mood, and reduces anxiety and depression (Hassmen et al., 2000). Palmer, 2005, Sarbadhikari et al 2006). During exercise endorphins are released into the bloodstream and bind

to the opioid receptors in the brain. They have an antagonistic effect on the receptors and therefore block the release the neurotransmitter molecules from the nerve terminal thereby blocking the signals of pain (Porth, 2004). The endorphin effect also causes a feeling of euphoria, reduces anxiety, tension, anger and confusion, all of which are present in individuals with AD.

It is of greater benefit to those with AD is repetitive exercises, such as walking, indoor bicycling, and activities such as folding laundry are encouraged. These exercises may decrease anxiety as there is no need for decision making or recalling what task comes next. In addition, it channels a tendency for restlessness and wandering, which are characteristics of the disease, into a beneficial activity.

Teri et al. (2003), which was previously discussed for it study on physical progression, also looked at how exercise affected the patients psychologically. It was found that at three months, patients in the exercise group had improved Cornell Depression Scale for Depression in Dementia (designed for assessing depression in elderly residents with dementia) scores and showed a trend for less institutionalization due to behavioral disturbance. For patients with higher depression scores at baseline, those in the exercise group showed significant improvement at 3 months on the Hamilton Depression Rating Scale (test measuring the severity of depressive symptoms in individuals) and maintained that improvement at 24 months. Those in the routine medical care group showed higher depression ratings and continued to show steady decline.

ROLE OF EXERCISE IN THE DELAY/PREVNTION OF AD

Over the past decade, a number of studies have shown the benefits of exercise on brain health and function, particularly in aging populations. Exercise participation has consistently emerged as a key indicator of improved cognitive function and lower risk of cognitive impairment, AD and dementia in general (Cotman 2002).

Smith & Friedland (1998) retrospectively examined the exercise habits of 373 people-126 with Alzheimer's and 247 healthy people. They found that individuals with AD has lower levels of physical activity earlier in life. More recent studies (Wang et al., 2006) followed a larger group of people (2,288) over the age of 65 for 6 years and came to the same conclusion. Lower levels of physical performance were associated with an increased risk of dementia and AD, whereas higher levels of physical function appeared to play a protective role and delayed the onset of AD.

An interesting observation was also made by Wang et al (2006), which may help predict AD before any cognitive signs actually appear. Researchers observed that among subjects without apparent cognitive deficits, those with poor balance, gait disturbance and poor handgrip were more likely to develop dementia, which is a form of AD. Therefore, the study suggests that a simple way of predicting Alzheimer's risk in the future might be to test how an elderly person walks, the strength of their grip, and their level of balance when standing.

Exercise may also delay or prevent AD, as it has been shown to decrease the amyloid plaques in the brain, as suggested by a recent study (Aldard et al., 2005). This study used TgCRND8 mice, transgenic for the human amyloid precursor protein, to directly examine the interaction between exercise and AD. The study found that five months of voluntary exercise resulted in a delay of the progression of AD, as evidenced by a decrease in beta amyloid plaques

in the frontal cortex, the cortex at the level of the hippocampus and the hippocampus, the brain region central to learning and memory.

Lifestyle changes are key to slowing the onset and progression of AD. The good news about this is that it might not be too late for those who have lived sedentary lifestyles. Lifestyle changes do not necessarily have to occur in the early years of life in order to see the benefits of exercise on AD, as observed by Rovio et al. (2005). This study looked at the long term association between midlife (mean age of 50 years) leisure-time physical activity and risk of AD. Upon follow up (mean age 71.6 years), it was found that individuals who participated in a leisure-time physical activity at least twice a week had 60% less chance of developing AD compared with sedentary people, even after adjusting for a wide array of potential factors.

There are several possible ways in which physical activity may protect against AD. First, the effect could be mediated through various vascular risk factors (e.g., hypertension, hypercholesterolemia, diabetes, obesity) that have been found to contribute to the development of AD. Physical activity is important in promoting overall and vascular health. There may also be several neurobiological mechanisms linking leisure-time physical activity to AD. Recent studies have indicated that physical activity affects several gene transcripts and neurotrophic factors that are important for the maintenance of cognitive functions. (Rovio et al., 2005, Sarbadhikari et al. 2006).

These findings are supported by animal research which demonstrates that exercise can increase neuronal survival and resistance to brain insult, promote brain vascularization, stimulate neurogenesis, enhance learning and contribute to maintenance of cognitive function during aging (Cotman, 2002).

Although many studies have shown the benefits of exercise on AD, no exercise prescription has yet to be established. Based on the research articles obtained, this paragraph will review the findings of how much, or rather how little, exercise is necessary to prevent or delay AD.

It was found that 15 minutes of moderate exercise 3 times a week was the least amount of exercise required to show a beneficial effect on AD (Larson et al, 2006). Exercise in this study reduced the risk of developing dementia by 30%. The study suggests that even a short brisk walk ever day might ward off the disease. Rovio et al (2005) reported that 20 minutes of exercise that caused sweating and strained breath, biweekly reduced the risk of AD by 60%. The most common forms of exercise participated in during this study was walking and cycling. Stevens et al. (2006) had his subjects exercise for 30 minutes three times a week for a total of 12 weeks and found that exercise slowed the rate of cognitive decline as well as physical decline related to dementia.

In a Harvard study completed in 2004, women who walked at a pace of 21-30 minutes per mile for two to three hours a week did better on cognitive tests than inactive women. To get the same benefits, it recommends: walking 1-2 hours each week at a pace of 15 minutes per mile, bike, and swim laps or play tennis for 1 hour each week, or jog for 30 minutes- 1 hour each week at a pace of 10 minutes per mile. Studies show that as the amount of time spent exercising increased the protective effect of exercise increased proportionately (Larson et al., 2006).

CONCLUSION

From this paper we can conclude that exercise plays a significant role in preventing or delaying Alzheimer's disease. However, if this conclusion is so true, why don't we have a set exercise prescription and why isn't the public aware of this? Could it be that some of these studies have somewhat of a subjective nature to them that make the validity questionable?

The problem that may arise with some studies is that measurements of exercise are self-reported. Everybody knows that overall exercise is good for you and that in order to maintain our health we are supposed to partake in it. This fact may influence some people to exaggerate about how much exercise they partake in so that they don't look bad to the public eye. In addition, what may be considered moderate exercise for one person may be high or low intensity for the next. Moderate exercise should be a defined and measurable factor so it can be reproduced to get the same beneficial outcome.

Despite all this, exercise still remains an important part of a healthy physical and psychological lifestyle. Even if the accuracy of some of these studies are in question, overall, there were too many positive outcomes for those with AD to disregard it. Therefore, let us continue exercising our way to a sound mind and body.

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Gestational Diabetes and Its Effects on the Fetus

Todd Pollack

ABSTRACT

Although the common effects of gestational diabetes on the fetus are known, the outcome of a diabetic gestation is ambiguous. There is concern for complications in the fetus at delivery due to enlarged size of the fetus, as well as concerns for birth defects, fetal distress, diabetes and obesity. Yet, although there is a correlation in the previous disorders and gestational diabetes, many of the mechanisms that cause these complications are unknown. By studying the pathology of gestational diabetes, researchers have learnt that the placenta controls fetal growth and provides a great deal of protection for the fetus. Therefore, many of the effects of diabetes are masked by the placenta. The problems that arise from gestational diabetes are often the result of a subtle disturbance of the fetuses' metabolism or from some obstruction of the placenta's ability to protect the fetus. Therefore, to properly understand the effects of gestational diabetes, it is necessary to know not only the effects of diabetes but also the mechanics of the placenta, the changes it undergoes during gestational diabetes and the effects of these changes on the fetus.

INTRODUCTION

Gestational diabetes is a complex disorder arising from glucose intolerance during pregnancy. It affects two to three percent of pregnancies in developed countries. Although gestational diabetes is rarely dangerous for the mother, the fetus can develop many problems that make detection and treatment of this disorder very important. The risks to the fetus include stillbirth, macrosomia, hypoglycemia, jaundice, respiratory distress syndrome, polycythemia, hypocalcemia, congenital abnormalities, obesity and diabetes (Kjos and Buchanan, 1999).

Although in a diabetic patient the effects of diabetes are easily predicted and understood, for the fetus of a diabetic gestation, the mechanisms of disease are poorly understood and rarely predictable. The reason for this is that the placenta has an amazing ability to protect the infant from the diabetic effects of the mother.

GENERAL EFFECTS OF INSULIN

To properly understand the effects of diabetes, it is necessary to understand one of the most important hormones that regulate glucose metabolism. Insulin is secreted by beta cells located in the islet of Langerhans in the pancreas. Insulin is a synthesized in the beta cells in three stages: firstly a large molecule, pre- proinsulin, with a molecular weight of 11,500, secondly as proinsulin with a weight of 9,000, and finally insulin with a weight of 5,808. Insulin has a very short half-life of six minutes in the blood. Therefore, since the half-life is so short, insulin's important effects are short lived (Guyton & Hall, 2005).

Insulin enhances glucose utilization and storage. It is required to store glucose for later use, while limiting high glucose levels in the blood. The body, specifically the brain, requires glucose as a primary source of energy. Without glucose the central nervous system becomes depressed; when glucose levels drop below 70 mg/dl, the patient will become extremely nervous, tremble,

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often break out in a cold sweat, and may hallucinate; when blood glucose levels drop below 50 mg/dl, the patient will lose consciousness and, as the blood glucose drop further, the patient will enter a coma which could cause permanent damage to the central nervous system. However, exceedingly high levels of blood glucose result in hyperglycemia, which results in cellular dehydration- because glucose causes a large amount of osmotic pressure in the extracellular fluid. Exceedingly high levels of glucose in the blood cause the kidneys to release glucose in the urine, which can further deplete the body of it fluids and electrolytes because a large amount of water will be released in the urine with the glucose (Guyton & Hall, 2005).

One of the primary stimulators of insulin is glucose. This directs high levels of glucose in the blood into cells throughout the body for use, lowering blood glucose levels. Additionally, gastrointestinal hormones also stimulate insulin production and secretion at the time of consumption before glucose even reaches the blood. These hormones are released through signals to the pancreas as the stomach is stretched when food enters the stomach. This is important so that a bolus of insulin is available immediately once glucose enters the blood to be stored in the liver as glycogen. Without insulin, glucose in the blood does not enter the cells. Insulin causes eighty percent systematic cells to be permeable to glucose within seconds of its release. This is the primary mechanism that causes the blood glucose level to drop to normal levels. Once insulin binds with the alpha subunit of insulin receptors, the beta subunit of insulin receptors, the beta subunit of the receptor undergoes tyrosine phosphorylation. This then causes a signal transduction cascade which activates glucose transport vesicles allowing glucose to enter the cell. Sixty percent of the glucose from a meal is immediately stored in the live as glycogen. Insulin has a direct effect on many important enzymes in the liver. Insulin inhibits liver

phosphorylates, which breaks glycogen into glucose. Insulin enhances activity of glucokinase, which phosphorylates glucose so that once the glucose enters the cell it gains a negative charge preventing it from diffusing out of the cell. Insulin also activates glycogen synthase which is active in the polymerization of glucose to glycogen (Guyton & Hall, 2005).

When insulin is absent from the blood, the reverse effects occur. Finally, glycogen is broken down by the enzyme phosphorylase, and then the glucose phosphate molecule is separated from the phosphate by the enzyme glucose phosphate which allows glucose to diffuse into the blood. Also lack of insulin causes the body to transport less glucose into the cell and inhibit use of glucose for metabolism. These mechanisms elevate the blood glucose levels to within a normal range for use by the central nervous system (Guyton & Hall, 2005).

In line with insulin's role in reducing excess glucose from the blood, insulin also inhibits metabolism of lipids. Additionally, when the liver has absorbed the maximum amount of glucose that it can convert into glycogen, insulin activates the glycolytic pathway and splits the glucose into pyruvate which is then converted into acetyl coenzyme A (AcCoA) and then further synthesized into fatty acids. This process is further enhanced by excess amount of citrate and isocitrate formed during glucose utilization (which is heightened when insulin is present) which activates acetyl-CoA carboxylase, a necessary step in lipid synthesis (Guyton & Hall, 2005).

Conversely, when there is a lack of insulin, the primary source of energy in the body is from fats. When insulin is not present in the blood, hormone-sensitive lipase is activated in adipose tissues which cause large amounts of triglycerides and fatty acids to be released into the blood. One effect of these excess fatty acids is that the liver will convert these free fatty acids into phospholipids and cholesterol. Lack of insulin can raise the percentage of cholesterol and lipids in the blood from .6% to above 3.5%, which is implicated in the clogging of the arteries. Additionally, increase use of fatty acids for energy causes a rise on acetoacetic acid acetone and Beta-hydroxybutyric acid, collectively called ketone bodies, which in large quantities cannot be metabolized by the body and is a condition known as ketosis, its effects will be discussed in connection to the pathology of diabetes (Guyton & Hall, 2005).

PHYSIOLOGY OF DIABETES MELLITUS

As discussed above, when insulin is not available or not effective, there is a decrease in the amount of glucose used by the body, and, therefore, an increase in the amount of glucose in the blood, which causes dehydration of cell tissue. This is primarily because glucose is not readily diffused through the cell membrane, causing an increase in the osmotic pressure in extracellular fluid; therefore, the extracellular fluid retains water and retracts water from the cells. The excess glucose in the circulation is excreted by the kidneys in the urine, which enhances dehydration because heightened osmotic pressure in the renal tubules prevents reabsorption of water from the urine (Guyton & Hall, 2005).

The absence of insulin increases the use of fatty acids for metabolism. This causes an extreme amount of ketone bodies to be formed. The amount of keto acids can rise tenfold when the body is not deriving energy from glucose. These acids will destroy the bicarbonate component of the body which is necessary to serve as a buffer. This will lead to acidosis which will lead to coma and death if the blood pH falls below 7.0. The acidosis is increased because the excess of keto acids are excreted by the kidneys only in the non-acidic form, commonly when the acids are combined with sodium. The depletion of sodium from the blood leads to larger

amounts of hydrogen ions in the blood, thereby increasing the effects of acidosis. An additional concern because of increased fatty acid metabolism is arteriosclerosis. Since there is an excess amount of fatty acids and cholesterol present in the blood, arteries can become clogged (Guyton & Hall, 2005).

PATHOLOGY OF GESTATIONAL DIABETES

Perkins, Dunn and Jagasia (2007) explain that even normal pregnancies portray an increasing resistance to insulin throughout the pregnancy. The placenta's production of human chorionic somatomammotropin (HCS) inhibits insulin's effect on the mother's peripheral tissues to uptake glucose, providing an abundance on nutrients for the fetus. Furthermore, HCS stimulates insulin production in the fetus causing an increased uptake of nutrients. As the fetus and placenta grow, more HCS is produced until a normal pregnancy the mother's insulin sensitivity decreases by fifty percent by the third trimester. Additionally, Catalnao et al. (2006) show the effct of insulin desensitization on lipid metabolism. Normally, insulin causes adipogenesis- an increase in lipid storage- however, placenta hormones cause the adipocytes to lyse, contributing to the available nutrients for the fetus.

Pregnant women with gestational diabetes have an additional forty percent decrease in insulin control from non-diabetic pregnant women, according to Catalano et al. (2003). They note defects in both the response of beta cells in the pancreas and in the signal transduction pathways of the insulin receptors as the cause of the glucose intolerance associated with gestational diabetes. There may be several different causes for the decreased response preventing beta cells from producing sufficient insulin: the additional stress on the body to pregnancy may "unmask" a predisposed genetic propensity to Type 2 diabetes. Alternatively, studies have shown the presence of antibodies obstructing beta cell function suggesting a possible link to Type 1 diabetes. Studies have shown that the increased desensitization of insulin on peripheral tissues in gestational diabetes is caused by a marked decreased ability of the beta subunit of the insulin receptor to undergo tyrosine phosphorylation. Additionally, there is a marked decrease in up-regulation of insulin receptors, decreasing the effect of insulin. These defects contribute a large amount of nutrients to the mother's blood, which produces a hyperglycemic environment, as well as the additional effects of insulin resistance, a primary use of fats for metabolism, which can clog arteries and lead to acidosis.

CONCERNS OF GESTATIONAL DIABETES FOR THE MOTHER

Interestingly, the major concerns associated with diabetes are not a strong concern for the mother in a case of gestational diabetes. Whereas a long-term diabetic patient may have clogged arteries and signs of acidosis, the mother does not normally develop such grave complications, probably because of the relatively short duration of gestational diabetes. However, the effects of hyperglycemia, dehydration and release of glucose from the urine- decreasing dehydration, are a concern.

The most serious complication associated with gestational diabetes is hypertension, according to Kjos and Buchanan (1999). There is a positive correlation to the mother's glucose levels and blood pressure. Therefore, careful monitoring and control of the mother's blood glucose levels and blood pressure is important to avoid complications associated with preeclampsia.

The most serious postpartum complication for mothers who develop gestational diabetes is an increased chance of developing diabetes unrelated to pregnancy. There is a significantly high chance of developing diabetes if the gestational diabetes developed in the first trimester or if the mother is obese (Kjos and Buchanan, 1999).

DETECTION AND CONTROL OF GESTATIONAL DIABETES

Kjos and Buchanan (199) suggest that an initial screening for signs of gestational diabetes be administered at the time of the first visit. Only women who are at risk to gestational diabetes need to be administered a glucose intolerance test. Again, at the end of twenty-four weeks, women should be reassessed for indications of gestational diabetes.

Gestational diabetes is usually treated through the mothers' diet (Kjos and Buchanan, 1999). Monitoring of blood levels is important to ensure that the blood glucose levels do not rise to levels that may harm the fetus. If the blood glucose levels are not controlled with diet or if there are indications of fetal complications to diabetes, it may be necessary to treat the mothers with insulin.

MACROSOMIA

Macrosomia is the most common problem associated with gestational diabetes. Because of the fetus' large size, there are complications during labor and delivery, which can lead to death. The simple cause of excess fetal growth is due to excess amount of nutrients available from the mother's hyperglycemia. This view is, indeed, substantiated through studies which show a correlation between the degree of fetal size and the degree of maternal blood sugar levels. However, fetal response to gestational diabetes varies tremendously among different racial and ethnic groups. In fact, only twenty to thirty percent of infants born to mothers affected by maternal diabetes are affected with macrosomia. This suggests that gestational diabetes causes a complex metabolic disturbance which can have many effects on the fetus (Kjos & Buchanan, 1999).

Alonso et al. (2006) explain that the placenta undergoes many changes due to a diabetic gestation. Since the placenta is the primary means of providing nutrients to the fetus, these changes are thought to protect the fetus. Osmond et al. (2000) show that transport of glucose across the placenta, uptake of nutrients from the mother, and utilization of glucose by the placenta is reduced as a result of gestational diabetes. This would explain the poor correlation between gestational diabetes and macrosomia. Therefore, he suggests that the fetus is affected with macrosomia and other conditions when this protection is not working properly. Growth of the fetus and placental must be regulated by some endocrine control. Insulin is the probable hormone to regulate pathways in the placenta, since it has already been determined that insulin receptors are located on the placenta, even though insulin is not necessary for glucose to pass through the placenta. Furthermore, the locations of these receptors change from the maternal side of the placenta during the first trimester to the fetal side of the placenta, suggesting that both the mother and the fetus control functions of the placenta.

Interestingly, Alonso et al. (2006) report that the average weight of fetuses from a diabetic environment had a smaller mass than the control group while the weight of the placentas from the diabetic group was much larger. His theory is that insulin activates the "diabetic placenta" by first increasing its weight and then to start a signal pathway to protect the placenta

and fetus from the diabetic environment. He further supports his theory by showing that there are additional insulin receptors on the placenta, showing that insulin plays a primary role in the protection of the placenta. However, he does not explain what function enlargement of the placenta has or how the placenta protects the fetus.

GREATER OCCURRENCE OF DIABETES AND OBSESITY AS ADULTS IN CHILDREN

The focus of many studies is what the effect of early life in the uterus has on a person's entire life. Perhaps, conditions such as obesity, hypertension and diabetes arise from the conditions in utero. Holness et al. (2000) suggest that a number of important metabolic and biochemical functions that are important for regular control of glucose later in life begin as a fetus, and therefore, the conditioning of how to properly maintain these system work is learned first in the uterus as well. Any disruption in the life of the fetus can cause a predisposition to later metabolic conditions. Holness et al. (2000) assumes that a child born with a low birth weight will have developed to compensate for the lack of nutrients available. The consequence of this is periods of impaired growth in tissues necessary to properly function later. Bo et al. (2003) also believe that a decreased growth rate of a fetus will lead to metabolic abnormalities later in life that may cause an increased risk of diabetes and obesity. Their study confirmed that fetal growth is correlated to risk of diabetes later in life. The understanding is that a fetus' metabolism is conditioned to the environment and this has an effect for the fetus' entire life.

Malee and Wu (1999) conducted a study of the changes in level of adrenocortical steroids as a result of gestational diabetes. Since maternal diabetes often increases a fetus' hormone levels, they hypothesized that the increased incidence of diabetes later in life of children born from a mother who was affected with diabetes could be a result of increased glucocoritisol which inhibits insulin efficacy. They assert that any function altered in the uterus can be "imprinted" in the fetus and have an effect throughout the life of the fetus, similar to the theory of Bo et al. (2003).

Although they did not find higher levels of corticosteroids in fetuses from diabetic gestations or normal gestations, there was an observed increase in adrenal activity. They found elevated levels of aldosterone, which is also produced in the adrenal cortex, and may indicate that diabetes has an effect later in the fetus' life on corticosteroids as well. Furthermore, even though the level of corticosteroids did not rise, some of the mRNA was found in abundance. Although increased levels of mRNA do not always cause increased hormone levels, it could indicate an "imprint" on the fetus' adrenal cortex activity due to gestational diabetes. Therefore, there is reason to believe that children born from a diabetic gestation may be affected with diabetes later in life due to a malfunction of the fetus' corticosteroid control of insulin.

Schroeder et al. (1997) conducted a study on a rat skeletal and myocardial tissue to determine the effects of gestational diabetes. They learned that while the hyperglycemic environment of the fetus did not produce more insulin, the glucose transporters in the muscle tissue decreased by sixty-five percent. They assume this process is a defense to limit the amount of glucose entering the cells. Schroeder et al. (1997) further predict that perhaps this may cause a predisposition for the gestational diabetic fetuses to develop diabetes later in life, since the low levels of glucose transporters develops insulin resistance in the skeletal muscle tissue. This may

be another mechanism in human fetuses that cause metabolic change in utero that affects the adult life of the fetus.

HYPOXIA AND HYPOXIA-RELATED CONDITIONS

Hypoxia, a decreased percentage of oxygen in the blood of the fetus, has been noted as a concern during a diabetic pregnancy in the third trimester. While no studies have been done to address the specific cause of hypoxia in gestational diabetes, Lassus et al. (2003) note that vascular endothelial growth factor is significantly lower in the cord blood of fetuses' from diabetic pregnancies. Vascular endothelial growth factor is important to vascular development. In the absence of this growth factor, mice die. In acute hypoxia expression of vascular endothelial growth factor occurs rapidly within a few hours. It seems that hypoxia occurs to the fetus because it is unable to regulate proper vascular maintenance and growth due to some inhibition of vascular endothelial growth factor.

Loukovaara et al. (2004) preformed an experiment to determine the cause of hypoxia related conditions, such as periventricular leukomalacia and cerebral palsy. It is known that inflammation is correlated to these two conditions and the study's aim is to determine if hypoxia causes the inflammation. They measured C-reactive protein (CRP) which rises in response to inflammation or stress. They found that CRP is positively correlated to hypoxia, and therefore that hypoxia is a likely cause of inflammation. Therefore, not only is hypoxia a danger to the fetus of a diabetic gestation but also periventricular leukomalacia and cerebral palsy.

Loukovaara et al. (2004) further wanted to ascertain whether the diabetic pregnancy will cause inflammation in the fetus. Furthermore, since inflammation is a risk factor for diabetes, perhaps this is the reason that children from diabetic pregnancies are at greater risk to diabetes later in life. They concluded that the cord serum CRP was no different in gestational diabetic pregnancies than normal pregnancies, and therefore, there is no evidence that increased risk to diabetes later in life is due to chronic inflammation. However, their study does not exclude the possibility of chronic inflammation in cases where the gestational diabetes was not controlled. Therefore, they conclude that more research on the relationship between chronic inflammation and diabetes is justified.

CONGENITAL ABNORMALITIES

Congenital abnormalities occur in six to ten percent of infants of diabetic gestations (Menegola et al. 1999). These deformities include "(in order of risk ratio): caudal regression syndrome, situs inversus, double uteter, renal agenesis, cardiac anomalies, and anencephaly" (Menegola et al., 1999, p.65). Menegola et al.'s study found that the earlier the embryo was exposed to a diabetic environment, the greater chances were of malformation. He even hypothesizes that after a certain period of development the effects of diabetes will not cause malformation.

Menegola et al. (1999) explain that embryos placed in a diabetic environment have a high level of free oxygen radicals and a low level of scavenging enzymes. Studies have shown that correcting the levels of free radicals also corrects the rate of mutations in the embryos. Rat embryos taken from a diabetic mother contained a significantly lower amount of glutathione is not produced by the embryo until a later stage than when mutations are likely to occur.

Therefore, Menegola et al. (1999) hypothesize that the effects of diabetes on the fetus may cause mutations are linked to low levels of glutathione. However, since whole rat embryos had to be implanted in a diabetic environment only after the chance of mutation had already passed (otherwise the control embryos would also have a high rate of mutation), Menegola et al. (1999) were unable to prove their theory in their experiment with rat embryos.

RESPIRATORY DISTRESS SYNDROME

One congenital abnormality that is a result of maternal diabetes is, as Malee and Wu (1999) note, delayed expression of surfactant proteins. Surfactants are produced by the septal cells in the alveoli and are important for proper function of the lung. The surfactants lower the surface tension of alveolar fluid so that the water molecules are not as strongly attracted by hydrogen bonding. If the surfactant was not present, the alveoli would collapse. This malformation is implicated in the greater incidence of respiratory distress syndrome in infants born from a mother gestational diabetes.

CONCLUSION

While the effects of diabetes have been known for quite some time, the effects that a diabetic environment has on a fetus are still unknown. It appears that complications due to diabetes are protected because of the protective role of the fetus, as Alonso et al. (2006) reported. However, although Alonso et al. (2006) do explain the activation of the protective role of the placenta, they do not explain how the placenta protects the fetus, what mechanism causes the rate of glucose transport across the placenta to decrease and what the role the enlarging of the placenta is. Clearly, much more research must be done to answer these important questions.

Another important question is whether the placenta plays a role in masking the effects of other complications due to gestational diabetes, for instance hypoxia and congenital malformations. Many studies of complications from gestational diabetes, such as Loukovaara et al. (2004) and Menegola et al. (1999) have difficulties studying the effects of gestational diabetes in the fetus, because the effects are so unpredictable; it seems that this would be due to the protection of the placenta. Perhaps a better understanding of the changes that the placenta undergoes during gestational diabetes will lead to better studies of the effects of gestational diabetes, and an understanding of the factors that predispose human infants to complications due to gestational diabetes. One approach would be to examine the placentas of fetuses which are affected with complications from gestational diabetes and compare them to the placentas from a diabetic environment that did not produce those same complications.

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