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A DIVISION OF TOURO COLLEGE IN FLATBUSH

Where Knowledge and Values Meet

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The cover illustration, created by Professor Antony O'Hara of the Digital Multimedia Design Program, refers to papers by Etta Rubin, Moshe Baitelman, and Maddie Nierman that discuss topics related the the brain.

The Relationship between Vertigo, Vestibular System Disorders, and Therapy

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Abstract

The relationship between vertigo and vestibular system disorders has been the subject of much research in recent years. The aim of this study is to review and analyze the relevant literature regarding this relationship, with emphasis on determining what causes the dizziness, how to test for vertigo, and specifically how to treat it. Additionally, while there may be many ways to prevent vertigo, the focus will be on how the role of therapy is vital in the future of healthcare, serving as prevention of anxiety and reoccurring vestibular disorders.

Introduction

About forty percent of Americans, will at some point of their lives have a balance problem that is disturbing enough that they consult a doctor, according to the National Institute of Health (Better Balance, 2017). Symptoms of dizziness from seasickness, fear of heights, or alcohol consumption were mentioned as early as 730 BC in Roman, Greek, and Chinese texts. However, due to the lack of scientific evidence at that time, an explanation for this dizziness was not present until mid-19th century scientists began to study the somewhat camouflaged vestibular system and research its possible correlations to vertigo. There are different forms of treatment available for vertigo and vestibular disorders. This review will investigate and determine which form of treatment is the most effective for treating vertigo and vestibular disorders.

Methods

The information gathered in this paper has been collected from numerous sources including databases such as PUBMED, Google Scholar, Touro Library, and Nature.com. The information was read, analyzed and compared to determine each study's validity and standpoint.

Discussion: Defining the Vestibular System

One's balance system helps one stand, walk, run, and move without falling. The eyes, inner ear, and body's muscles and tendons coordinate with the brain, which help one control balance (Tamaki, 2016). Together the organs that control or sense the signals throughout the body are known as the vestibular system. The vestibular apparatus found in the inner ear of humans, provides a sense of balance and awareness of spatial orientation. It is made up of three semicircular canals, anterior, posterior, and lateral, that are sensitive to angular accelerations (head rotations: up-down, side to side, and tilting movements). It also includes two labyrinth organs, the utricle and the saccule, that are sensitive to linear (or straight-line) accelerations by sensing gravity (Tamaki, 2016). Information from the vestibular organs of each ear, the eyes, and the rest of the body are then integrated in the brain.

The semicircular canals originate from the utricle and are positioned at a 90° angle from one another, with the horizontal canal tipped backwards 20-30 degrees (Figure 2). They are filled with endolymphatic fluid which exerts pressure against the 10–50 micrometer stereocilia, the canal's sensory hair cell receptors (Better Balance, 2017). Calcium carbonate crystals called otoconia are attached to both the medial wall of the saccule and floor of the utricle. Thus, under the influence of acceleration of a car, for example, it can cause stimulation of the hair cells by their movement relative to the endolymphatic fluid. The receptors respond by sending impulses to the brain about movement from the specific canal that was stimulated. When the vestibular organs on both sides of the head are functioning properly, they send symmetrical impulses to the brain and let the brain know that the car has accelerated.

Additionally, the Vestibulocochlear Nerve, also known as Cranial Nerve VIII, is responsible for a human's sense of hearing and equilibrium (Patel et. al., 2017). It transmits balance-related information from the semicircular canals and the labyrinth organs to the central nervous system. The superior portion of the nerve innervates anterior and horizontal canals and utricle, while the inferior portion innervates posterior canal and saccule. When, for example, a rotational head movement occurs, it causes the fluid in the semicircular canals to move which causes the stereocilia in the saccule, utricle, and the cristae of the semicircular canals to bend. When the hair cells bend, they exert a mechanical force into electrical nerve action potentials stimulating cranial nerve VIII. However, if one is jumping up and down, descending in an elevator, or accelerating on the highway, the two labyrinth organs of each ear are activated. Consequently, it causes the fluid in the labyrinth organs to move, while otoconia, the calcium carbonate crystals, lag behind. The lagging behind of the otoconia causes hair cells in the labyrinth organs to bend, and the bending of the hair cells stimulates cranial nerve VIII as well. Through the help of the cochlea and the vestibular apparatus, cranial nerve VIII sends messages from the inner ear to the central nervous system to empower the body's sensation of hearing and balance (Gill-Body, 2001).

History of Vertigo

Descriptions of vertigo and dizziness as a result of seasickness, fear of heights, and alcohol consumption were found in Roman, Greek, and Chinese texts as early as 730BC –600CE (Huppert et al, 2018). One of the earliest references to vertigo and the idea of dizziness in relation to fear of heights is found from the 5th century BC in the Hippocratic Corpus. In a collection of around 60 early Ancient Greek medical works strongly associated with the physician Hippocrates and his teachings, the

Hippocratic Corpus uses Latin keywords of vertigo such as caligo, caligare, altitudo, magnitudo, and celsitudo, describing relationship between heights and dizziness (Huppert et al, 2013).

During the sixteenth century the middle ear was described in detail and further progress was made between the sixteenth and eighteenth century in describing the inner ear.

Knowledge of the vestibular system and its functions began to grow with nineteenth century scientists such as Jan Evangelista Purkinje, Ernst Mach, Josef Breuer, Hermann Helmholtz, and Alexander Crum-Brown (Huppert et al, 2018). The first textbook on neurology (Lehrbuch der Nervenkrankheiten des Menschen, 1840) by Moritz Romberg contained general descriptions of signs and symptoms of various conditions with the key symptom of vertigo, but lacked a definition of vestibular disorders. However, in the nineteenth century technological advancement permitted a description of the cells and structures that constituted the cochlea. Von Helmholtz made progress in hearing physiology when he postulated his resonance theory, and later Von Békésy made progress as well when he observed a traveling wave within the cochlea of human cadavers. Brownell later made a major advance when he discovered that the ear has a mechanism for sound amplification, via outer hair cell electromotility (Hachmeister, 2003).

Vertigo as a Symptom

Vertigo and dizziness are among the most common complaints in neurology clinics. They account for about 13% of patients entering emergency units and are reported as the third most common major medical symptom in general medical clinics (Brandt, Dieterich, 2017).

Dizziness and vertigo are complex neurological symptoms, traditionally categorized as one of four "types" based on symptom quality: (1) vertigo, an illusion of spinning or motion, (2) presyncope, feeling faint, (3) disequilibrium, a loss of balance or equilibrium when walking, and (4) lightheadedness, wooziness, and giddiness, etc. (Newman-Toker, 2007).

Vertigo is a symptom, not necessarily a disease, but it is an outcome of a physiological or uncontrolled processes, characterized by sudden dizzy spells causing a lot of discomfort (Srinivasan, Jebasingh, 2007). Vertigo is often caused by an inner ear problem. The sensations from the inner ear, eyes, and throughout the body (somatosensory) are mismatched. Humans need the above three to regulate their balance (Better Balance, 2012). Any abnormality in one or more of these can trigger vertigo.

Vertigo can also be caused by decreased blood supply to certain areas of the brain. This can follow mini-strokes called transient ischemic attacks (TIAs) or can happen as a result of a permanent stroke (Seiden, 1989). Similarly, head trauma, even a minor blow to the head, is a risk factor to cause vertigo (Naguib et al, 2012).

There are different types of vertigo. Rotatory vertigo mimics the sensation of being on a merry-go-round, like in vestibular neuritis and other disorders, while postural vertigo resembles the sensation of riding in a boat. Yet many patients use the term "dizziness" for lightheadedness without any sensation of movement (e.g., in drug intoxication). Lightheadedness is the most common type of vertigo; it mainly affects older patients and it is characterized by brief attacks of rotational vertigo, accompanied by vertical positioning nystagmus that rotates toward the lower of the two ears and beats toward the forehead. The attacks are triggered by reclining the head, or by lateral positioning of the head or body, with the affected ear downward. After a change in position of one of these types, rotational vertigo and nystagmus arise after a latency of a few seconds and last a total of 30 to 60 seconds (Strupp, Brandt, 2008).

Importance of Treating Vertigo

According to news from Munich, Germany by NewsRx editors, research states, "Vertigo and dizziness are among the most common complaints in neurology clinics, and they account for about 13% of the patients entering emergency units (Nervous system diseases and conditions, 2017). "Some vertigo is so violent a person can't get out of bed, sometimes for several days at a time. They can't hold a job or take care of the family or just be with the family. Some balance problems can be very debilitating," says Browning, a certified clinical audiologist at Rocky Mountain Hearing and Balance in Salt Lake City (Collins, 2007)

The NIH also reports that balance-related falls cause more than half of the accidental deaths among the elderly. About forty percent of people over age sixty five fall each year, totaling more than thirteen million falls a year, resulting in at least 1,600 senior citizens' deaths as a direct or indirect result of falls (Collins, 2007).

Dysfunction of the vestibular system in humans can be severe and extremely debilitating, leading one to become housebound and lead a very restricted life, with high rates of anxiety disorders and depression. In the United States of America, approximately 35% of people aged 40 and over have suffered from some form of vestibular dysfunction [based on a sample of 5,086] (Smith, 2017).

Vestibular Disorders and Vertigo

Humans can become disoriented if different sensory input received from their eyes, muscles, tendons, or vestibular organs conflict (Brennan, 2012). For example, Physiological vertigo is Vertigo while in moving vehicles, colloquially known as motion sickness. This is due to a "sensory conflict" between one's vision and the actual movement sensation. In motion sickness, it is caused by multi-sensory motions that do not correlate to the expected pattern of movement. Many feel better sitting in the front seat of the car or bus as it gives them more visual input than at the back seat. These individuals are encouraged to keep their eyes open as this will let the brain gain more information of the movement of the vehicle and decrease the

vertigo. Additionally, signal conflicts may occur when a person is standing next to a bus that is pulling away from the sidewalk. The visual image of the large moving bus may create an illusion for the pedestrian that he, rather than the bus, is moving. However, at the same time the information from his muscles and joints indicates that he is not actually moving. Sensory information provided by the vestibular organs helps override sensory conflicts (Bedford, 2012). In addition, higher level thinking and memory might suggest the person to glance away from the moving bus to look down in order to seek visual confirmation that his body is not moving relative to the pavement.

One of the most commonly diagnosed vestibular disorders is Benign Paroxysmal Positional Vertigo, also known as BPPV (Smith, 2017). BPPV is a mechanical problem in the inner ear that is the most frequent cause of dizziness. It occurs when a tiny crystal of calcium breaks free from the wall of one of the semi cicular canals and moves into the canal of the inner ear, causing disruptions in the inner ear signals that are eventually sent to the brain about head and body movements (Najeeb, 2016). Typically, the crystals fall into the posterior semicircular canal every time the patient lies down with the affected ear down. As a result, the crystals move the fluids of the inner ear, activating the sensor of the semicircular canal inappropriately. This is perceived as vertigo or spinning by the patient and lasts less than a minute until the crystals settle down and stop moving (Vestibular.org). It can occur suddenly while the sleeping person gets into a particular position, yet, the after effects of uneasiness and unsteadiness are even more disturbing than the original pain (Baloh, 1998). BPPV is called "benign" because it usually resolves spontaneously within a few weeks or months; in some cases, however, it can last for years. If left untreated, it persists in about 30% of patients (Strupp et al, 2008).

The treating physician will perform a simple maneuver called the Dix Hallpike to test for the condition. The doctor will ask the patient to sit on the exam table and he'll turn the patient's head 45 degrees to one side and then will help him or her lie back quickly so his or her head hangs slightly over the edge of the table. This movement may make the loose crystals move within the semicircular canals. The doctor will then ask if the patient feels symptoms of vertigo and watch his or her eyes to see how they move, checking for nystagmus, an involuntary movement of the eyeballs, which is a major symptom of vertigo.

Once vertigo is proven, the follow-through procedure would be either an Epley maneuver involving rotation of the patient in the reclining position with their head hanging down, or Semonts or Brandt Daroff maneuvers, which are all equally effective. The cure rate for these procedures is more than 95% within a few days, as shown by multiple controlled studies (Strupp et al, 2008). The purpose of these procedures is to displace abnormal calcium deposits within the inner ear to an area which prevents them from stimulating the nerve cells, which causes vertigo.

Canalith Repositioning Maneuvers, also known as Epley maneuvers, can be used to treat vertigo. It is a repositioning maneuver involving sequential movement of the head into four positions, staying in each position for approximately thirty seconds to subsequently move the displaced crystals out of the affected area (American-hearing.org).

With a series of specific head and body movements for BPPV recommended by the American Academy of Neurology, the movements are done to move the calcium deposits out of the canal into an inner ear chamber so they can be absorbed by the body. One will likely have vertigo symptoms during the procedure as the canaliths move. The movements are safe and often effective. Commonly, this will be followed by Brandt-Daroff exercises, which will consist of shaking the crystals within the fluids by alternating body positions and ultimately dissolving them.

The treatment of benign paroxysmal positioning vertigo with the Semont maneuver is as follows: In the initial sitting position, the head is turned forty five degrees to the side of the unaffected "healthy" ear. Then the patient is laid on the right side, i.e., on the side of the affected ear, while the head is kept in forty five degrees of rotation to the other side. This induces movement of the particulate matter in the posterior semicircular canal by gravity, leading to rotatory nystagmus toward the lower ear that stops after a brief interval. The patient should maintain this position for about one minute. While the head is still kept in forty five degree of rotation toward the side of the healthy ear, the patient is rapidly swung over to the side of the unaffected ear, so that the nose now points downward. The particulate matter in the semicircular canal now moves toward the exit from the canal. This position, too, should be maintained for at least one minute. The patient returns slowly to the initial, sitting position. The particles settle in the utricular space, where it can no longer induce rotatory vertigo. The above sequence should be performed three times in a row three times per day, in the morning, at noon, and at night. Most patients are free of symptoms after doing this for three days (Strupp et al, 2008).

If one is diagnosed with BPPV, one should get treatment from a physical therapist. Physical therapy has a success rate with the symptoms usually resolving within one or two treatments. The physical therapist guides the patient through a series of movements that will relocate the crystals to their place of origin or to an area of the ear that is not affected by their presence (Kelowna Capital News, 2008).

Another kind of vertigo is vestibular vertigo. Vestibular vertigo is the leaking of inner ear fluid, vestibular failure, infection of the vestibule, and tumors. Vestibules are sensitive to the movement of the head, and any type of irritation or damage to the vestibules can cause confusion in the brain, resulting in vertigo. Vestibular nerve inflammation, which is mostly viral in nature, is self- limiting and almost always resolves by itself.

Similarly, Labyrinthitis and Vestibular Neuritis are vestibular

disorders resulting from an infection that inflames the inner ear or the nerves connecting the inner ear to the brain. This inflammation disrupts the transmission of sensory information from the ear to the brain. Vertigo, dizziness, and difficulties with balance, vision, or hearing may result. This is explained from the inner ear organs and the balance signals sent through the vestibulocochlear nerve. When one ear is infected, it sends faulty signals and thus the brain receives mismatched information, resulting in dizziness or vertigo.

Lastly, Meniere's disease is a chronic, incurable vestibular disorder defined in 1995 by the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology. It produces a recurring set of symptoms as a result of abnormally large amounts of endolymph fluid collecting in the inner ear. Meniere's disease can develop at any age, but it is more likely to happen to adults between 40 and 60 years of age (Agrup Et. Al., 2007).

Vertigo is often triggered by a change in the position of one's head. Within seconds, one can experience an awful sensation, as if the entire environment has begun to spin or that one is spinning against a stationary environment. One may feel nauseated, have a headache, and have abnormal jerky eye movements (Brandt, 2000). Ringing in the ears or hearing loss symptoms can last a few minutes to a few hours or more and may come and go. Another symptom may be nystagmus, which is involuntary movement of the eyeballs. One will not see it in the mirror because once one fixes their vision in the mirror, nystagmus disappears. Additionally, hemi spatial neglect (failure to be aware of one side of space), room tilt illusion, loss of spatial memory, and ultimately pusher syndrome, a loss of postural balance are all symptoms of vertigo. It is important to note that most of the peripheral vestibular disorders have a clinical diagnosis, and therefore medical history is important.

Testing for Vertigo

The Head Impulse Test (HIT) is one of the many tests to do to find the possible cause of vertigo. During the Head Impulse Test, the patient is asked to fix his or her eyes on a target (e.g. the examiner's nose). The examiner will then generate a rapid head impulse while monitoring the patient's eyes for a corrective or compensatory saccade (CS) response. Individuals with normal vestibular function should not generate a compensatory saccade after a head impulse, rather the eyes should stay fixed on the target.

Ocular Motor Testing looks at the systems responsible for integrating balance, vision, and movement. Four categories of ocular motor functions: stability of gaze; smooth pursuit eye movements,optokinetic nystagmus; and saccadic eye movements are used to determine spontaneous and gaze-evoked nystagmus. Nystagmus often results in reduced vision and depth perception and can affect balance and coordination. This testing can also be used to determine skew deviation- wherein the eyes move upward (hypertropia), but in opposite directions. Skew deviation is caused by abnormal prenuclear vestibular input to the ocular motor nuclei, most commonly due to brainstem or cerebellar stroke.

Frenzel goggles are extremely useful in evaluation of patients with vestibular disorders. They were termed by the German otolaryngologist Herman Frenzel from Gottingen, Germany in the 1950's, and have been used as an examination tool to disable the patient's ability to visually fixate on an object while at the same time allowing the examiner to adequately visualize the eye. In essence, they consist of the combination of magnifying glasses and a lighting system. When Frenzel goggles are placed on the patient and the room lights darkened, nystagmus can easily be seen because the patient's eyes are well illuminated and magnified, and because fixation is removed as the patient can hardly focus through magnifying glasses on a dark room. The Frenzel goggles reduce visual fixation by means of the magnification glasses of about sixteen diopters (a unit of measurement of the optical power of a lens or curved mirror), and also allow a better examination of the eye movements, specifically nystagmus (Strupp, et. Al., 2014).

Despite these tests, sometimes one may feel dizzy or light-headed but it's not truly vertigo. This is common in people with panic disorders or low blood sugar count, or when one suddenly stands up from a lying or seated position or is intoxicated with drugs or alcohol. Alcohol interferes with the communication between nerve cells and cell receptors that send messages between the body and the brain. As a result, the cerebellum, the part of the brain that usually creates nerve impulses that control an individual's balance and other fine movements for balance, cannot function properly due to the uncoordinated nerve signals. Ultimately, the muscle movements become uncoordinated and one can lose his or her balance (Susanto, 2014).

Treatment

Vertigo is a symptom, so finding its cause is the key to treating it. For some, treatment from an audiologist, physical therapist, or occupational therapist for balance treatment is needed. In many cases, vertigo goes away without any treatment. This is because one's brain is able to adapt, at least in part, to the inner ear changes, relying on other mechanisms (Phillips, 2011). For example, if one is at a very loud concert, the brain adapts and contracts the inner ear muscles to protect the inner ear from damage. Similarly, the brain relies on sight as well as the musculoskeletal system to help with balance when one is walking along a beach. Stepping on sand may not be as sturdy as pavement, but with the help of the eyes and muscles, one can maintain his or her balance even on uneven surfaces.

"Vestibular Rehabilitation" may be recommended if one has recurrent bouts of vertigo. This is a type of physical therapy aimed at helping strengthen the vestibular system. It helps train the other senses to compensate for vertigo or learn ways to

turn one's head or move without getting dizzy. It includes improving balance and stability while in motion, improving neuromuscular coordination and decreasing anxiety due to vestibular disorientation, and minimizing falls (Merlingolda, 2013).

Medicine can be another treatment towards vertigo discomfort. In some cases, medication may be given to relieve symptoms such as nausea or motion sickness associated with vertigo. Antihistamines such as meclizine (Antivert) may offer short-term relief from vertigo in addition to anti-nausea medications (Donaldson et al, 2008). If vertigo is caused by an infection or inflammation, antibiotics or steroids may reduce swelling and cure the infection.

In a few cases when medical treatment is not effective in controlling vertigo, surgery may be considered to repair or stabilize the inner ear function (Strupp et al, 2008). There are destructive treatments that are designed to eliminate vertigo, possibly sacrificing hearing. These procedures are appropriate for consideration when medical treatment and vestibular rehabilitation has failed to control vertigo symptoms. For example, labyrinthectomy is a destructive procedure used for Ménière's disease. Labyrinthectomy is appropriate for patients in whom there is no hearing in the ear which is causing vertigo and thus offers excellent control of vertigo (Hain 2012). The balance end organs, namely the semicircular canals and two otolith organs, are removed so that the brain no longer receives signals from the parts of the inner ear that sense gravity and motion changes. It is important to note the hearing organ, the cochlea, is also sacrificed with this procedure. Ultrasound surgery at times may also be used to destroy the balance end organs so that the brain no longer receives signals from the parts of the ear that sense gravity and motion changes (vestibular.org).

After an estimated half a million people have received cochlear implants by 2016, several decades since the first cochlear implant was performed in 1961 (Smith, 2017), the success of cochlear implant technology has naturally encouraged researchers to consider other forms of bionic implants that can substitute for lost sensory function. The development of the 'vestibular implant or prosthesis', which aims to replace a missing or dysfunctional vestibular system, has been given to groups of patients since 2007 and considerable progress has been made in this area.

It is important to note, if vertigo is caused by a more serious underlying problem, such as a brain tumor or injury to the brain or neck, initial treatment for those conditions may help to alleviate its chain reaction vertigo as well.

Conclusion

Through the analysis and research shown throughout the paper, it leaves one empowered by what may be the best future healthcare treatment for one who has vertigo and other vestibular disorders. There are many types of treatment for vestibular disorders, but where does the future for best healthcare treatment of vertigo and vestibular disorders lie? According to the above research discussed, doctors and healthcare providers should be weary of surgery and medication as solutions to vertigo, for many times the vertigo symptoms may prove to be invalid or subside over time and these treatments are short term and destructive to nearby organs in the body. As a healthier and longer lasting alternative, Vestibular Rehabilitation via Epley, Sermont, or Brandt Daroff Maneuvers will not only help control the dizziness, but also strengthen other senses in the body to compensate for vertigo. As a result of the therapy, it will not only prove strengthening of the vestibular system, but will also decrease the anxiety and the symptoms of disorientation that occur as a result of vestibular disorders.

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www.american-hearing.org

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New Medical Indications for Thalidomide and its Derivatives

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Abstract

Thalidomide is an anti-inflammatory and an anti-angiogenesis drug that is being used around the world for a variety of malignant and inflammatory diseases. Is it justified to continue prescribing and developing thalidomide given the discovery of many well-known side effects including catastrophic birth defects? To answer this question, this paper will discuss the pharmacology and history of thalidomide, as well as many of its proposed mechanisms of action. The medical indications for the current use of thalidomide as well as for several newer and more potent derivatives are mentioned for their therapeutic results, as well as their adverse side effects.

Introduction to Thalidomide

"Thalidomide remains, arguably, the greatest disaster in medical history" (Greener, 2011). Thalidomide was introduced in 1957 by a German pharmaceutical company as a sedative and hypnotic drug. It was also widely used in several countries to alleviate morning sickness in early pregnancy. It was sold in more than forty-six countries without any testing for teratogenicity, as was common practice for new drugs at that time. More than 10,000 children around the world developed severe birth defects, especially shortening or absence of limbs. There was also an increase in miscarriages and infant mortality. Remarkably, the United States Food and Drug Administration did not approve sale of thalidomide despite five requests from manufacturers. As a result, only seventeen babies were affected in the United States, probably from thalidomide purchased in Canada (Huang, et. al. 2008).

By 1961 production and distribution of thalidomide were banned worldwide. Due to the risk of teratogenicity, almost no research into the use of thalidomide took place for the next decade. The first new indication for thalidomide was in treating a complication of leprosy known as erythema nodosum leprosum (ENL) (Sharma, et. al. 2007). Beginning in 1967, anecdotal reports of the use of thalidomide in cancer treatment began to be published. Some of the diseases treated were astrocytoma, multiple-myeloma, chronic lymphocytic leukemia, and malignant melanoma. New research is being done to investigate the mechanism of action of this class of immunomodulatory drugs in various malignancies and in non-malignant conditions such as Alzheimer's Disease and persistent erythema multiforme (He, et. al. 2013).

Methods

Articles and studies researched in this paper were obtained through the EBSCO and ProQuest databases with access provided by the Touro College Library. Additional research articles were obtained through the National Center for Biotechnology Information (NCBI) website and the Jackson Laboratory website. Images and diagrams that are used throughout the paper were obtained from the research articles cited.

Chemical Analysis

Thalidomide is a racemic glutamic acid analogue also known as [+] alpha-[N-phthalidimo]-glutarimide. It consists of two linked rings: a glutamic ring and a phthalic ring. Because it contains a chiral carbon the molecule is unstable and can switch back and forth between two enantiomers which are mirror images of each other. This reaction takes place rapidly in water or body fluids. While only one state, the S-enantiomer is thought to be teratogenic, the safer state, the R-enantiomer, is not stable and cannot be preserved in the body. (Bartlett, et. al. 2004)

Thalidomide has traditionally been synthesized by a multistep process which uses expensive ingredients and produces a very low yield. A recent research study by chemists at Stockton University in New Jersey reports on a new rapid synthesis of thalidomide and its analogs using easily obtained reagents which rapidly interact with the assistance of a microwave. It produces relatively high yields. The reaction of the corresponding anhydrides using DMAP (4-N, N- dimethylaminopyridine) as a base catalyst takes place in ten minutes at 150 degrees Celsius. The chemists developing this new method of production describe their innovation as a, "novel green one-pot synthetic technique." (Benjamin, et. al. 2017)

Pharmacology

Thalidomide has an active half-life of 8 to 12 hours. It is broken down by hydrolysis in tissue fluids and metabolized by the liver using the cytochrome p450 system. Although it is absorbed slowly, it has a high oral bioavailability. The plasma concentration peaks after at least two hours. Little drug is bound to protein in the plasma. The average elimination half-life of both enantiomers is five hours. (Vargesson, et. al. 2015)

According the British Columbia Cancer Drug Manual accessed online, high fat meals increase the time to peak concentration. Thalidomide is distributed mostly in the internal organs such as the gastro-intestinal tract, liver and kidneys. It is found to cross the blood brain barrier and is also found in the ejaculate.

In 2004, a new derivative of thalidomide was introduced for treatment of multiple myeloma. Lenalidomide, under the trade name Revlimid is considerably more potent than thalidomide. It has an added amino group at position 4 of the phthaloyl ring and removal of a carbonyl group from the phthaloyl ring. Pomalidomide (3-aminothalidomide) was the second thalidomide analog to be used in treating multiple myeloma. It is even more potent than the analogs before it. A third thalidomide analog, Apremilast is now showing effectiveness as an oral treatment for psoriasis and psoriatic arthritis. (Bartlett, et. al. 2004)

Mechanism of Action: Innate immunity: Effects on Epidermal Regeneration, Soluble Mediators, and Natural Killer Cells

Studies of epidermal regeneration have shown that thalidomide increases human keratinocytes migration and propagation. Testing was done with a motility assay and thymidine incorporation assays to better understand how thalidomide promotes this proliferation. In addition, the chemokine IL-8 that promotes migration of neutrophils and keratinocytes significantly increases with thalidomide treatment. Because of this, researchers have theorized that the mechanism with which thalidomide aids in wound healing is by promoting keratinocyte production and movement. This may be useful in explaining its ability to treat ulcerative diseases, such as Behcet's (a rare disorder causing inflammation in blood vessels with symptoms that include mouth and genital sores, inflamed eyes, and rashes) and aphthous stomatitis (shallow sores inside the mouth or at the base of the gums that make it hard to eat or talk) (Paravar & Lee, 2008).

The exact mechanism of thalidomide's anti-inflammatory action via soluble immune mediators is still being investigated. However, researchers have linked thalidomide's anti-inflammatory action to its ability to speed up the degradation of messenger RNA in blood cells. These effects have been analyzed in both human monocytes and mouse macrophages (Paravar, Lee, 2008).

By reducing the half-life of the messenger RNA coding for TNF-alpha, (Tumor Necrosis Factor) it in turn reduces the blood serum level of TNF-alpha. TNF-alpha is a cell signaling protein (cytokine) involved in systemic inflammation. By reducing TNF-alpha, thalidomide is an effective treatment for inflammatory diseases such as erythema nodosum leprosum (ENL) and lupus erythematosus (LE). Many studies have been performed that confirm this reduction of TNF-alpha. For example, 48-68% of patients with ENL have a reduction of TNF-alpha in the serum from their pretreatment levels. Tuberculosis patients also show reduced levels in in-vitro and in-vivo studies and patients gained a significant amount of weight while on the medication. HIV-1 patients also had reduced TNF-alpha levels while under treatment with thalidomide. A further confirmation comes from studies using rodent models of pancreatitis in which thalidomide was seen to have similar effects (He, et. al. 2013).

Another proposed mechanism of action involves thalidomide's effects on Natural Killer (NK) cells which are vital in destroying tumor cells, intracellular pathogens, and cells infected by viruses. Using in-vitro studies, thalidomide has been observed to enhance NK-cell-mediated lysis of cancerous cells. This was demonstrated in a study that co-cultured NK cells with the same patient's cancerous cells. Additionally, thalidomide increases the secretion of NK cell activators such as IL-12 and specifically induces NK cell antitumor responses. In an in vivo study, the number of NK cells in a multiple myeloma patient were increased with thalidomide therapy. The results of these studies indicate that thalidomide's ability to enhance NK-cells is the mechanism by which it aids in the treatment of multiple myeloma (Paravar, Lee, 2008).

Adaptive immunity: B Cell Antibody Suppression, T Cell Stimulation

Thalidomide suppresses B cell antibody formation in studies primarily using New Zealand Black (NZB) and Murphy Roths Large (MRL) mice. These strains were chosen because they have a genetic predisposition to autoimmune disorders (The Jackson Laboratory Website, updated 2018). Thalidomide inhibits the usually increased production of splenic IgM in NZB mice and splenic and lymph node IgG1 in MRL mice. In clinical studies, leprosy patients receiving thalidomide and dapsone treatment had lower serum IgM levels than patients receiving dapsone alone. The combination of these studies suggests that thalidomide's action may be based on its downregulatory effects on antibody production (Paravar & Lee, 2008).

Thalidomide was shown to be able to co-stimulate T cells once they were already partially activated by the T cell receptor. Co-stimulation is an important mechanism for immune defense. A second signal is sent to naïve T cells which facilitates their initiation and further generation of an antigen-specific effector response. The co-stimulatory effect of thalidomide can be used as an immunological adjuvant, that is, it can enhance the response to tumor antigens in cancer patients (Bartlett, et. al. 2004).

Reduction of Tumorigenesis: Apoptosis and Restriction of Tumor Growth, and Antiangiogenic Activity

Tumors grow and expand due to their ability to evade apoptosis. In studies, thalidomide has induced apoptosis (G1 growth arrest) in human cancerous cells. It has also been shown to decrease the expression of the apoptosis-suppressing protein Bcl-2 in blood and bone marrow of patients with multiple myeloma. Another mechanism proposed is thalidomide's ability to induce monocyte apoptosis by involving the cytochrome c-dependent pathway. (Cytochrome c is known for its role in the mitochondria as a key contributor in ATP synthesis. In our case, we are more interested in thalidomide's stimulation of cytochrome c's other effect which is triggered when a cell receives an apoptotic stimulus. The cytochrome c is then released into the cytosol and triggers programmed cell death through apoptosis) (Paravar & Lee, 2008).

In 1971, Dr. Judah Folkman of Harvard Medical School formulated his hypothesis that tumor growth depends on angiogenesis, the formation of new blood cells in the malignant tissue. Thalidomide, as mentioned above, inhibits limb bud formation in the embryo. This led to the idea that the teratogenic and antiangiogenic actions may be related.

Dr. Folkman made the groundbreaking discovery that tumor

growth and angiogenesis were related when he was working on testing the efficacy of hemoglobin solutions as a substitute for blood transfusions. As a part of this study he injected isolated organs with mouse melanoma cells. He found that the tumors could not grow and spread in the isolated perfused organs, but when they transplanted these same tumor cells into mice, they were quickly vascularized and grew larger. After conducting more research trials to prove his hypothesis, Dr. Folkman published an article in the New England Journal of Medicine that stated that tumor growth depends on angiogenesis and that inhibiting angiogenesis can be used to treat certain cancers. From 1980 to 2005 Dr. Folkman's lab worked on testing twelve angiogenic inhibitors including interferon alpha, fumagillin, endostatin and, notably, thalidomide (Ribatti, 2008).

Angiogenesis, the development of new blood vessels, is vital to the growth and spreading of cancerous tumors. The cancer degrades basement membranes and extracellular matrix and brings endothelial cells towards an angiogenic stimulus. It also involves pericytes and smooth muscles cells as well. Various growth factors are also needed to form new blood vessels from preexisting micro vessels. These growth factors include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) (Fialho, et. al. 2013).

One proposed mechanism for thalidomide's antiangiogenic effect is its ability to inhibit VEGF and bFGF production, which thereby inhibits vessel growth. Studies using human endothelial cells showed that thalidomide inhibits VEFG secretion and cell migration and lowers the total number of capillaries. In studies using chicken embryonic chorioallantoic membrane (CAM) thalidomide inhibited VEGF and bFGF induced vessel growth. In one trial done by the Department of Cellular and Molecular Biology and Pathogenic Agents of the University of Sao Paulo, twelve CAMs from chicken eggs were incubated with thalidomide implants and analyzed after two days to review the percentage of vessel deterioration. The results showed significant regression of vessels in CAM that had thalidomide or thalidomide-loaded implants as compared to the negative control (NC) group and an implant not containing any drug (Fialho, et. al. 2013).

Another study proposed that thalidomide inhibits endothelial cell proliferation by inhibiting the binding of the SP-1 transcription factor, which has many binding sites on the VEGF promoter. This protein usually binds to the VEGF promoter to initiate and activate the transcription of the gene directly. When its binding is inhibited, it causes less VEGF to be produced and therefore limits the cells' growth (Yabu, et. al. 2005).

A third mechanism of action being researched is thalidomide's ability to suppress the VEGF gene by downregulating the VEGF receptors Flk-1 and neuropilin-1. By reducing the productivity of the VEGF receptors, thalidomide lessens the effects of the vascular endothelial growth factor in general. This causes less signals for angiogenesis to be sent, so the body forms less vasculature. With a smaller supply of blood, the growing limbs do not receive enough oxygen and nutrients, and they subsequently do not grow properly and result in under developed or malformed limbs (Yabu, et. al. 2005).

A study done using zebrafish embryos and Human Umbilical Vein Cells (HUVEC) showed promising results in this area. The thalidomide treated embryo was shorter in length than the control. In addition, the lumens of the dorsal artery and posterior cardinal vein were clearly seen (with hematoxylin and eosin staining) in the control but were so reduced in the embryo treated with thalidomide that they were barely visible (Yabu, et. al. 2005).

Teratogenesis

Over the past fifty years, many separate models for thalidomide embryopathy have been proposed, however a full understanding of its mechanism of action is still incomplete. The proposed theories are not necessarily mutually exclusive. And it is possible that multiple mechanisms of action are each involved to some extent. Additional theories include nerve toxicity, inhibition of cell adhesion molecules and effects on chondrogenesis.

One breakthrough discovery revealed that Cereblon (CRBN), a protein encoded by the CRBN gene, may be the primary target for binding by thalidomide. The authors found that thalidomide binds directly to CRBN, a substrate receptor for the CUL4A-DDB1 E3 ubiquitin ligase, a protein that employs an E2 ubiquitin-conjugating enzyme that carries ubiquitin, recognizes a protein substrate, and assists the transfer of ubiquitin from the E2 to the protein substrate. The ubiquitin is used to tag specific proteins to be broken down by proteasomes. This binding inhibits the activity of the assembled E3 ubiquitin ligase complex so that it does not tag the specific proteins, and an unknown substrate is allowed to accumulate. This in turn effects the expression of the fibroblast growth factor eight (FGF8) and causes growth defects. Thus, the action of the E3 ubiquitin ligase complex is necessary for limb outgrowth (particularly in zebrafish) and by disrupting this complex, thalidomide induces teratogenic effects. Consequently, they demonstrated that the deformities caused by thalidomide were directly mediated through stopping its inhibition of CRBN. In zebrafish and chickens, when they used an overexpression of a CRBN mutant that does not bind thalidomide, they did not notice the defects usually caused by this drug, proving that it is the binding of the drug to CRBN that is responsible for the embryopathy effect (Ito. et. al. 2010).

Following this discovery, CRBN was also shown to be crucial for the mechanism of action of thalidomide's anti-myeloma properties, as well as other immune-modulatory drugs (IMiDs). One of the downstream targets of CRBN was shown to be interferon regulatory factor 4 (IRF4). This protein which regulates the transcription of interferons is essential for myeloma cell survival and is downregulated by IMiD therapy (Zhu, et. al. 2013).

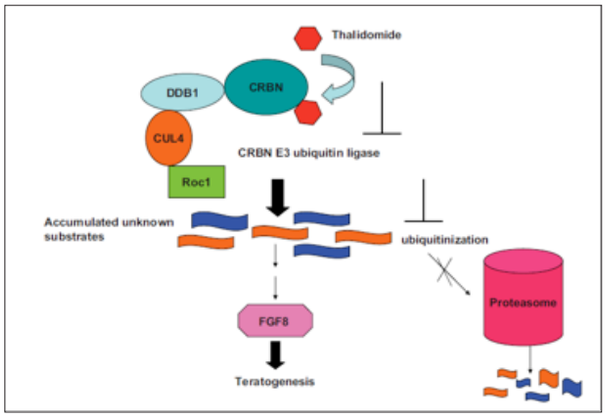

Figure 1. Depiction of the downstream targets of thalidomide that may lead to its teratogenic properties (Zhu, et. al. 2013).

A recent series of experiments supported in part by the National Cancer Institute further confirmed the hypothesis that antiangiogenesis and teratogenesis are related. The researches tested a variety of angiogenesis inhibitors, including thalidomide and its analog CPS49, using zebrafish and chicken embryo models. They focused on assessing the developmental defects and teratogenic effects that the drugs caused. They concluded that different classes of angiogenesis inhibitors, regardless of the molecular target or specific mechanism of action, are teratogenic to chicken embryos. This study also proved that using chicken embryos and zebrafish embryos is a valid way to screen new drugs for teratogenic effects before they are used in clinical trials on women of childbearing age (Beedie, et. al. 2016).

New Research in Treatments Using Thalidomide and Its Derivatives: Multiple Myeloma (MM)

Multiple Myeloma is a type of blood cancer that affects plasma cells. In multiple myeloma, malignant plasma cells amass in the bone marrow crowding out the normal plasma cells that help fight infection. This disease can damage the bones, immune system, kidneys, and cause anemia. A randomized trial compared the costs and benefits of using a melphalan (chemotherapy) and prednisone (steroid) treatment in conjunction with thalidomide (MPT) as opposed to melphalan and prednisone (MP) alone. The testing was done on 135 elderly patients over the age of 65 with MM who were ineligible for stem-cell transplants. The results showed a significant increase in the median progression-free survival (PFS) of patients using a combination of drugs that included thalidomide. PFS is the length of time during and after the treatment of a disease that a patient lives with the disease but it does not get worse. Also, the overall survival (OS) rate was 52 months as opposed to only 32 months for patients using only melphalan and prednisone. The following graph compares the progression-free survival rate of patients using MPT versus MP treatment. The MPT group has a significantly higher PFS rate. The Y axis is the fraction of living patients who began in the study. Both the MPT and MP groups start at 1.00, meaning that 100% of participants are alive. The X axis is the months of the treatment. The MPT group has a higher PFS rate during the forty months of the trial. The P value is 0.02, meaning the results are statistically significant (Sacchi, et. al. 2011).

One downside of adding thalidomide to the treatment regiment is the adverse side-effects that were more frequent in patients using thalidomide, which will be discussed in more detail in the "Adverse Effects of Thalidomide" section of this paper. Researchers observed a significant increase in neutropenia, deep venous thrombosis, infection, and peripheral neuropathy. The incidence of toxicity correlates with the drug dosage. The study concluded that while including thalidomide in the therapy showed an increase in activity against MM, it came with a substantial cost, which will be discussed later.

Trials have also been conducted using the second-generation thalidomide derivate, lenalidomide. In a randomized, controlled clinical trial, lenalidomide therapy (in combination with dexamethasone, a corticosteroid, or with melphalan and prednisone) significantly improved PFS in patients with newly diagnosed MM who were not eligible for stem-cell transplants as compared to MP treatment alone. These improvements in progression-free survival were also reflected in patients' health-related quality of life (McCormack, 2015).

In general, the analog was found to have improved efficacy and increased tolerability as compared to thalidomide. Although the treatment regiments had similar efficacy results, lenalidomide was observed to have fewer toxic side-effects than its parent drug. The continuous use of lenalidomide did not have a negative impact on the drug's tolerability. It also did not increase the cases of neutropenia as compared with shorter-term use of the drug. With new analogs, melphalan and prednisone plus a thalidomide-type drug could be considered the new standard of care for the treatment of patients with MM over age 65 years and for younger patients who are transplant-ineligible (McCormack, 2015).

Recent studies are working on understanding lenalidomide's mechanism of action. They propose that it affects signal transduction, also known as cell signaling, which leads to the suppression of COX-2 but not COX-1. This can partly explain its selective efficacy on cells. Meaning, it is able to be more selective in the cells it targets so it can have a greater potency with less side effects. Even though the exact molecular targets of lenalidomide are not well known, its activity across a spectrum of conditions highlights the possibility of multiple target sites of action (Kotla, et. al. 2009).

Malignant Melanoma

Malignant melanoma is the most aggressive and life-threatening skin cancer. It develops in the melanocytes and has a very high

tendency to spread to other parts of the body. Symptoms can include a new, unusual growth or a change in an existing mole. Brain metastases will develop in almost half of the patients with advanced melanoma and in 15-20% of these patients, the central nervous system is the first site of relapse. The overall survival rate is very short, only two to four months (Vestermark, et. al. 2008).

In one study, the antitumor activity and toxicity of thalidomide was evaluated in patients with phase II brain metastases related to metastatic melanoma. Thalidomide was administered orally to patients, with the dose increasing over a one-month period from 100 mg per day to 400 mg per day. Twenty-five men and eleven women with a median age of forty-eight years were enrolled in the study. The average survival rate for the study group was unchanged from historical data. Median PFS was 1.7 months and OS was 3.1 months. Although thalidomide showed limited activity against the metastatic melanoma in the central nervous system, minor effects on peripheral tumor manifestation were noted. This led researchers to conclude that thalidomide may one day be part of the treatment plan for patients with that form of metastasis in the future. Researchers also concluded that more investigation should be done using thalidomide in combination with Temozolomide, a cytotoxic chemotherapy drug (Vestermark, et. al. 2008).

Persistent Erythema Multiforme

Erythema multiforme is a common, usually self-limited disease which predominantly affects patients in their 20s and 30s. Its symptoms include target shaped lesions (circular red patches with central clearing) on the skin and mucous membranes. There are three clinical subgroups of erythema multiforme: classical, recurrent, and persistent erythema multiforme. The most severe form is the persistent erythema multiforme which can leave a patient stricken with continuous lesions. No potential cause has been found yet, so this subgroup is defined as idiopathic.

Thalidomide can be effectively used as a treatment for this skin condition. It was initially introduced as a treatment for persistent erythema multiforme as early as the 1980's because of its immunomodulatory and anti-inflammatory effects. The following case report in the recent literature illustrates its effectiveness. A fifteen-year-old boy in the Republic of China experienced a sudden onset of target-like lesions on his trunk and limbs. Although the wounds healed within two weeks, new eruptions continued to appear. After unsuccessful treatment attempts with oral corticosteroids and topical agents as well as the antiviral drug valaciclovir, thalidomide was prescribed to the patient. After two weeks of administering 100 mg per day, the lesions gradually healed, and no new ones developed. After two months of slowly reducing the dosage, there was no recurrence of disorder. The researchers noted that because of the risk of neuropathy associated with taking daily doses of this medication, clinical vigilance and regular neurological exams are advised (Chia-Wei, et. al. 2008).

Cutaneous Lupus Erythematosus (CLE)

Cutaneous Lupus Erythematosus is an autoimmune disease, which affects multiple organ systems in the body. In this disease an individual's own immune system attacks various cells causing a wide variety of symptoms. Typically, it causes extensive, disfiguring lesions. Although these lesions are not life threatening, they can be very itchy or painful. Many patients respond to standard treatment methods such as sunscreen, topical corticosteroids, or oral antimalarial drugs. However, for cases in which these treatments did not prove useful, or if the CLE was severe, thalidomide can be an important therapeutic option (Sharma, et. al. 2007).

In a study done by the Department of Dermatology at the University Hospital of Leuven, Belgium, thirty patients received thalidomide treatment for refractory CLE over fifteen years (from February 1998 to August 2013.) Each of these patients had previously tried at least two different drug treatments with no success. All of them were required to follow vigorous contraceptive methods because of thalidomide's known teratogenic properties. Patients received an initial dose of 50 mg per day, which was increased to 100 mg if the CLE was extensive. Although six patients prematurely stopped treatment due to the negative side-effects, all patients who continued in the study experienced improvements within 1-9 weeks after beginning treatment. A high rate of relapse (73%) was observed in patients who stopped thalidomide treatment. In addition, five patients were not able to be weaned off the drug due to flare-ups of CLE when they attempted to taper off the doses. The researchers concluded that while thalidomide treatment for CLE does have strong efficacy, because of its considerable risk of polyneuropathy, it should only be considered as a possible therapy for severe cases of CLE or for patients who have exhausted other treatment options without significant relief (Baret, et. al. 2015).

In another study done on twenty-five patients in India suffering from various inflammatory skin diseases, including discoid lupus erythematosus, thalidomide was found to be an effective treatment. Of the seven patients with LE, four had excellent response, two had partial response and one discontinued treatment due to deep vein thrombosis (DVT). The authors recommended thalidomide as an effective treatment but advised physicians to be watchful of thrombo-embolic events (Sharma, et. al. 2007).

Erythema Nodosum Leprosum (ENL)

When the author's father, Stanley Newfield MD, was a young dermatology resident training at the United States Public Health Service Hospital in Staten Island, New York he was involved in diagnosing and treating many cases of leprosy, also known as Hansen's disease. This research interest was perhaps especially appropriate since he is a kohen, the traditional caretaker for

people suffering from *tzoras* (leprosy) in the Torah.

As a federal institution, his hospital was responsible for caring for patients with transmissible infectious diseases. Leprosy patients were referred from a large area of the north-eastern United States for treatment. One of the complications of leprosy is the immune-system reaction known as Erythema Nodosum Leprosum. ENL is characterized by the presence of many inflammatory skin nodules and symptoms such as fever, arthritis, eye inflammation, neuritis, and swollen lymph nodes. It was observed that many patients suffered from ENL, which was even more distressing to them than some of the other leprosy symptoms such as loss of sensation in the extremities and/or exaggerated skin folds on the face.

Dr. Newfield explained that he was surprised, back in 1979, to find that the infamous drug, thalidomide, was the miraculously effective treatment for ENL. He was well-aware of the teratogenic effects of the drug which he learned about in detail in medical school. Now this same drug was resurrected for a new use, albeit with strong precautions to avoid administration to pregnant women. Due to his involvement in leprosy treatment, he was coauthor of a research report on the epidemiology of leprosy in New York City, which was published in the prestigious Journal of the American Medical Association.

Many people have a fear of leprosy since it is transmissible through contact with an infected patient. However, this seems to require prolonged exposure, usually in a household setting. The research verified that very few leprosy patients acquire the disease in the continental United States, rather they brought the disease with them when they immigrated to this country. The average latent period from entering the United States until onset of symptoms was 4.8 years, with a range of 0 to 38 years (Levis, et. al. 1982).

Almost forty years have passed since thalidomide was first used for ENL treatment, and it remains the drug of choice for this condition, as evidenced by two current research reports originating from India, a country with a large number of leprosy patients. The first paper reports on eleven patients with ENL. Six of those patients had excellent response to thalidomide and five had to stop treatment prematurely due to side-effects (most commonly DVT but also rashes and tremors.) (Sharma, et. al. 2007) In the second study following one hundred patients diagnosed with ENL, the group of fifty patients treated with thalidomide had a faster and longer lasting clinical response than the control group of fifty patients treated with prednisolone, a strong steroid medication. Patients on thalidomide also experienced fewer relapses of their cutaneous symptoms (Kaur, et. al. 2009).

Animal Models of Alzheimer's Disease (AD)

Alzheimer's Disease is a degenerative disease that destroys memory and other important mental functions. This is an incurable condition that afflicts an estimated 5.7 million people in the United States, mostly over the age of sixty-five. Research studies are currently being performed on a mouse model of AD. In one study, healthy mice were injected with streptozotocin (STZ), a chemical that causes AD-like cognitive deficits. In this study, one group of mice were pre-treated with thalidomide. Learning and memory behaviors were evaluated on the seventeenth, eighteenth and nineteenth days of the study using the Morris water maze test. In this test, a mouse is placed in the center of a circular pool and must find the hidden platform that allows it to escape. Mice that are not treated with the drug will improve their time to escape after doing the test multiple times. The SZT injections caused a significant decrease in the mice's improvement in their performance on the test. In comparison, the learning and memory behaviors of the thalidomide treated mice were significantly better preserved and they were able to complete the tests with improved timing, although they were not as improved as the mice who did not receive any drugs. This positive result appears to be due to the anti-inflammatory effect of TNF-alpha inhibition (Elçioğlu, et. al. 2013).

A second study used a strain of mice with the human APPswedish transgenic (APP23) mutation. This mutation is associated with Alzheimer's Disease. These mutated mice show deficits in spatial memory which become severe with age. (The Jackson Laboratory Website. 2018) This report showed that thalidomide treatment improved memory and learning ability in the mutated mice. The level of TNF-alpha in the thalidomide treated mice was decreased. This in turn reduced the amount of amyloid (A beta 1-42,) a harmful protein, which accumulated in their brain tissue. The control group mice treated with the inactive vehicle had 750 units of harmful amyloid, while the thalidomide group had only approximately 100 units (He, et. al. 2013).

In mice brain tissue, the control group (treated with the vehicle) developed a large number of harmful senile plaques but the thalidomide treated group showed very few.

Adverse Effects of Thalidomide

This section summarizes the adverse effects of thalidomide, some of which have also been mentioned previously. Thalidomide was withdrawn from widespread use shortly after the devastating effects on developing embryos was discovered. The drug came back into limited use for malignant and inflammatory diseases in non-pregnant women. It can still have serious harmful effects: peripheral neuropathy (pain and tingling in the hands and feet), venous thrombosis (blood clots in the veins), skin rashes, constipation, somnolence (excessive daytime sleepiness), weakness, and bradycardia (BC Cancer Drug Index. updated 2018).

Peripheral neuropathy is a common side effect that is associated with prolonged use of the drug, but there is no clear mechanism of action that explains its correlation with a cumulative dose. The risk for nerve damage is highest after 6 months or more of therapy. The symptoms are usually reversible when the

patient stops thalidomide treatment, although some patients do suffer long term nerve damage (Rajkumar, et. al. 2002).

Constipation caused by thalidomide can vary from mild to severe. As many as 90% of patients can develop mild constipation. This effect is thought to be secondary to thalidomide's action on the autonomic nerve endings in the gut. Severe constipation usually occurs in patients who are taking high doses of thalidomide. It is especially prevalent among patients who are already prone to developing constipation, such as those who lead an inactive lifestyle. Change of diet and exercise can help alleviate this side effect (Hall, 2003).

Deep vein thrombosis and pulmonary embolisms only occur in one to three percent of the patients receiving thalidomide as treatment for myeloma. There is not enough research done yet to determine whether the risk of DVT in these patients is higher than usual for patients receiving other types of treatment for this severe malignancy, due to the nature of the disease or the side effect of immobility that it can cause. Patients confined to bed rest tend to have higher rates of DVT. The risk is also elevated when patients receive thalidomide treatment in combination with dexamethasone or other chemotherapy drugs, have an inherited thrombotic predisposition, or are over the age of sixty-five (Hall, 2003).

A recent case report documents a thirty-eight-year-old female from Puerto Rico with a history of prenatal thalidomide exposure. She suffered from phocomelia, the dramatic birth defect in which the hands or feet are attached close to the trunk. An MRI scan revealed that the uterus and vagina were also absent. These internal anomalies have been less appreciated by physicians because they are not noticeable without special imaging, however they can cause serious symptoms such as pain and malignancy (Dotters-Katz, et. al. 2013).

The analog drug, lenalidomide, has not been found to be teratogenic in rabbits, a sensitive species used to detect birth defects. However, it may still have adverse effects such as morbilliform rashes (Huang, et. al. 2008). In a study done at Mount Sinai Medical Center in New York City, doctors found that 7.2% of the 806 patients receiving IMiD treatment developed rashes. In almost all cases, the rashes could be managed without having to discontinue treatment. Thus, it appears that lenalidomide may lack some of the adverse effects of thalidomide, while still having superior immunomodulatory and antiangiogenic efficacy than its parent drug (Barley, et. al. 2016).

An additional analog, Pomalidomide, is now an approved drug for MM. A research study conducted in Aberdeen, United Kingdom analyzed its effect on zebrafish and chicken embryos. The tests showed no detectable teratogenic, antiangiogenic or neurotoxic effects. Despite having less side effects, it has more anti-inflammatory properties than either thalidomide or lenalidomide (Mahony, et. al. 2013).

Conclusion

The psalmist wrote, "The stone that the builders rejected became a cornerstone." (Psalm 118:22) This report illustrates this concept as it pertains to the drug, thalidomide. This drug was universally banned due to its harmful side-effects. Years later it was found to have unique healing properties in several serious diseases.

It appears that the continued use and development of thalidomide treatments is justified. Although it can have many serious side effects, specifically crippling teratogenesis, it is often the last resort as treatment for patients suffering serious diseases. Care should be taken to prevent pregnant women from using this drug to avoid causing birth defects. Given the significance of these effects, the future of thalidomide is not in the drug itself but in the derivatives that are now being tested. Lenalidomide and pomalidomide are more effective and have less toxic effects. As scientists continue to discover their specific mechanisms of action they will be able to alter the drug to have even less adverse effects and help more patients worldwide. The prognosis for PFS and OS for patients with MM and other forms of cancer will decrease even more and give the people suffering with these diseases hope for their future. With this new and exciting research thalidomide and its analogs have justly become important medical treatments.

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Nonsurgical Approaches to Glioblastoma

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Abstract

Due to the sensitivity of location, brain cancer is one of the most difficult and deadly known cancers. There are various forms of cancer in the brain with many shared characteristics as well as unique manifestations in each. While cancers originating in the central nervous system present in several ways, the most common forms are high grade gliomas generally, and glioblastoma or anaplastic astrocytomas specifically. With the advent of technology, researchers have been able to propose and refine extensive profiles of these relentless tumors, enabling greater and more successful treatment profiles to be developed. Where treatments used to consist primarily of chemotherapy and surgery, research has enabled the development of immunotherapy and gene therapy techniques as well as alternative treatments to take on the caustic disease.

Introduction

The foremost distinction of brain tumors is between primary; those that originated within the brain itself, and secondary; those that developed beyond the blood brain barrier and migrated to the brain under metastatic conditions. Secondary tumors account for the majority of all brain cancers and approximately 50% of these tumors metastasize from lung cancers (Gallego, 2015). According to the 2014 World Cancer Report, patients diagnosed with glioblastomas were measured to have a median survival time of 12-15 months post diagnosis with fewer than 5% of patients living beyond 3 years (Patchell, 2003). Under the World Health Organization's grading protocol for central nervous system (CNS) cancers, glioblastomas (GBM) are a grade IV glioma (Bleeker, et al., 2011). This is the most common form of primary brain tumor and the most fatal found in humans, accounting for 15% of all intracranial neoplasms (Bleeker, et al., 2011; Young, et al., 2015). The average age at diagnosis is 64 years old and the approximate annual incidence of GBM per 100,000 population is 3.19 cases, making an understanding of the disease and its treatment of paramount importance (Ostrom et al., 2017). GBMs develop rapidly de novo in astrocytes, star-shaped neuroglial cells suspected to play a role in blood brain barrier maintenance and neurotransmitter management (Kolb & Whishaw, 2009). This swift development occurs without any previous primary or lower grade lesion development that may indicate or preclude the GBM onset, making early diagnosis and adequate treatment more difficult.

Since 2005, the first-line standard of care has been established as surgery followed by chemotherapy, most often with temozolomide (TMZ), and radiotherapy. Despite the established longevity of this standard, its efficacy is highly questionable and there is still recurrence in the majority of cases (Patchell, 2003). To complicate matters further, there is no second-line standard of care for glioblastomas and recurrence is consistently fatal (Roy, et al., 2015).

Discussion: Difficulties with Conventional Treatments

One of the significant factors contributing to the aggressive character of GBMs is their infiltrative nature. Astrocytes have extensive networks of processes forming from their cell bodies, creating an intricately branched structure extending throughout the nervous system. Where some cancers merely invade their host tissue, the cells of grade IV astrocytomas penetrate their host with protrusions. These processes weave a complex margin with host tissue that blurs the border between invasive and host cells. This makes complete surgical resection nearly impossible as practitioners are unable to differentiate between, and separate, the healthy and malignant tissue. As a result, recurrence is extremely high in GBMs and alternative treatments must often be considered post-surgery when complete resection is not achieved (Patchell, 2003).

Beyond the inability to properly remove gliomas, the benefit of surgery is questionable. In a study of recurrent brain tumors, researchers found no significant benefit to a second resection, though a first resection did confer an increased overall survival (Suchorska et al., 2015). Nonetheless, the nature of the neurosurgical procedure lends itself to various complications. Along with the wound and postsurgical medical complications, brain surgery runs the risk of systemic and cortical injuries, damaging physical and mental health with the most common risk of neurological impairment (Jackson, et al., 2016). Research also found that the acquisition of motor and language deficits post-surgery is linked to decreased survival rates compared to patients without surgical brain damage (McGirt et al., 2009). The results of these studies indicate that the benefit of surgery is questionable.

Chemotherapy treatments generally follow the attempted resection procedures but have limited success due to the impermeability of the blood brain barrier to foreign chemicals (Deeken & Loscher, 2007). TMZ is a common chemotherapy drug for gliomas and has demonstrated mild success against GBM and anaplastic astrocytoma but its overall effectiveness is slim and is accompanied by a cocktail of undesirable side effects (Friedman, et al, 2000). The established first-line standard of care is precarious and insufficient. As a result, research has turned to alternative methods by which to treat and manage this deadly.

Genetic Analysis

In order to purposefully theorize and discuss possible solutions and treatments, GBM manifestation must first be understood. Occurring primarily in older patients, GBM progresses quickly and with low survival rates. A minority of cases have been seen in younger patients with a history of epilepsy connected to progressive low-grade gliomas (Ostrom et al., 2015). To address the lack of understanding, research continues to look for commonalities in tumor onset. As genomic instability is an enabling characteristic

of cancer, efforts have been made in the field to source a primary effector with genetic basis. As of yet, there is only one confirmed molecular predictive factor for GBM - methylation of the O6-methylguanine-DNA methyltransferase (MGMT) promoter (Bleeker, et al, 2011). The relative dearth of information on the molecular basis for GBM requires additional research.

Whole-genome single nucleotide polymorphism (SNP)-based array analysis of gene expression in GBM patients has indicated several genetic changes that shed some light on the nature of the illness. Notably, loss of heterozygosity frequently occurs on the 17p gene, which contains the tumor protein 53 (p53) (The Cancer Genome Atlas (TCGA), Research Network, 2008). Loss of heterozygosity refers to the deletion of a chromosome portion with the corresponding homologous segment being duplicated to compensate for the loss of gene neutrality (Bleeker, et al, 2011). The significance of this mutation is reflected in the loss of genetic code for p53. p53 encodes a nuclear phosphoprotein involved in regulation of cell proliferation. In other words, wild type p53 is a tumor suppressor gene. Mutations in p53 not only cause loss of tumor suppression, they can activate p53 to an oncogene in a negatively dominant fashion implicating these mutations in a number of cancers (Finlay, et al., 1989). Further, loss of heterozygosity of chromosome 10q is associated with poor rates of survival and is the most common genetic alteration found in primary and secondary GBM's. This chromosome contains various tumor suppression codes, most notably the region containing ANXA7, an epidermal growth factor receptor (EGFR) inhibitor, and deletions contribute to a proliferation of cell growth; a distinguishing behavior of cancerous tumors (Yadav et al., 2009).

Additional research focuses on genetic amplifications associated with GBM. Specifically, amplification of the gene for EGFR on chromosome 7 has been shown to be a consistent characteristic in glioblastomas (Finlay, et al, 1989; Bleeker, et al, 2011). Focal amplifications (amplifications containing a small concentration of genes) correlate with overexpression or mutation of EGFR, leading to subsequent activation of the PI3K/AKT pathway, another indicator of poor prognosis (Beroukhim et al., 2007; Phillips et al., 2006). General amplification of entire chromosomes, specifically chromosome 7, has shown correlation with the activation of the Met axis, a codependent cycle with Hepatocyte Growth Factor (HGF) which furthers the occurrence of cell proliferation (The Cancer Genome Atlas (TCGA), Research Network, 2008).

Another genetic component considers IDH1 mutations. The IDH1 gene codes for isocitrate dehydrogenase 1, a critical component of the citric acid cycle, catalyzing the conversion of isocitrate to α-ketoglutarate. Mutations of this gene have been discovered in 12% of glioblastomas (Watanabe, et al., 2008). Mutations of this enzyme do not appear to cause loss of function, rather, cancer-associated IDH1 mutations alter the reaction of the enzyme, enabling it to catalyze the NADPH-dependent reduction of α-ketoglutarate to R(-)-2-hydroxyglutarate (2HG). As many humans have a reduced capacity to dispose of 2HG in an efficient manner, overaccumulation of the metabolite has been implicated in the formation of malignant brain tumors (Dang et al., 2009). 2HG also has a penchant to activate NF-κB, a prominent protein complex that controls cytokine production, cell survival, and DNA transcription, implicating it in many cancers, including various leukemias (Chen et al., 2016).

Ultimately, the strongest evidence of a genetic aspect to GBM is that of the MGMT promoter. While TMZ treatment in general has shown some benefit, much of its effectiveness is diminished by this molecular occurrence. TMZ acts by modifying the O6-position in guanines. This translates to DNA lesions leading to DNA cross-links preventing cell replication. MGMT can remove the alkyl groups and contribute to TMZ resistance but not when methylated. Because about 50% of patients exhibit MGMT methylation, the effectiveness of TMZ is considerably lessened in approximately half of all cases (Bleeker, et al., 2011). Research is being done particularly in this area to better understand the environment surrounding methylation and prospectively harnessing the power of methylation to increase TMZ effectiveness (Lee et al., 2018).

Additional aberrations in genetic behavior exist in GBM with varying degrees of frequency. With more tools becoming available, it is apparent that the picture of GBM is not clear and further genetic research is required. Nonetheless, these tools enable researchers to identify the most prevalent deviations from typical behavior. For example, many alterations occur within three primary pathways; the p53, RB, and PI3K/AKT pathways, and appear to occur in a mutually exclusive fashion (The Cancer Genome Atlas (TCGA), Research Network, 2008). This defines a narrower scope of application, hopefully resulting in the development of rational therapeutic techniques and drug design. These genomic approaches may ultimately contribute to more individualized therapies for greater patient longevity.

Immune Evasion and Immunotherapy

In addition to the complications involved with gene aberrations in malignant gliomas, the impressive rate at which GBMs evade natural immune response opens another point of study for prospective treatments. Avoidance of the immune system was added as a defining characteristic of cancer in 2011 and an examination of the natural immune response has helped develop vaccinations and treatments for various diseases (Hanahan & Weinberg, 2011). Nonetheless, the ability of tumors to dodge these receptors requires a better understanding, specifically in the brain. In line with original theories, the CNS is immunologically privileged. Unfortunately, it is not quite as privileged as once suspected. While early theories assumed this privilege was a result of isolation of the brain by the blood brain barrier,

newer research refutes this. Peripheral immune cells do in fact cross the barrier, but CNS neurons and glia regulate immune responses in a different fashion than other cells (Carson, et al., 2006). Researchers have demonstrated this deviance from the standard immune system using experimental foreign grafts to rabbit brain. These grafts were rejected at a much slower rate than grafts to less unique locations (Medawar, 1948). An additional indication of CNS immune system inadequacy is the immune response of the brain in multiple sclerosis patients. The immune system of affected individuals appears to contribute directly to the proliferation of the disease rather than fighting it (Hemmer, et al., 2015). Despite these deficiencies, analysis suggests proper manipulation of the immune system can assist in the fight against brain cancers, achieved through understanding the mechanism by which tumors evade it.

ICT-107 is a multiple-antigen-pulsed dendritic cell vaccine containing multiple tumor-associated antigens (HER2, TRP-2, gp100, MAGE-1, IL13Rα2, and AIM-2) known to interact with glioma stem cells. These antigens may be ideal for vaccination as their introduction to the dendritic cell tumor environment stimulates T-cells to generate glioblastoma specific cytotoxicity (Huang et al., 2017). This immunotherapy was the first such trial to reflect significant results in terms of overall, progression-free survival in newly diagnosed GBM (McGranahan, et al., 2017). A phase III trial is awaiting additional funds to further investigate the effectiveness of this vaccine (Phuphanich et al., 2012). Another vaccine in phase II trials is Gliovac. This vaccine utilizes autologous antigens from the patient subjects combined with allogeneic antigens (antigens with a dissimilar genetic makeup from that of the patient) derived from other GBM patients. Beyond using tumor lysate, the Gliovac vaccine is designed similarly to ICT-107 to encourage the development of cytotoxic T-cells to enhance the struggling immunological response (Schijns, Virgil E J C et al., 2015). Further studies examine the link between the CTLA-4 receptor, a constitutively active receptor on the Treg gene, and its downregulation of the immunologic response in active T cells (Leach, et al., 1996). By blocking CTLA-4, researchers have seen considerable success with enhancing antitumor immunity, rejecting both artificially introduced and pre-established tumors. The rapid growth of immunotherapeutic treatments stresses the importance of a more comprehensive understanding of the immune system and its reactions to foreign in vivo developments. More specific information on marker antibodies will go a long way in furthering treatments, not only for GBM but also for other immuno-evasive diseases.

Treatment with Infection

A seemingly counterproductive method of treatment has been considered to address incomplete resection of glioblastoma tumors. While the actual number of surgical site infections after malignant brain tumor resection is not well established, data indicates the rate to be about 3.4% (Uzuka et al., 2017). A speculative theory supported with anecdotal evidence presented the idea that patients who developed postoperative bacterial infections and survived had a radically increased overall survival. A study reported four cases of patients with surgical site infections living well beyond the expected range of survival for glioblastoma (Bowles, et al., 1999). This theory has been subjected to several trials and though not statistically significant, results showed that many infected patients live longer on average though reverse logic may be cloud results. Infection may correlate with increased life span because patients that live longer are more likely to develop an infection, not because infection lengthens survival (De Bonis et al., 2011).

Antiangiogenic Treatments

Antiangiogenic treatments have shown a lot of promise in cancers management and particularly in that of GBM as second-line treatments. The rationale for antiangiogenics is strong; as cancers are highly vascular, preventing the growth of blood vessels to supply the tumor should help deplete tumor resources and severely retard malignancy. Tumors are known to exploit the body's nutrient and oxygen supplies and require enhanced networks of blood and lymphatic vessels to facilitate this resource abuse (Nishida, et al., 2006). Vascular endothelial growth factor (VEGF) is a signaling protein that stimulates the creation of blood vessels and has received increased attention as an indicator of oncogenesis (Pavlova & Thompson, 2016). To address this, researchers developed Bevacizumab, a monoclonal antibody that counters the effects of VEGF and has been approved for trials in the United States for some time. Dosed intravenously, Bevacizumab has been used independently as a monotherapy and in pairs or groups of antiangiogenics for a multi therapeutic approach in various cancer types. In early trials, a statistically significant number of patients demonstrated an improvement in overall survival, but randomized phase III trials have been unable to demonstrate an appreciable improvement in overall survival required to move this form of therapy forward (Bleeker, et al., 2011).

Retroviral Delivery

A promising treatment technique addresses issues with alternative treatments. Retroviral replicating viruses (RRVs), are a novel way of treatment delivery and contain a wide variety of unique characteristics that allow for highly specific gene transfer in an efficient manner. Pharmaceutical companies are combining many of the above therapies into a single, comprehensive treatment method for recurrent high-grade gliomas with the goal of extending these concepts to further treatments.

Retroviruses are positive-sense RNA viruses. These obligate parasites cannot reproduce or complete their life cycle without a host cell. Once within a host, these viruses use the enzyme reverse transcriptase to produce DNA from the viral RNA

sequences. This DNA strand is then incorporated into the host cell genome with an integrase enzyme. By transferring it's genetic data into the host, the virus ensures it will be reproduced when the host reproduces. The applications of these viruses can allow scientists to produce desired proteins and genetic products for treatment purposes. These viruses confer many advantages to the treatment of cancers and in particular, of glioblastoma. One such benefit of RRVs is the selective acceptance within cells. It has previously been established that tumors exhibit a distinct loss of immunity and that a central aspect of cancer development is the evasion of the immune system. Cell populations naturally assess tissue to prevent proliferative, uncontrolled growth, and cancers evolve to avoid the anti-tumor response. This evolution evades immune checkpoints and regulators but also enables immunoediting, the process by which cancers read, react, and adapt to immune processes. By disabling the anti-tumor response, cancer cells can replicate continuously unchecked (Butt, et al., 2004). The lack of immunity that permits cancers to exist also creates an opportunity for medical intervention. RRVs are generally recognized by healthy cells and dealt with accordingly by the normal immune system, but the reduced defense mechanism of cancer cells allows the RRV to incorporate genetic components into the cancer gene pool. This selective acceptance safeguards healthy tissue while priming cancer cells. Another key feature of RRVs is the ability to spread non-lytically and without an exceptional inflammatory response. Gene products are not produced until the virus is activated and this avoids an inflammatory response from the innate immune system (which is not heavily compromised). Coupled with a nonlytic retroviral infection, this delivery system enables researchers to target specifically cancerous or immunocompromised cells (Logg, et al., 2012).

The Tocagen therapy relies on an engineered retrovirus known as Toca 511 (vocimagene amiretrorepvec) for delivery. Toca 511 is a murine leukemia virus that infects dividing cells, preferentially integrating into cells that are immune-deficient — in this case GBM cells. In addition, the retrovirus has no trouble crossing the blood brain barrier, an issue that arises with other treatments. The virus contains the genetic code for an altered cytosine deaminase (CD), a protein enzyme that catalyzes the reaction converting cytosine residues to uracil (Ostertag et al., 2011). In trials, after several cycles of RRV treatment, researchers found a high concentration of CD present in tumor cells, but none or insignificant levels expressed in quiescent cells. The retrovirus delivery method efficiently targets tumor cells and integrates its genetic contents into the DNA of the host cell without affecting the genetic integrity of healthy cells in the body.

While the success of the genetic transfer is significant, the expression of optimized CD within tumor cells is of little consequence. Further action is required to have an impact on cancerous cells. 5-fluorocytosine (5-FC) is subsequently administered as a prodrug, a drug in an inactive form. Despite widespread uptake of 5-FC by the majority of cell types, this inactivity prevents any notable response from quiescent or typical cells. However, in the Toca-511 pretreated tumor cells, 5-FC is converted by CD to 5-fluorouracil (5-FU), a potent antineoplastic cancer drug used as the drug of choice for colorectal, esophageal, gastric, and many other cancers. While the precise mechanism of 5-FU is unknown, it is suspected to interfere with DNA synthesis, protein synthesis, and RNA processing (Pinedo & Peters, 1988). Collectively, these contribute to an incompatible state for the tumor cell leading to apoptosis and subsequent removal by the immune system. Furthermore, 5-FU has also been shown to destroy myeloid-derived suppressor cells. These cells help evade the immune system and elimination of these suppressor cells allows the patient's natural immunity to assist in tumor destruction (Cloughesy et al., 2016). An additional advantage of the engineered 5-FU produced in Toca-511 allows the active drug to diffuse through the cell membrane, giving the potential to have therapeutic effects reaching beyond the cytosol of the parent cell (Hemmer, et al., 2015). There is strong evidence to suggest this phenomenon, called the bystander effect, occurred in preliminary trials and it resulted in an improved overall survival rate as well as an improved recurrence free outcome. The Tocagen drugs are undergoing clinical trials but the progress they signify in science is considerable and opens the door to a proliferation of improved treatment techniques.

Multi-Therapy

The multi hit model of cancer, also known as the Knudson hypothesis, is the theory originally proposed in 1953 hypothesizing a stepwise component to oncogenesis. The theory suggests that cancer onset is not the result of a single mutation, rather the outcome of many mutations, creating conditions for the perfect storm that is cancer (Nordling, 1953). Knudson's hypothesis has greater validity after "Hallmarks of Cancer" was published by Weinberg and Hanahan (Hanahan & Weinberg, 2000). The hallmarks reiterate the idea that cancer is multi-faceted and more complex than a single aberration in physiology and development. The theory gives rise to an alternate approach to treatments; if the disease is multifaceted, then so must be the treatment plan. Various treatment proposals utilize this theory for multi-hit therapy. Some antiangiogenic treatments are being applied in conjunction with others for a comprehensive approach. Several attempts have been made, or are now in trials, that couple effective treatments like that of TMZ with newer medications to enhance their effectiveness. In concurrence with TMZ, patients have been tested with a wide variety of pharmaceutical cocktails, including gefitinib and erlotinib, a pair of EGFR inhibitors (Bleeker, et al., 2011). Patients have also been treated with radiotherapy and Gliadel wafers. These wafers are intracranial implants containing carmustine, a nitrosourea

alkylating agent, and give a steady treatment of chemotherapy to their host after being inserted into the brain (van den Bent et al., 2008). Direct insertion prevents the need to circumvent the blood brain barrier and offers a continuous administration of the drug for the duration of the treatment. As a nitrosourea, carmustine alkylates DNA strands, preventing normal gene expression in cancer cells and dampening tumor development. Further, contrary to previous research, resistance to a single alkylating agent does not predict resistance to alternate alkylating agents, allowing for a multi-agent approach to prevent cancer gene expression (Schabel, 1976).

The development of chimeric antigen receptor T-cell therapy (CAR-T) builds on the success of above-mentioned treatments such as retroviral genetic manipulation and immune system enhancement. This technique utilizes engineered T-cells to recognize specific markers in cancer cells and induce apoptosis. One lab at the Memorial Sloan Kettering Cancer Center focused on B cell acute lymphoblastic leukemia and is examining options to extend this therapy to gliomas. In the leukemia study, patients with relapsed B cell acute lymphoblastic leukemia had T-cells isolated from plasma and treated with a vector. The vector programmed T-cells to produce chimeric antigen receptor, a receptor that can be engineered to enable the T cells to recognize specific proteins found in cancers (CD19 in this case) (Brentjens et al., 2013). One particular advantage of CAR-T therapy is the use of the patient's own immune cells. Using autologous material decreases the incidence of rejection or other complications that frequently occur with the introduction of foreign cells (Almasbak, et al., 2016). CAR-T therapy has demonstrated high rates of success with above an 88% long term survival rate and is being evaluated for expansion to a greater variety of cancers, including high- and low-grade glioma (Wilkins, Keeler, & Flotte, 2017).

The range of ideas employed with multi-hit therapies and techniques takes advantage of the advances made in genetic and molecular research. Introducing therapies that make use of immunotherapy and genetic recombination, or that address multiple facets of angiogenesis are particularly useful in tackling the multidimensional aspects of cancer. Combination treatments allow scientists to develop a more comprehensive blend that can address the particular deficiencies associated with each individual medication and enable an extensive treatment plan for the greatest chances of survival.

Conclusion

Glioblastoma multiforme is a high-grade malignancy with a poor prognosis. While additional preventative research is required to improve diagnostic methods and screening, management methods are under rapid development to improve not only survival but also quality of life. A greater focus on intermolecular functioning within GBM helps provide a better understanding of how to effectively diagnose and recognize the cancer. Additionally, such information allows the introduction of periodic screening in higher risk individuals as determined by these advances. By analyzing intermolecular pathways such as kinase receptors and genetic modifications, researchers can develop steps or procedures to counter the cancer. This basic research extends itself heavily to the applied sector and allows for the creation of therapies and treatments that respond to the characteristics and actions unique to this disease and the complexities of its location.

Many treatments have already been proposed in an attempt to contain the rapid tumor proliferation associated with glioblastomas. With the knowledge gained from the success and failures of these treatments, adjusted multi-therapy approaches and novel replacement techniques have been developed. From genetics and immunological studies to bacterial and retroviral infections, these alternatives offer increased variability and possibility for patients. Despite the grim prognosis that typically accompanies the diagnosis of GBM, there is the potential for the cancer to no longer be as debilitating as it currently is or once was. Particularly through immunotherapy and gene therapy, as well as the use of antiangiogenics and nitrosourea alkylating agents, GBM can soon be combatted beyond the standard of surgical resection and chemotherapy.

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Neuraxial Analgesia and its Effects on Neonatal and Maternal Outcomes

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Abstract

Neuraxial analgesia is one of the most popular and effective forms of pain relief for labor and childbirth used by more than 50% of women in North America. Despite the standard use of the procedure, there is much that is still inconclusive about the side effects on mother and baby. Current studies show it has an impact on some outcomes and seemingly no effect on others. While the extent of the impact is still unclear, there are some adverse side effects of neuraxial analgesia, which include instrumental deliveries, respiratory distress and lower Apgar scores. Further research is required to conclude how and to what extent neuraxial analgesia impacts mother and baby.

Introduction

Labor and childbirth are known to be extremely painful experiences. Over the years there have been multiple methods developed which provide pain relief. Neuraxial analgesia, known colloquially as 'epidural', is one of the most popular forms of pain relief and is used by 50-70% of women in North America. It became a standard form of labor analgesia during the 1970s (Silva and Halpern 2010).

When neuraxial analgesia is administered, a small amount of anesthetic is inserted into the epidural space of the spine to prevent pain signals from traveling from the spine to the brain (Schrock and Harraway-Smith 2012). The drugs used most often in North America are bupivacaine and ropivacaine (Silva and Halpern 2010). There are few forms of epidurals used. For the epidural procedure, a catheter is inserted directly into the epidural space of the spine and the patient receives a continuous flow or multiple injections of local anesthetic. Spinal anesthesia refers to local anesthesia inserted into the subarachnoid space and one injection is usually sufficient. Combined spinal-epidural analgesia includes an initial spinal intrathecal injection as well as an epidural catheter for additional drugs. Neuraxial analgesia is an umbrella term which includes all three methods: epidural, spinal and combined spinal-epidural.

This text includes all three types of neuraxial analgesia used in labor. When using the term 'epidural', it refers to any of the three forms.

During labor and delivery, the primary concern is the safety and health of the mother and neonate.

Despite the longevity and standardized use of the procedure, there is much that is still unknown about the side effects of epidural analgesia and its impact on labor progress and maternal and neonatal outcomes. This text aims to explore if there are any adverse effects of epidural on the process of labor and delivery and on the health of the mother and neonate.

Methods

Original studies and scientific papers found through TouroLib access to databases such as PubMed, Proquest, and EBSCO were used fir this review. Google scholar was also used. Many of the studies are retrospective since readily available data greatly increases the population sample size. To ensure the research included pertains to current techniques, most studies used were published within the last ten years.

Oxytocin Augmentation

There are several ways in which epidural may have an effect on the mother during labor. Epidural has been linked to increased need for oxytocin augmentation during labor and delivery. Oxytocin is a hormone secreted by the pituitary gland. During labor, it stimulates uterine contractions and dilates the cervix. Oxytocin is continually secreted during labor through a positive feedback loop. Contractions push the fetal head towards the cervix which stimulates additional oxytocin to be produced. If labor does not progress at a proper rate, synthetic oxytocin can be administered intravenously to strengthen the contractions. This process helps progress the labor, however, it increases the risk of multiple adverse outcomes for the neonate (Bell et al. 2014).

A retrospective case-control study was done using clinical records of all mothers admitted to Riga hospital between January 1, 2013, and December 31, 2013. Infants included in the study were singleton pregnancies, at least 37 weeks of gestation, in cephalic position and vaginally delivered.

Epidural analgesia was given on demand, and the birth had to occur more than one hour after the epidural analgesia was used. Eight hundred and thirty-two women were included in the study, 304 who received epidural and 528 in the control group. Women who had epidural analgesia had higher rates of augmentation of their first stage of labor with oxytocin than women who did not receive epidural analgesia (Krievina et al. 2015). These findings are consistent with a study which also found increased oxytocin use in women receiving epidural analgesia (Decca et al. 2004).

Another study of 160 women, 80 receiving epidural analgesia and 80 in the control group found no difference in the percentage of patients who received oxytocin, but the maximum rate of oxytocin infusion was significantly higher in the epidural group, compared to the control group [P=.001] (Mousa et al. 2012).

These studies have exhibited an increase in the relationship between epidural and the need for synthetic oxytocin administration, which increases the risks for complications during labor and delivery.

Length of Second Stage Labor

Epidural has also been studied in association with its effect on the second stage of labor. The second stage of labor is also known as the pushing stage. Prolonged second stage labor has been associated with adverse effects on neonatal outcome, including

an increased relative risk of birth-asphyxia related complications and increased chances of NICU admission (Sandstrom et al. 2017). The longer the second stage of labor, the lower the infants one-minute Apgar score was and the higher the incidences of asphyxia. Prolonged second stage labor also has adverse effects on the mother. Li et al., discovered that the longer the second stage of labor progressed, the higher the incidences of cesarean section and postpartum hemorrhaging (Li et al. 2011). A study conducted across Washington state found that in nulliparous women, there was a two-fold increase in the median duration of second stage labor in women who received epidural analgesia when compared to those who did not. It also recorded a two-hour increase in second stage labor in the 95th percentile for women who received epidural analgesia (Souter et al. 2018).

A similar retrospective analysis was performed on all live, singleton, full term, vaginal deliveries in a university hospital between the years 2012-2014. Exclusion criteria included operative deliveries due to fetal distress, major fetal anomalies and chromosomal abnormalities The inclusion criteria were met by 15,499 deliveries. In this sample, women delivering with epidural analgesia were associated with an additional 82 minutes of second stage labor in the 95th percentile for both nulliparous and multiparous women. The study examined the effects of epidural analgesia on both nulliparous and multiparous women, and within each of those groups, there were women who received oxytocin augmentation were compared to those who did not. There was a significant effect found in all four groups. This study found epidural analgesia to be overall associated with over a one-hour increase in second stage labor (Shmueli et al. 2016).

A retrospective cohort study done on all live, singleton births in the University of California, San Francisco between 1976 and 2008 studied the effects of epidural on labor length. The study excluded pregnancy complications and cesarean sections that took place during the first stage of labor. The study included 42,268 women, where 49.9% had received epidural analgesia and 50.1% did not. Statistical analysis showed that women in the 95th percentile of the second stage of labor who received epidural analgesia had an average difference of more than two hours in duration of second stage labor when compared to women who did not receive an epidural. This number was statistically significant in both nulliparous and multiparous women (Cheng et al. 2014).

Instrumental or Operative Vaginal Delivery

Epidural has also been studied in its association with the need for instrumental or operative vaginal delivery. Instrumental or operative vaginal delivery is when forceps or vacuum extraction is used to extract the fetus from the vagina. This has been associated with adverse neonatal outcomes.

One study conducted on 2052 women in San Diego studied the risk of instrumental vaginal deliveries and cesarean sections in nulliparous and multiparous women when epidural analgesia is administered during the first stage of labor. The study excluded multiple births, inducements and preterm births. In nulliparous women, the relative risk of operative vaginal delivery was 2.5 when compared to women without an epidural. For multiparous women, the crude relative risk was 11.5 for operative deliveries (Nguyen et al. 2010).

A retrospective study on 350 female patients who received epidural analgesia and 1400 controls showed that vacuum extraction and cesarean sections were more frequently performed in the epidural group than the control group [$p<0.001$]. The epidural analgesia slowed down the progress of labor, which led to an increased rate of instrumental deliveries. The instrumental delivery appeared to affect the neonatal outcome more than the epidural analgesia itself (Hasegawa et al 2013).

A study conducted on 100 neonates born with epidural analgesia and 100 without had results consistent with other studies regarding epidurals and instrumental deliveries. Instrumental delivery, which included both forceps and vacuum extraction, had taken place during 13 births, 11 of which were from the epidural group [$p<.010$], making this a statistically significant study (Shrestha et al. 2014).

Krievina et al. 2015, whose population size was 832, discovered no difference between epidural analgesia groups and the non-epidural groups in regards to the use of vacuum extraction. The study recorded vacuum extraction only used in first-time mothers (Krievina et al. 2015).

While not indisputably conclusive, there is evidence in the research of a correlation between epidural analgesia and an increased need for instrumental deliveries.

Cesarean Section

A few studies noted the association between epidural analgesia and increased rate or risk of cesarean sections. Nguyen et al., (2010) conducted a study showing that administration of epidural analgesia during the first stage of labor increased cesarean section rates (Nguyen et al. 2010). Hasegawa et al., (2013) also recorded that cesarean sections were more frequently performed on the group of women receiving epidural analgesia than the control group [$p<.001$] (Hasegawa et al., 2013). Mousa et al. and Decca et al. both recorded no increased cesarean section rates (Mousa et al. 2012) (Decca et al. 2004). The results of the studies disagreed but both Nguyen et al. and Hasegawa et al. studies consisted of significantly larger populations than either Mousa et al. or Decca et al.

Maternal Fever

Epidural is known to increase maternal temperature. Maternal temperature during labor and delivery is associated with effects on the neonate. Intrapartum fever is related to infectious and noninfectious etiology. It is a well-known risk factor for adverse

neonatal outcomes including neonatal sepsis, seizures, asphyxia and mortality (Petrova et al. 2001). It is also known to effect obstetric outcomes; intrapartum fever significantly increases the rates of cesarean sections and operative vaginal deliveries (Lieberman et al. 1999). Whether increased fever related to epidural has the same adverse effects as other intrapartum fever is a question that has been studied.

One study was performed to investigate the relationship between combined-spinal epidural and increased maternal intrapartum temperature. Seventy women were included in the study, 35 receiving combined spinal-epidural anesthesia and 35 who did not receive any pharmacological form of pain relief. At the start of the study, the median temperature of the women was the same in both groups; after the first hour, there was an increase of intrapartum maternal temperature in the women receiving epidural analgesia. The difference in temperature between the two groups remained until the sixth hour and onward, where no statistically significant difference was found.

Five of the 35 women receiving combined-spinal epidural developed intrapartum fever while no fever was found in the group receiving non-pharmacological pain relief (P=0.027). However, there were no statistically significant differences in neonatal outcome. None of the infants born to mothers who had intrapartum fever developed neonatal sepsis, required antibiotic treatment, nor were they evaluated for neonatal infection. This study showed that combined-spinal epidural had an impact on intrapartum maternal temperature and can cause fever. It does not, however, have any adverse effects on neonatal or maternal outcome (de Orange et al. 2011).

A statewide study was conducted in Colorado on the association between epidural analgesia, maternal fever and neonatal antibiotics. Antibiotic administration to neonates who are at risk of infection is essential in preventing neonatal sepsis. However, unnecessary exposure to antibiotics is associated with significant risks.

There was no difference found between epidural and non-epidural groups in the administration of antibiotics to newborns born to mothers with fever. Women who received epidural analgesia were 5.4 times more likely to develop intrapartum fever. However, the infants had antibiotics administered equally in all cases of fever, regardless of what the cause was. This indicates that infants born to mothers who received epidural analgesia are more at risk of receiving unnecessary antibiotic treatment (White et al. 2017). While epidural-related fever did not seem to have a physiological effect on the neonate, the fever did lead to unnecessary antibiotic exposure.

Apgar Scores

One of the first assessments performed on a neonate is the Apgar score. The Apgar scoring system is a rapid method of assessing the clinical status of a newborn at one and five minutes of age. The Apgar score comprises of five components: 1. Color 2. Heart rate 3. Reflexes 4. Muscle tone and 5. Respiration. Each component is given a score of 0, 1, or 2. This is an accepted and standard method for reporting the status of an infant immediately after birth. While the Apgar score cannot be used to predict outcomes or diagnose alone, it is a useful tool in assessing the newborn. Because this is the standard test performed on all infants born in Western countries, it is simple data to collect and study and is one of the foremost things to examine when determining a baby's status (acog.org). Since most studies are conducted retroactively and all infants are scored on the Apgar system, it is an easy and commonly studied component.

A study was conducted in Spain on all full-term infants excluding inductions, elective cesarean sections and major pregnancy complications. The mean Apgar scores at one and five minutes were slightly but statistically significantly lower in the group of infants exposed to epidural analgesia [p=0.001] (Herrera-Gomez, 2015).

A different study conducted by Krievina et al. had a similar infant population, however with only singleton infants included. There was no statistically significant difference in the Apgar scores of infants whose mothers received epidural analgesia and those whose mothers did not. In this case, the birth had to be at least one hour after the epidural analgesia was administered. Although the outcome was different than in the previous study, the population size was much smaller in this study than in the study conducted by Herrera-Gomez (Krievina et al. 2015).

Another study recorded the Apgar scores were significantly lower in neonates who were delivered via vacuum extraction when compared to those delivered via spontaneous vaginal delivery and cesarean sections, regardless of whether the mother had an epidural or not. However, this study also found that epidural analgesia significantly increased the incidences of operative delivery. This leads to an indirect association between epidural analgesia and lower Apgar scores (Hasegawa et al. 2013).

Respiratory Distress

One of the variables that is observed when a child is born is the respiratory state of the newborn. A case-control study discusses the effects that epidural analgesia can have on the respiratory state of newborns in the immediate neonatal period. The case consisted of newborns who had gone into respiratory distress. The control group was comprised of site matched neonates of similar gestational age who did not go into respiratory distress. Infants included were late preterm or term. Exclusion criteria for this case group were major congenital malformations associated with newborn respiratory distress, culture-proven sepsis within 72 hours of birth or elective cesarean sections. Two hundred and six infants and 206 matched controls were included in the study. Seventy percent of infants who experienced respiratory distress had mothers who were exposed to epidural analgesia. In the control group 63% of infants had mothers who were exposed

to epidural analgesia. After statistical adjustments, the difference between the two groups was statistically significant [P=.04]. This study shows an association between epidural use and subsequent respiratory distress in the infant (Kumar et al. 2014).

A study conducted in Spain with 2399 infants studied the association between epidural analgesia and need for resuscitation. From the infants born to mothers who used epidural analgesiac, resuscitation was required in 28.7% versus 17.6% in the infants whose mothers did not have epidural analgesia [p=.001]. This study shows a correlation between epidural analgesia and the risk of resuscitation in newborns (Hererra-Gomez 2015).

Breastfeeding

The benefits of breastfeeding both on infant and mother have been well documented. In infants, breastfeeding is associated with reduced incidences of respiratory and gastrointestinal tract infections. Breastfeeding is also shown to reduce the long-term risk of diseases, obesity and diabetes. Maternal benefits include reduced bleeding after delivery, more rapid involution of the uterus and lower risks of future diseases (Eidelman 2012).

A study was conducted on the relationship between epidural analgesia during labor and onset of lactation in healthy women. Those included in the study were all singleton, full-term, vaginal deliveries and pregnancies that had no other pathological conditions. Women who experienced complications during labor were excluded. Only healthy babies with Apgar scores of greater than eight and those that did not require intensive care were included. All the women who participated in the study intended to breastfeed. There were 209 women who received epidural analgesia and 157 who did not. There was a five and twenty day follow up with the mother. There was no difference found in the onset of lactation between the two groups. Onset of lactation was determined as the time between birth and the peak intensity of the three major symptoms of lactation: breast tension, heat, and pain. At 20 days postpartum, more mothers who received epidural analgesia were formula feeding their babies than the mothers who did not receive epidural analgesia (Mauri et al. 2015).

Conflicting results were obtained in a retrospective study conducted by Herrera-Gomez et al. 2015 on all full-term infants born in San Juan de la Cruz Hospital between March 2010 and March 2013. Exclusion criteria included induced labors, elective cesarean sections and high-risk pregnancy factors. The study included 2399 infants. One of the variables studied was the onset of early breastfeeding, which was defined as within the first two hours of birth. Early breastfeeding onset was observed in 82.4% of infants in the epidural group and in 91.1% of infants in the non-epidural group, a significant difference with the odds ratio (OR) factor of 1.96 (Herrera-Gomez et al. 2015).

A study by Dozier et al. (2013) which observed the relationship between epidural analgesia and breastfeeding cessation, found similar results to Mauri et al. 2015. This study included 722 full term, singleton vaginal deliveries in which the mother-initiated breastfeeding. A significant crude relationship was found between epidural analgesia and breastfeeding cessation within 30 days [p<.01]. It was also found that the overall interaction between epidural analgesia and intravenous oxytocin negatively affected breastfeeding (Dozier et al. 2013).

Another study conducted on 100 women who received epidural analgesia and 100 women who did not. Exclusion criteria were cesarean sections, preterms, low birth weight and other complications. The timing of the initiation of breastfeeding was divided into three groups: within six hours of birth, six to 24 hours postpartum and after 24 hours. In the epidural and non-epidural group, 96 and 98 infants in each group respectively had started breastfeeding within six hours. Only one in each group established breastfeeding between six to twenty-four hours. Two newborns in the epidural group and one in the non-epidural group started breastfeeding after 24 hours. The P value was .06 not a statistically significant value (Shrestha et al. 2014).

Most studies show no significant difference in the onset of lactation between mothers who had epidurals and those who did not. Women who did not have epidurals were more likely to be breastfeeding for a longer period. However epidural analgesia was not the primary factor in predicting the duration of breastfeeding.

Conclusion

The research discussed in this paper covered a variety of topics such as oxytocin, length of labor, increased need for instrumental-assisted deliveries, Apgar scores and breastfeeding. There are, however, many other areas of impact that have not been covered, such as hypertension, NICU admission and urine passage. The exact impact that epidural analgesia has on maternal and neonatal outcomes is not conclusive. However, studies show that neuraxial analgesia is associated with increased risk of some maternal and neonatal outcomes such as instrumental deliveries, respiratory distress and lower Apgar scores, while having less of an effect on variables such as breastfeeding. Due to the considerable number of variables that determine maternal and neonatal health, it is difficult to identify which outcomes are due solely to epidural analgesia. Most of the research focuses on whether neuraxial analgesia causes side effects, but there is less research on the reasons why it does. Perhaps if the mechanism could be better understood, then more may be understood as to who is at greater risk for experiencing side effects and clinicians can then have the possibility and tools to better treat and educate mothers to be.

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Exercise Can Potentially Cure Parkinson's Disease: A Comprehensive Review

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Abstract

Parkinson's disease is the second most common neurodegenerative disease that negatively affects many American lives. It is characterized by the degeneration of dopamine-secreting neurons in the Substantia Nigra Pars Compacta (SNpc). Individuals with Parkinson's Disease lack motor coordination and experience severe motor impairments. Unfortunately, there is currently no treatment method available that can successfully cure the disease. In fact, all of the mainstream available treatments only eliminate some PD symptoms, and they cause many negative side effects. Although nontraditional, exercise is a side effect free treatment method that can potentially slow the progression of the disease and alleviate some symptoms. This paper first gives a comprehensive overview of the pathology and current treatments of Parkinson's disease. Then this paper reviews the benefits of exercise therapy for PD patients and the potential biological mechanisms that drive the success of exercise therapy.

Introduction

Although not as prevalent as Alzheimers, Parkinsons disease(PD) is the second most common age-related neurodegenerative disorder (Paillard, et. al. 2015, Maiti, et. al. 2017). It occurs in about 1-2% of the population over 65 and has been found to occur even less in younger populations (Maiti, et. al. 2017). Thus, age is considered to be one of the most prevalent risk factors for the development of the disease. Although Parkinson's disease does affect multiple parts of the brain, it is mainly characterized as a neurodegenerative disease that causes the degradation of dopaminergic neurons located in the Substantia Nigra Pars Compacta (SNpc) (Maiti, et. al. 2017, et. al. 2013). In a healthy person, the axons of these neurons extend to the Striatum where they help relay inhibitory signals by secreting dopamine which in return causes the basal ganglia to send inhibitory signals to Globus Pallidus and thalamus. These inhibitory signals help regulate movement by preventing the thalamus from over stimulating the motor cortex and causing impairment of motor coordination (Maiti, et. al. 2017). Due to the degradation of SNpc neurons, patients with Parkinson's disease have a significant decrease of dopamine secretion in the Striatum and as a result they experience significant motor impairments (Petzinger, et. al. 2013, Maiti, et. al. 2017).

Symptoms

PD is a neurodegenerative disorder that slowly worsens over time, thus, individuals with Parkinson disease experience a wider array of symptoms as the disease progresses. It takes around 15 to 20 years for PD symptoms to progress and it is estimated that the first visible symptom is seen only once 80% of the SNcp neurons have died out. Due to the progressive nature of the disease, early symptoms are subtle and often unidentifiable (Sveinbjornsdottir, 2016, Maiti, et. al. 2017). At first, most individuals will experience twitching in their arm, leg, jaw, lips or chin (Maiti, et. al. 2017). This symptom is called a resting tremor and is observed in the early stages of the disease in about 70% of PD patients (Maiti, et. al. 2017) (Moustafa, et al., 2016). It is most commonly found in the arms and the legs, and it usually starts off only on one side of the body. Gradually, this tremor develops bilaterally affecting both sides of the body (Maiti, Manna, & Dunbar, 2017). The second most common symptom is rigidity or stiffness of the muscles and joints (Maiti, et. al. 2017) (Moustafa, et al., 2016). This side effect causes individuals with PD to feel week and have severe cramping pain (Maiti, et. al. 2017). Individuals with PD also lose their ability to move quickly and experience a symptom called bradykinesia (Moustafa, et al., 2016, Maiti, et. al. 2017). Bradykinesia is characterized by loss of automatic movements, slow handwriting, decreased eye blinking, lower speech volume, difficulty initiating movement and difficulty in stopping continuous movements. In addition, PD patients also experience balance and coordination problems and will often fall. All of the above symptoms are considered to be primary symptoms of Parkinson's Disease and are essential for the diagnoses of the disease (Sveinbjornsdottir, 2016, Maiti, et. al. 2017).

In addition to the above symptoms ,some secondary symptoms may develop. These include continued changes in speech including the slurring of words, the repetition of words and excessively slow or abnormally fast speech. Urination and defecation become exceedingly hard due to the slow movement of smooth muscle in the digestive tract. Swallowing and chewing food becomes very difficult because of the bodies inability to perform peristalsis properly. In addition, PD patients often have a hard time staying asleep at night due to "insomnia, Rem sleep behavior disorder, sleep apnea, sleep attacks and restless leg syndrome (Maiti, et. al. 2017)." Some people with PD also experience depression, mood problems, anxiety, cognitive dysfunction, apathy and Dementia (Chauduri & Schapira, 2009) (Maiti, et. al. 2017).

Mechanisms of Parkinson's Disease

Parkinson's Disease can manifest due to external factors or as a result of inherited genetic mutations. Most cases of PD are considered to be non-genetic forms of PD, that result from environmental stimuli (Maiti, Manna, & Dunbar, 2017). Both genetic forms and sporadic forms of PD follow similar mechanisms of neuronal degeneration. Three of the most probable pathways of neuronal degeneration are death through mitochondrial dysfunction, A-synuclein aggregation, malfunctioning chaperone proteins and Autophagy lysosomal pathway damage.

Mitochondrial dysfunction can be caused by both genetic and environmental factors. Some environmental factors that cause PD include pesticides, herbicides, fungicides, and insecticides. Consequently, farmers and individuals who live in rural areas

are considered to be more susceptible to PD due to direct exposure to these substances through the air and drinking water. Other environmental factors that cause PD included bacterial toxins, viruses and illegal street drugs such as synthetic forms of heroin and rotenone. These environmental factors "inhibit complex one activity and cause dysfunction in the electron transport chain which causes oxidative stress (Maiti, et.al. 2017, p. 12)." Gene mutations of the DJ-1 and Pink 1 genes also cause mitochondrial dysfunction that can result in oxidative stress by inhibiting the formation of mitochondrial protective proteins. Once initiated by either environmental or genetic factors, oxidative stress causes the phosphorylation of tau proteins which eventually aggregate to form Lewy Bodies. These Lewy bodies accumulate in the cell making it exceedingly hard for the cell to complete basic tasks needed for neuronal function . Lewy bodies also make pores in the cell membrane resulting in "neuronal death via oxidative stress, energy failure, excitotoxicity, and neuronal inflammation. Eventually, the infected neurons begin to die due to their inability to perform tasks essential for survival". In addition to oxidative stress, mitochondrial dysfunction can also directly lead to energy failure. Energy failure refers to the cells inability to produce the chemical energy needed for cell survival. Energy failure causes the degeneration and death of dopaminergic neurons (Maiti, Manna, & Dunbar, 2017).

Mutations in the a-synuclein gene cause an alternative mechanism of neuronal degeneration. This mutation leads to the creation of misfolded α-synuclein proteins that eventually aggregate and develop into Lewy Bodies . Lewy bodies block up the cell making it close to impossible for basic cell functions to be completed. In addition, the Lewy bodies can make holes in the cell membrane which cause cell death by initiating, oxidative stress, energy failure, excitotoxicity, and neuroinflammation (Maiti, et. al. 2017). Thus, the α-synuclein gene mutation directly affects neuronal cell death.

Mutations of the parkin and UCHL1 genes can lead to the death of SNpc neurons by causing the destruction of ubiquitin-proteasome system(UPS) and molecular chaperones (Maiti, Manna, & Dunbar, 2017). The UPS actively brakes down short polypeptides into intracellular plasma membrane proteins, and it also helps with degeneration of "misfolded or damaged proteins in the cytosol, nucleus or endoplasmic reticulum (Maitiet. Al. 2017, p. 9)." Chaperone proteins play an important role in ensuring that proteins are folded properly. Damage to these two systems lead to the accumulation of misfolded proteins and to the impairment of the Autophagy Lysosomal pathway(ALP). The ALP helps degrade large protein debris that cannot be degraded by the UPS. When the UPS, chaperone proteins and ALP fail to complete their respective jobs, misfolded proteins begin to aggregate in the cell and form Lewy bodies (Maiti, Manna, & Dunbar, 2017). The Lewy bodies go on to disrupt cell function leading to the degeneration SNpc neurons.

The Spread of the Disease

Many researchers believe that once Parkinson's disease is initiated, Lewy body infected neurons transmit the disease to adjacent uninfected neurons (Maiti, Manna, & Dunbar, 2017). This method of proliferation is known as the prion hypothesis. Interestingly, many studies have found that α-synuclein begins to aggregate in the enteroendocrine cells of the gastrointestinal tract and then spreads to the brain via prion infection. Although this may be the method of proliferation used in some cases of PD, it is definitely not used in all PD cases since Lewy bodies are not necessary for initiation of PD (Maiti, et. al. 2017).

Inflammation may also be responsible for the spread of Parkinson's disease (Zhang, et al., 2011) (Phania, et. al. 2012). Neuronal inflammation occurs due to head trauma or the development of pathogenic disease such as PD. Inflammation in the PD brain is characterized by activated microglia cells, astrocyte cells and imported T-cells that crossed the blood-brain barrier (Cebrián & Sulzer, 2017). In the case of Parkinson's disease, the immune response is unbalanced, and instead of eliminating the disease it aids in the progression of the disease.

Microglia cells are activated as a result of α-synuclein accumulation (Cebrián & Sulzer, 2017). Once activated they act as phagocytic cells and participate in the removal of toxins and dead neurons (Vivekanantham, et al., 2015). They also produce "a plethora of pro-inflammatory mediators including prostaglandins, cytokines, chemokines, complement, proteinases, ROSs and RNSs (Vivekanantham, et al., 2015)." These substances initiate cell death by directly causing oxidative stress. In addition, microglia phagocytose extracellular neuromelanin that was released by dying neurons (Cebrián & Sulzer, 2017). During this process cytokines and hydrogen peroxide are released and cell death via oxidative stress is initiated (Zhang, et al., 2011).

Astrocytes are supposed to help regulate the immune response by protecting neurons from oxidative stress. In theory this would allow the microglia cells to participate in the immune responses without initiating neuronal cell death. However, because Parkinson's disease affects a part of the brain that has almost no astrocytes, this regulatory system fails to occur. In addition, many of the astrocytes present in the PD brain contain α-synuclein aggregations and release harmful substances such as cytokines, chemokines, and IFN-T (Cebrián & Sulzer, 2017). Thus, although astrocytes have the potential to be neuroprotective in Parkinson's disease, they are neurodestructive.

In addition to the damage that both astrocytes and microglia cells cause independently, they also work together to proliferate the disease by increasing the permeability of the blood-brain barrier. They accomplish this task by releasing cytokines which actively increase the permeability of the blood-brain barrier. This allows CD4 T cells and CD8 T cells to enter the central nervous system easily. Once the T cells reach the SNcp, they are activated by microglia cells that present antigens on their surfaces

using MHC II molecules and MHC I molecules (Kannarkat, Boss, & Tansey, 2013). This activation causes the release of more cytokines which leads to cytotoxicity and further activation of microglia cells (Kannarkat, et. al. 2013, Federoff, 2014). Thus, infiltrating T cells aid in the progression of Parkinson's disease (Phania, et. al. 2012).

Treatment

Traditional treatment for Parkinson's disease is limited to medications, surgeries, stem cell implantation and gene therapies that are only effective in eliminating side effects and cannot cure the disease. Most of these treatment methods have a multitude of side effects and only work for a limited amount of time or on a small population of Parkinson's patients. Various tratmentoptions and their success in treating PD are reviewed below with a particular focus on their success in treating PD and their adverse side effects.

Medications

Levodopa, a precursor form of dopamine, is perhaps the most common PD medication (Connolly & Lang, 2014) because it can cross the blood-brain barrier where it is converted to dopamine. By increasing dopamine levels in the brain, this medication successfully reduces resting tremors (Maiti, et. al. 2017). Unfortunately, Levodopa has also been found to cause vomiting, nausea, restlessness, drowsiness, low blood pressure, sudden onset of sleep and impulsive control disorders (Connolly & Lang, 2014) (Maiti, et. al. 2017). Additionally, chronic use of levodopa results in dyskinesia and motor fluctuations (Smith, et. al. 2012). Levodopa also has been found to quickly lose its effectiveness because it is converted immediately upon arrival into the central nervous system and by the time it reaches the target area enzymes have already started breaking it down (Maiti, et. al. 2017). Thus, to increase the potency of levodopa, many dopamine agonists are administered in combination with levodopa.

Monoamine oxidase-B (MAO-B) inhibitors such as selegiline and rasagiline are examples of dopamine agonists that are used in combination with levodopa (Maiti, et. al. 2017). MAO-B is an enzyme that participates in the breakdown of dopamine (Kay et. al. 2013). MAO-B inhibitors help prevent the breakdown of dopamine and increase the potency of levodopa (Smith, et. al. 2012) (Maiti, et. al. 2017). Although these medications have been successful in prolonging the effects of levodopa, they also cause a multitude of negative side effects. Which include, "Dizziness, dry mouth, insomnia, muscle pain, rash, nausea, constipation, severe headache, tachycardia, arrhythmia, hallucinations, chorea, or difficulty in breathing (Maiti, Manna, & Dunbar, 2017, p. 19)."

Another commonly used dopamine agonist is Catechol-O-methyltransferase (COMT) inhibitors (Maiti, Manna, & Dunbar, 2017). These drugs prevent the breakdown of dopamine and increase the efficiency of levodopa (Smith, Wichmann, Factor, & DeLong, 2012) (Maiti, Manna, & Dunbar, 2017). Entacapone and tolcapone are two examples of commonly used COMT inhibitors (Smith, Wichmann, Factor, & DeLong, 2012). Along with increasing the lifespan of dopamine these drugs cause hepatotoxic, nausea, orthostatic hypotension, urine discoloration, dizziness and mitochondrial dysfunction (Maiti, Manna, & Dunbar, 2017).

Dopamine agonists such as pramipexole and ropinirole are examples of drugs that are used instead of levodopa to treat the early stages of Parkinson's disease. They help alleviate Parkinson's symptoms by increasing dopamine levels in the brain. These drugs are not as effective as levodopa, and they cause similar side effects as levodopa. Some of the side effects commonly observed while administering these drugs are hallucinations, low blood pressure, nausea, dizziness, drowsiness, dry mouth, swollen legs and feeling faint upon standing (Maiti, et. al. 2017).

Anticholinergic drugs are also used to treat Parkinson's disease. These drugs inhibit the release of acetylcholine, which is overproduced in the brains of Parkinson's patients due to diminished dopamine inhibitory signaling (Maiti, et. al. 2017). They are successful in alleviating tremor and rigidity in about 50% of patients (Smith, et. al. 2012) (Maiti, et. al. 2017). Their adverse side effects include memory loss, confusion, hallucinations, constipation, urination problems, dry mouth, dry eyes and blurred vision (Smith, et. al. 2012). Due to the limited population that can be helped with anticholinergic drugs and the adverse side effects of these drugs they are less commonly used for the treatment of PD.

Surgical Treatments

Deep brain stimulation(DBS) surgery is a common surgery used to treat the advanced stages of Parkinson's disease. This surgery is only performed once all the medications mentioned above begin to lose their potency (Smith, et. al. 2012). Additionally, this surgery can only be performed on individuals who had success in using levodopa and show no signs of dementia or psychiatric abnormalities (Smith, et. al. 2012) (Okun, 2012). During DBS surgery electrodes are implanted into GPi and STN of the brain (Okun, 2012) (Maiti, et. al. 2017). These electrodes are attached to two batteries that are implanted in the chest directly under the color bone (Maiti, et. al. 2017). Electrical signals are then generated by the implanted batteries and sent to the electrodes in the brain where they stimulate inactive neurons (Okun, 2012) (Maiti, et. al. 2017). Once implanted this device is controlled by an external handheld device (Maiti, et. al. 2017). DBS has been shown to successfully eliminate many motor abnormalities including motor fluctuations. Additionally, because DBS eliminates the need for levodopa it successfully reduces dyskinesia and dystonia (Smith, et. al. 2012) (Maiti, et. al. 2017). Unfortunately, DBS entails a surgery that can cause stroke, hemorrhage, infection, speech issues and balance problems (Okun, 2012) (Maiti, et. al. 2017) (Smith, et. al. 2012). DBS has also been found to

increase depression, mania and suicide risk (Okun, 2012). Thus, although DBS can be helpful in treating PD, it may also cause adverse symptoms that can lead to death.

Another two surgeries which are used to treat Parkinson's disease are pallidotomy and thalamotomy. Pallidotomy is a surgery in which a part of the globus pallidus is destroyed . As a result of this destruction, "the synaptic connections with thalamus or striatum are altered in a way which decreases tremor, rigidity, bradykinesia and posture abnormalities in PD patients (Maiti, et. al. 2017, p. 21)." Thalamotomy is the destruction of the thalamus, which disrupts the connection between the basal ganglia and the motor cortex. This procedure restores neurotransmitter balance and reduces tremor . However, thalamotomy is unsuccessful in alleviating other symptoms such as bradykinesia, rigidity, and dyskinesias (Maiti, et. al. 2017).

Stem Cell Implantation

Stem cell implantation therapy involves the implantation of human fetal-derived dopaminergic tissues into the striatum. Implantation of these tissues into the brain results in higher levels of dopamine, which indicates that these stem cells mature properly and are able to create synapses. When porcine-derived dopamine-producing cells are used moderate improvements in symptom control are observed. However, when allogenic human fetal ventral mesencephalic cells are used much greater symptom relief is observed (Maiti, et. al. 2017). "These cells survive and make appropriate synaptic connections, while increasing DA levels within the host cells (Maiti, et. al. 2017, p. 22)." Although stem cell implantation seems promising, it causes many safety issues including unchecked proliferation and tumor development (Smith, et. al. 2012). Thus, more research is needed to develop this treatment technique and increase its safety.

Gene Therapy

Although most cases of Parkinson's disease are sporadic, there are some genetic forms of the disease. Scientists are now starting to develop gene therapy techniques that can cure the genetic forms of PD. Some of the techniques that are being tested are viral vector-mediated gene delivery, AADC-TH -GCH therapy, RNA interference-based therapy and CRISR-Cas-9 gene editing system. Most of these therapies have been successful in animal models, and some have also been used to treat humans successfully. However, most of these therapies must be tested further in order to ensure their safety (Maiti, Manna, & Dunbar, 2017).

Exercise as a Treatment Method for PD

There is no treatment method for Parkinson's disease that is symptom-free. In fact, all of the treatments available for PD are not successful at curing the disease, and when used they generate adverse side effects. Thus, Patients diagnosed with Parkinson's disease often feel helpless and experience depression because until recently, their diagnosis meant awaiting and experiencing the impending uncontrollable loss of movement until death.

However, in the past two decades, athletic programs such as Boxing, Tai Chi, dancing, treadmill training and forced bicycling have begun to bring hope to Parkinson's patients. These programs are designed in a way that teaches Parkinson's patients physical skills that helps stop the progression of the disease and even allows some individuals to regain full range of movement. Studies have proven the success of these programs, but there is no significant scientific work that can explain how exercise alleviates Parkinson's symptoms on a biological level. In the past couple of years, scientists have turned their attention to discovering the underlying biological mechanism that allows the above exercise programs to be so successful in treating Parkinson's disease. Based on the current scientific research the most probable mechanisms are that exercise promotes neuroplasticity and neuroprotection.

The Two Main Components that make an Exercise Program Successful

Most of the Successful exercise programs incorporate goal based learning and aerobic exercise. The inclusion of these two components helps promote neuroplasticity, which is defined as,

A process by which the brain encodes experiences and learns new behaviors… the modification of existing neural networks by addition or modification of synapses in response to changes in behavior or environment, which can encompass exercise. Neuroplasticity includes a wide range of structural and physiological mechanisms including synaptogenesis, neurogenesis, neuronal sprouting, and potentiation of synaptic strength, all of which can lead to the strengthening, repair, or formation of neuronal circuitry (Petzinger, Fisher, McEwen, Beeler, & Walsh, 2013).

Goal-based learning refers to the incorporation of tasks that are aimed at improving specific skills that are impaired in PD. Intensity, specificity, complexity, repetition, and difficulty are all important aspects of goal-based exercise that help drive. Goal-based exercise also stimulates cognitive engagement of the prefrontal cognitive circuits. These circuits are involved in early motor movement and the development of automaticity. Thus, activation of prefrontal circuits helps patients with Parkinson's disease relearn skills that were previously automatic. Aerobic exercises are similar to goal-based exercise in the fact that it too promotes cognitive engagement and neuroplasticity. "Aerobic exercise is defined as vigorous and sustained physical activity that leads to increased cardiopulmonary function resulting in improved oxygen consumption (maximum oxygen uptake) and blood flow to the brain (Petzinger, et. al. 2013, p. 719)." By increasing blood flow to the brain, aerobic exercise stimulates neuroplasticity and improves cognitive prefrontal cortex function. When goal based and aerobic exercise are combined their effects are compounded, and significant changes in neuroplasticity are observed (Petzinger, et. al. 2013).

The Successful Programs and how they Incorporate Goal Based Learning and Aerobic Exercise

"Tai Chi, as a mind-body exercise, consists of a series of dance-like movements linked in a continuous sequence, flowing slowly and smoothly from one movement to another that emphasizes weight transfer and movement of the body." (Yan Yang, 2015, p. 2) It has been shown to improve dynamic postural control, balance, gait, and quality of life (Petzinger, et. al. 2013, Yan Yang, 2015). In one study, scientists observed improved stride length, stability and maximum excursion in patients who had participated in tai chi classes twice a week for 24 weeks (Petzinger, Fisher, McEwen, Beeler, & Walsh, 2013). Tai chi incorporates cognitive engagement by practicing combinations of movements and control of an individual's center of gravity (Yan Yang, 2015). However, it does not include intense aerobic exercise, and it is not clear if this lowers its success rate compared to other exercise debilitation programs.

Dance is an aerobic form of exercise that has also shown promise as a potential treatment for Parkinson's Disease. Dance enhances motor learning by making people pay attention to music and rhythm (Blandy, Beevers, Kerry, & Morris, 2015) (Petzinger, Fisher, McEwen, Beeler, & Walsh, 2013). In addition, partner dancing further stimulates cognitive engagement by teaching people partner coordination (Petzinger, Fisher, McEwen, Beeler, & Walsh, 2013). By incorporating both cognitive and aerobic exercise, dance has been found to improve gait, motor impairment and balance (Blandy, Beevers, Kerry, & Morris, 2015).

Boxing therapy has become a very popular form of exercise therapy for PD in the past couple of years. In 2006 The Rocksteady boxing foundation located in Indiana opened the first noncontact boxing program for PD Patients. Since then, Rocksteady boxing programs have been established across the United States. Boxing therapy takes a whole-body approach by including dynamic balance activities and multi-directional reaching and stepping. These programs also include jump rope and framework activities that help improve people's ability to initiate movement (Combs, et al., 2011). Boxing helps improve balance, gait, parkinsonian symptoms and quality of life (Combs, et al., 2011) (Petzinger, et. al. 2013). Scientists have reported that the benefits of boxing last for a long time even if patients stop participating in as many classes as they originally did. The boxing programs included aerobic exercise and goal-based exercise by incorporating fast-paced multi-directional body movement (Combs, et al., 2011). Thus, boxing is one of the most promising forms of exercise therapy available for PD patients.

Cycling training is another popular form of exercise rehabilitation. Both forced cycling and voluntary cycling programs have been found to help improve PD symptoms (Nadeau, et al., 2017). In forced cycling PD patients are placed on an electric bike and forced to pedal at a speed that is above their comfort level (Ridgel, et. al. 2009). This system helps recruit cognitive involvement and has been shown to improve neuroplasticity in the human brain. In addition, forced cycling helps improve tremor and bradykinesia. Voluntary cycling has been shown to improve cardiovascular capacity, executive function, motor learning and walking speed (Nadeau, et al., 2017). Both voluntary and forced cycling are promising treatment options for Parkinson's Disease, and scientist still have not determined which form of cycling is more successful.

Treadmill training, when used to treat mild to moderate PD, helps improve, "velocity, postural stability, gait rhythmicity and joint excursion (Petzinger, Fisher, McEwen, Beeler, & Walsh, 2013, p. 717)." Some studies have reported treadmill training to be completely infective at treating Parkinson's disease. This inconsistency may be a result of improper feedback during workouts, which is supposed to promote cognitive engagement and enhance motor learning (Petzinger, et. al. 2013). Thus, treadmill training is a prime example of an exercise program that must include both goal based and aerobic exercise in order to be effective.

Neuroprotection

Neuroprotection is the driving force behind the neuroplastic effects that both goal based and aerobic exercise exert on the PD brain. Exercise provides neuroprotection by increasing dopamine release and dopamine receptor expression, decreasing dopamine clearance from the synaptic terminals, stimulating the release of neuroprotective factors and initiating an anti-inflammatory response.

Exercise therapy promotes the release of dopamine, decreases dopamine clearance from the synaptic cleft and facilitates the binding of dopamine to D2 receptors in the dorsal striatum. Exercise has been found to increase dopamine synthesis and release in both human and mice models (Horak & King, 2009). One theory for how this is accomplished is that exercise increases tyrosine hydroxylase (TH) levels in the PD brain. TH is an enzyme that increases dopamine levels by converting L-tyrosine to L-dopa (a precursor form of dopamine) (Morgan, Corrigan, & Baune, 2015). TH has also been found to decrease aggregated a-synuclein proteins, which play a primary role in neuronal death in PD (Petzinger, Fisher, McEwen, Beeler, & Walsh, 2013). In addition, exercise decreases DAT levels, which participates in the degradation of dopamine in the synaptic clefts. This increases the dopamine available to bind to dopamine receptors. D2 receptors in the Putman are activated with intensive exercise and are available to bind to dopamine (Jakowec, Wang, et. al. 2016). D2 receptors play an important role in cortical-striatal glutamatergic modulation and are essential for motor learning (Jakowec, et. al. 2016) (Petzinger, et al., 2015).

Exercise plays a role in modulating the inflammatory response in the Parkinson's diseased brain. It is well known that exercise

reduces inflammation and oxidative stress (Shu, et al., 2014). One of the ways that physical activity reduces inflammation is by increasing anti-inflammatory cytokine interleukin 10. Exercise also increases interleukin 6 which usually acts as an inflammatory substance, but in the case of exercise, its effects are anti-inflammatory. Interleukin 6 accomplishes its job by eliciting, "an anti-inflammatory response that includes increased expression of several factors including interleukin 10 and interleukin 1 receptor antagonists, and inhibition of factors such as tumor necrosis factor alpha (Petzingeret, et. al. 2013, p. 722)." In addition, exercise reduces the proliferation of astrocytes and microglia cells which secrete harmful inflammatory cytokines. Physical activity also may drive the conversion of activated microglia cells from M1 myeloid cells which secret harmful cytokines to M2 myeloid cells which helpful secret cytokines (Jakowec, et. al. 2016). This may mean that exercise not only reduces inflammation but also stimulates the development of a positive immune response.

Exercise also increases the secretion of neuronal protective factors, which include brain-derived neurotrophic factor (BDNF), Galia cell-derived neurotrophic factor (GDNF) and hypoxia-inducible factor 1 alpha (HIF-1α). Both BDNF and GDNF promote neuronal growth, prevent neuronal death and help the neurons function properly (Paillard, Ronlland, & Barreto, 2015) (Morgan, Corrigan, & Baune, 2015). BDNF, in particular, is activated by aerobic exercise and has been found to reduce rigidity and muscle stiffness (Morgan, Corrigan, & Baune, 2015) (Jakowec, Wang, Holschneider, Beeler, & Petzinger, 2016). Skilled based exercise increases cortical-striatal function, which results in an increased demand for oxygen. The lack of oxygen activates transcription factor HIF-1α. This transcription factor increases neuronal health by regulating genes that control, "Metabolism, mitochondrial integrity … and signaling cascade pathways involved in nitric oxide synthase and glutamine synaptogenesis (Jakowec, Wang, Holschneider, Beeler, & Petzinger, 2016, p. 41)." Thus, both aerobic and skill-based exercise help increase the release of neuroprotective factors.

Conclusion

Parkinson's Disease is the second most common neurodegenerative disease, and its prevalence is expected to double by 2040 (Ridgel, et. al. 2015). There is no cure for Parkinson's Disease. All the available mainstream treatments cause adverse side effects and are only successful at eliminating a small number of symptoms. However, alternative therapies such as exercise have been found to reduce targeted symptoms, without causing negative side effects. Unfortunately, exercise therapy although growing in popularity is not being used by most primary doctors to treat Parkinson's Disease. Educating affected individuals and medical practitioners on the benefits of exercise programs can potentially help improve the lives of many individuals. Why should medications that cause a plethora of side effects be prescribed when we can use exercise programs instead? Why tell patients that there is no hope in sight for them, when in fact exercise can potentially stop the progression of Parkinson's disease and in some cases eliminate all Parkinson's disease symptoms. It is time for medical professionals to change their outlook on Parkinson's Disease, instead of relaying a message of helplessness they should relay a message of hope.

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The Association Between Type of Fat and the Risk of Developing Cardiovascular Diseases

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Abstract

Objective: To determine the significance of various types of dietary fat in the progression of atherosclerosis leading to heart disease. Methods: Study inclusion criteria constituted relevance to the discussion topic and peer reviewed literature. Age of the published material was taken into account as well, with greater preference being given to more recent research as the topic of nutrition and its relation to chronic disease remains an emerging science.

Results: This research review found an overall significant relationship between the type (monounsaturated, polyunsaturated, saturated and trans-fat) of dietary fat intake and the incidence of cardiovascular disease. While earlier research identified an increased risk of cardiovascular disease to be associated with an overall increased intake of dietary fat, newer studies recognized the increased risk to be associated more specifically with saturated and trans fatty acids. Substitution of other dietary fats in place of saturated fat in the diet also plays a role in cardiovascular disease. The studies reviewed here found a protective effect of polyunsaturated fatty acids when replacing saturated fat in the diet. On the other hand, replacing saturated fats with carbohydrates has no evidence of improved cardiovascular health. Studies looking at the implications of substituting saturated fat with monounsaturated fats remain inconclusive at this time. Aside from dietary fat intake, the progression of atherosclerosis leading to cardiovascular disease can be caused by oxidative stress such as in the case of hyperglycemia and diabetes.

Conclusions: As health care costs continue to rise in this country, preventive medicine plays an increasingly important role in the prevention and treatment of chronic disease. Research shows that decreasing the percentage of saturated fats in the diet and substituting these with healthier polyunsaturated fats, as well as minimizing oxidative stress in the body can decrease the incidence of cardiovascular disease, a leading cause of death in the United States.

Introduction

In February of 1980 the United States Department of Agriculture and Department of Health and Human Services jointly published the first set of Dietary Guidelines for Americans. During this time, health scientists were beginning to focus on the link between macronutrient intake and a correlated risk for chronic diseases. In these original guidelines, Americans were encouraged to keep their total fat intake levels below thirty percent of their diet, have less than ten percent of dietary fat come from saturated fat, and maintain a cholesterol intake that was under 300 mg per day (Canty, 2018).

The guidelines have been updated every five years since and have evolved based on accumulating scientific evidence, population patterns in health status, food consumption, and physical activity. The 2015 Dietary Guidelines have a stronger emphasis on healthy eating patterns and regular physical activity. However, there has been minimal change in the recommendations for fat intake for almost 40 years since the first set of guidelines. In 2015, the Dietary Guidelines for Americans recommended that fat be 20-35% of the diet with less than 10% coming from saturated fat and minimizing trans fats as much as possible. While the 2015 guidelines did not address cholesterol, the recommendations from 2010 remain the same as well (<300 mg/day) (U.S. Dept. of Health and Human Services, 2015).

Controlling fat intake has consistently been recommended to improve health for Americans with a specific focus on limiting saturated fat in the diet. However, heart disease, a form of cardiovascular disease, remains to be the leading cause of death for both men and women worldwide. Stroke, a complication of cardiovascular disease is the fifth leading cause of death in the Unites States. Dietary fat intake is a major modifiable risk factor in the development and progression of cardiovascular disease. (Center for Disease Control, 2017).

Methods

Studies reviewed in this paper were selected from journal databases, library catalogs, and Health and Nutrition Science professional websites. Papers were gathered from PubMed, CINAHL, Medline, and similar scientific search engines using advanced search phrases "PUFA," "MUFA," "dietary fat intake," "trans-fat," "oxidative stress" and "CVD".

The writer considered over 36 papers and over 20 papers were included in the final research review. Study inclusion criteria consisted of relevance to the discussion topic and peer-reviewed literature. Age of the published material was taken into account as well, with greater preference being given to more recent research as the topic of nutrition and its relation to chronic disease remains an emerging science.

To improve the writer and reader's understanding of the relation between dietary fat intake and disease, noted textbooks in advanced biochemistry topics were referenced and included in the discussion section of this paper.

Discussion

Fat is an essential macronutrient required by the body. In 1927, the effects of fat deficiency on growth and development inspired researchers Evans and Burrs to dub fat "vitamin F". Two years later, in 1929, Evans and Burrs found that a fat-free diet in weanling rats produced impaired growth, scaly skin, tail necrosis, increased mortality, and impaired fertility (Ross, et. al, 2014). These symptoms were reversed when provided with linoleic or linolenic acid and the term "essential fatty acids" was coined. Almost thirty years later, in 1958, Essential Fatty Acid Deficiency (EFAD) was first demonstrated in humans, when infants fed a milk-based diet lacking essential fatty acids (EFA) showed severe skin symptoms alleviated by the addition of linoleic acid. Total Parenteral Nutrition Support, lacking fat-soluble vitamins, was

also shown to produce EFAD in a 6-year-old girl maintained on total parenteral nutrition for five months (Burr, 1981).

We require fat and fat-soluble vitamins for energy reserves, cell signaling, synthesis of hormones, and their structural role in cell membranes (Canty, 2018). The average intake of fat in this country has remained relatively consistent since the 1990s.

The average American consumes 33% of his calories from fat and 10% from saturated fat. There were significant changes over the years in the consumption of monounsaturated fat (MUFA) (14.6% to 13%, p = 0.03) and polyunsaturated fat (PUFA) (6.9%-8.0%, p = <0.001) as well as the ratio of PUFAs to saturated fat (7.1%-8.1%, p = < 0.001) (fig. 1) (Vadiveloo, at. al. 2013).

Atherosclerosis is the narrowing of the arterial wall caused by a buildup of plaque. As the endothelial layer on the artery wall weakens, it becomes susceptible to plaque accumulation by a high concentration of Low-Density Lipoprotein (LDL) -cholesterol in the blood. As the plaque begins to build, monocytes and macrophages are attracted to the site of the plaque contributing to stenosis (Narrowing of the artery lumen). The layer of smooth muscle in the artery begins to grow a fibrous cap over the plaque. As the plaque continues to develop, this cap can rupture and form a blood clot that may cause a heart attack or a stroke (Escott-Stump, 2015, Mahan and Raymond, 2017).

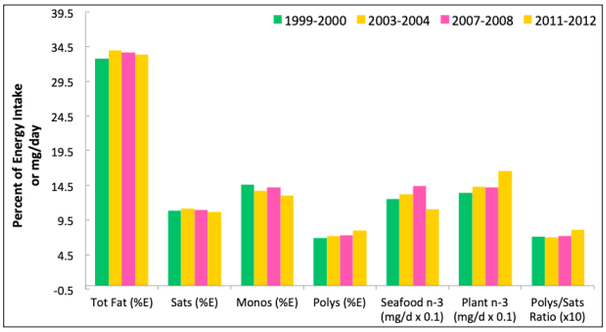

Figure 1: Trends in dietary fat consumption (NHANES)
Source: Canty, D. Advanced Nutrition: Protein, Fats and Carbohydrates. Spring 2018.

Types of Fats

As part of the Strong Heart Study, researchers examined the association between dietary fat intake and coronary heart disease in 2,938 Indian American adults ages 45-79 for an average of 7 years. Over 400 participants experienced Coronary Heart Disease (CHD) during this time, including 138 fatalities. The researchers divided participants into two age group categories, 47-59 years and 60-79 years. Across both age groups, individuals who developed CHD were more likely male, hypertensive and with lower High-Density LipoProteins (HDL) levels and higher serum triglycerides. Amongst the older group, those that developed CHD had higher LDL levels as well. Amongst the younger group, an increasing rate of mortality due to CHD was seen with greater quartiles of total fat intake, specifically saturated and monounsaturated fat (Lee, et. Al. 1990). This study suggests that abnormal lipoprotein levels (low HDL, high LDL) are associated with an increased morbidity in adults. Additionally, total fat, rather than a specific type of fat, is associated with increased mortality from CHD.

The Seven Countries Study (Keys, et. al. 1984) investigated the intake of various fatty acids as well as dietary cholesterol in relation to serum cholesterol and 25-year mortality from coronary heart disease. The study was carried out from 1956-1964 and constituted 16 cohorts across seven countries to include over 12,000 men between the ages of 40-59 years. Dietary intake data was collected and standardized by one dietitian. In 1960 total lipids were measured (at that time there was no instrument available to measure trans-fat, omega-3 or omega-6 fatty acids). Data was collected and reported for saturated, monounsaturated and polyunsaturated fats. Later, in 1987, total lipids were isolated and cis and trans fats, as well as omega-3 fatty acids, were identified. The individual saturated fatty acids, namely lauric acid and myristic acid, were significantly related to cholesterol levels ($p < 0.001$). Moreover, the researchers identified a strong positive relationship between the intake of saturated fats and death rates from CHD ($p < 0.001$) (fig. 2). It is worthwhile to note that a 25-year lapse in time from the collection of data to the analysis may have impacted the researchers' findings.

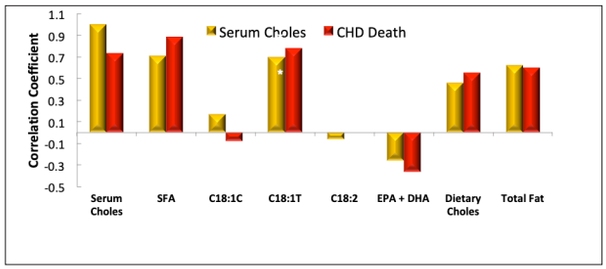

Figure 2: Correlations Between Fat Intakes (E%) and Serum Cholesterol and 25y CHD Death Rate
Source: Canty, D. Advanced Nutrition: Protein, Fats and Carbohydrates. Spring 2018.

More recent studies support these findings as well, indicating that the source of dietary fat influences heart disease. A 20 year follow up of the Nurses' Health Study looked at the association specific types of dietary fat intake as they relate to the risk of coronary heart disease. The study consisted of 78,778 US women who were without CVD or diabetes at the initiation of the study in 1980. At the follow up, 1,766 incidences of CHD were reported, 525 of which were fatal. Researchers found that intake of polyunsaturated fats was inversely associated with CHD (P = 0.004) (fig. 3). The results were substantial amongst women with an overweight BMI (>35 kg/m2). Trans-fat intake was associated with an increased risk of developing CHD (P=0.01), particularly in women under 65 years of age (Colditz, Hankinson, 2005).

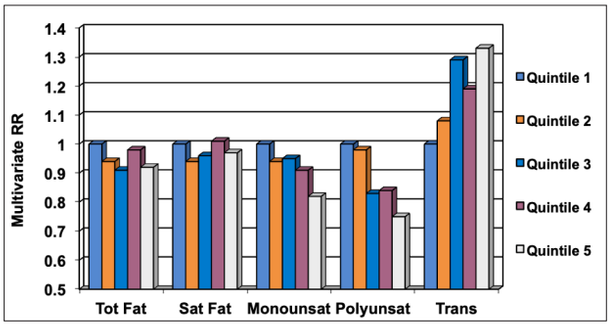

Figure 3: Fat intake and CHD risk in women; Nurse's Health Study 20y Follow-up
Source: Canty, D. Advanced Nutrition: Protein, Fats and Carbohydrates. Spring 2018.

These findings are consistent with a 2006 cross-sectional study of 1,123 adults between the ages of 20 and 98 explaining the possible connection between the inverse relationships. The researchers looked at the relationship between fasting plasma polyunsaturated fatty acid (PUFA) levels and markers of inflammation. They found a statistically significant, negative correlation between the intake of PUFAs, specifically omega-3 and omega-6 fatty acids and plasma levels of pro-inflammatory markers (IL-6, TNF, IL-10, CRP). This study suggests that the anti-inflammatory properties of polyunsaturated fatty acids are associated with lowering plasma levels of pro-inflammatory markers that play a role in the progression of atherosclerosis (Ferruci, et. Al. 2006)

Replacing Saturated Fat in the Diet

Current guidelines from the HHS and USDA recommend <10% of calories come from saturated fat and the American Heart Association recommends no more than 5-6% of intake should come from saturated fat to decrease LDL cholesterol. However, there are fewer studies and recommendations discussing what the recommended replacement for saturated fat in the diet should be. A meta-analysis of eight randomized control trials that included over 1,000 incidences of CHD amongst 13,614 participants, found that increased PUFA intake in place of saturated fat in the diet reduced CHD events by 19% (Mozaffarian, et. Al. 2010). More specifically, each 5% increase in PUFA consumption reduced the risk of CHD by 10%. The analysis suggests that these improvements in the incidence of CHD were likely the effect that PUFAs had on lowering LDL in the blood. The short-term studies part of this identified a 5% energy intake from PUFAs that replaced a 5% energy intake from saturated fat lowered LDL cholesterol by 10 mg/dl while having no significant impact on HDL cholesterol. This intake of PUFAs in place of saturated fats lowered the total cholesterol to HDL ratio by 0.16. Conversely, no change in the total cholesterol to HDL ratio was found when saturated fat in the diet was replaced with carbohydrates. The observational studies identified a 44% decrease in CHD risk associated with each unit decrease of total cholesterol to HDL ratio.

It is important to note that these studies measured the effects of replacing saturated fatty acids with PUFAs in the diet and therefore cannot ascertain whether the benefits were related to the decrease in saturated fatty acids or the increase in dietary PUFAs. It can be concluded that replacing saturated fats with PUFAs in the diet likely decreases the incidence of CHD while replacing saturated fat with carbohydrates does not. Consequently, it may be concluded that the effects of replacing saturated fat in the diet with monounsaturated fat are mixed. Future studies looking at the implication of replacing saturated fat with proteins and/or MUFAs would be beneficial.

Animal vs. Plant Fats

Emerging research looked at monounsaturated fatty acids to identify if the source of MUFAs had a relationship to its impact on heart disease. Researchers used data from the Nurses' Health Study that included 63,000 women and data from the Health Professionals Follow-Up study that included 30,000 men. Both of these studies utilized food frequency questionnaires administered every four years to evaluate participants' diets.

When analyzed, participants with a higher intake of MUFAs from plant sources showed a 16% lower risk of death (from any cause) compared with those that had a lower intake. In contrast, participants with a higher intake of MUFAs coming from animal protein exhibited a 21% increase in risk of death related to any cause. Replacing dietary MUFAs from animal sources for the equivalent per gram in plant sources may lower the risk for death by heart disease or any cause of death between 24% and 26% (Roeder, 2018).

Similar findings have been reported for with saturated fat. It is thought that plant sources of saturated fat may, in fact, play less of a role in the morbidity and mortality from coronary heart disease than animal sources. These findings can be explained by a recent study looking at the structural implications of fatty acids. An animal source of saturated fatty acids is located mainly at the sn-2 position of the glycerol backbone as shown in figure 5 and affects the metabolism, functionality, and physiological effects of the saturated fatty acids (fig. 4) (Nettelton, et. al. 2017). The Multi-Ethnic Study of Atherosclerosis aimed to determine the association between SFA intake from varying food service and in the incidence of CVD. The study included 5,209 participants between the ages of 45–84 that were followed for a

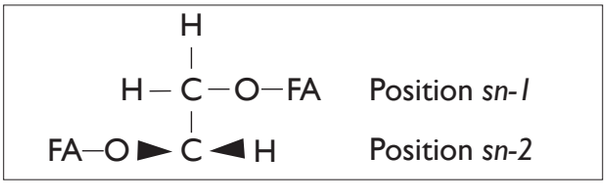

Figure 4: Implications of Structure and Position of Saturated Fatty Acids Source: Nettleton, 2017.

decade from 2000. Diet was assessed using a food frequency questionnaire and over 300 cases of CVD were assessed during follow up valuations.

After adjusting for confounding factors, researchers concluded that a higher intake of saturated fat from dairy sources was associated with a lower risk of CVD (95% CI for + 5 grams per day and 5% of energy intake from dairy sources of saturated fat). In contrast, meat saturated fat was associated with an increased risk of developing CVD with the same increase in grams per day and % energy intake (fig. 5).

Substituting 2% of energy intake from meat sources of saturated fat with dairy sources of saturated fat was associated with

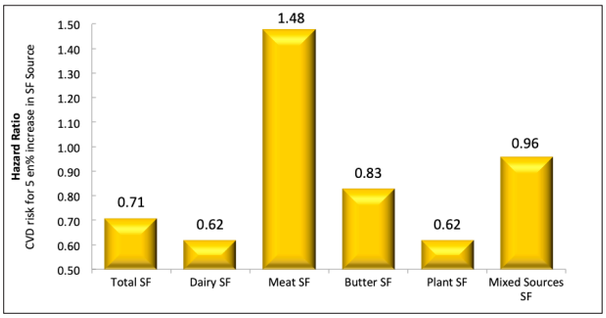

Figure 5: Dietary Source of Saturated Fat May Affect CVD Risk Source: Canty, D. Advanced Nutrition: Protein, Fats and Carbohydrates. Spring 2018.

a 25% decreased risk of CVD (Otto, et. al. 2012). This particular study found no association between CVD and intake of plant or butter saturated fats, however, intakes recorded in the Food Frequency Questionnaires were narrow.

Processed Fat: Trans Fat

Since in 2006, food manufacturers have been required to list trans-fat on all food labels. Trans fats (trans unsaturated fatty acids) are artificially produced solid fats. Liquid vegetable oils are heated with catalysts in a process called hydrogenation, which breaks double bonds in unsaturated fats and adds hydrogens, thereby solidifying the fat, making it a solid at room temperature.

The current dietary guidelines for healthy Americans recommend keeping trans-fat intake "as low as possible" (< 10% of total calories). These recommendations are based on studies that have found that trans fats increase LDL-cholesterol levels in the blood while decreasing healthy levels of HDL-cholesterol. One early study demonstrated this by providing 34 adult women and 25 adult men with three diets that each study subject followed for three consecutive weeks. All three diets were mixed, natural diets and identical in nutrient consumption except for 10% of energy intake coming from oleic acid (a monounsaturated fat with one cis bond), trans isomers of oleic acid, or saturated fatty acids. LDL cholesterol was significantly higher on the trans fatty acid diet compared to the other two diets (P <0.0001) with no significant differences found between men and women (Mensink, Katan, 1990).

A significant relationship has been reported between dietary trans-fat and coronary heart disease. Dietary data was studied from participants in the Nurses' Health Study and intake of trans fat was calculated using the questionnaires completed by more than 80,000 female participants, all of whom were without diagnosed cardiovascular disease at the time. Over the 8 year follow up period, 431 cases of new CHD were diagnosed. Adjusting for age and total caloric intake, the intake of trans fat was directly related to increased risk of CHD (P = 0.001). Margarines, cookies, cakes and refined white bread were each significantly associated with an increased risk of developing CHD, with a stronger association found for the participants who stated consistent margarine intake for the last 10 years (Willett, 1993).

More recently, the DASH diet (Dietary Approach to Stop Hypertension) has been found to reduce the risk of cardiovascular disease in women. The DASH diet focuses on consuming adequate servings of fruits and vegetables, lean proteins and limiting intake of saturated and trans fatty acids (2 a day where a serving is 1 teaspoon of margarine). A study followed more than 88,000 women between the ages of 34 and 59 years. Food frequency questionnaires were collected 7 times between 1980 and 2004. Over 2,000 cases of nonfatal myocardial infarction, over 3,000 strokes and 976 women died of CHD over the 24-year follow-up period. After adjusting for confounding factors, increased DASH scores were associated with decreased risk of acquiring some form of CVD (P <0.01) (Fung, et. al. 2018).

Do PUFAs Have any Faults in Relation to Disease?

Lipid peroxidation is the oxidative deterioration of lipids possessing double bonds by interaction with a reactive oxygen species (ROS). Given their higher content of double bonds, PUFAs are more susceptible to oxidation than SFAs and even MUFAs.

Antioxidants, such as vitamin E and vitamin C, in our diets and in our bodies, serve to detoxify the oxidative damage caused by ROS. In a healthy individual, the generation of reactive oxygen species is in normal balance with antioxidant activity. However, increasing amounts of oxidative damage and/or decreasing amounts of antioxidants leads to oxidative stress in which elevated levels of ROSs can lead to oxidative damaging of proteins, carbohydrates and DNA molecules.

Oxidative stress has been linked to disease states such as atherosclerosis and certain cancers. The question remains whether the disease state causes the increased lipid peroxidation and oxidative stress or if the oxidative damage leads to the disease state (Ross, et. al, 2014).

Coconut Oil: Celebrity Status Threatened

The American Heart Association published a 2018 Presidential

Advisory that had much of the health community involved in a conversation regarding saturated fats and their role in oxidative damage and chronic disease. The AHA's advisory mentioned a New York Times piece highlighting a recent health survey in the United States which found that 73% of Americans classified coconut oil as a "health food" compared to 37% of nutritionists. The AHA contributes the positive perception of coconut oil in its relation to health promotion and disease prevention to a result of good marketing by the coconut oil industry (Sacks, et. al. 2017).

Statistics from the USDA reveal that coconut oil consumption increased from 2013 to 2014 by 9.55% and then dropped in 2014 and 2015 to 6.23% and 13.47% respectively. This information sheds some light on the increased promotion of coconut oil as a health food by the coconut oil industry and the belief of Americans, that it is a health food. However, coconut oil's nutrition profile did not change during this time. It remains to be 82% saturated fat, and like all densely saturated fats, plays a role in cardiovascular disease.

A recent review looked at seven interventional studies comparing coconut oil with unsaturated fats in the diet and their individual influences on lipid profiles of study participants. All seven trials resulted in higher total cholesterol for those receiving coconut oil intervention, all of which were statistically significant. Six out of the seven studies found a statistically significant increase of LDL cholesterol for the intervention group. HDL levels were statistically significantly raised in five studies for those receiving the coconut oil intervention (Eyres, et. al. 2016). The AHA warns consumers that the HDL improvements seen with diet or drug treatment cannot be directly linked to changes in CVD occurrences and therefore encourages Americans to look at the LDL changes as effected by various fats alone in regard to their role in CVD (Sacks, et. al. 2017).

Atherosclerosis: Other Players

As the walls of the arteries get thicker, narrowing the lumen due to plaque buildup, patients with atherosclerosis experience worsening symptoms of CVD, including hypertension (Nelms et. al 2016) . During the initial stages of plaque buildup, the endothelial cells of the luminal lining, which provide the barrier between the lumen and the vascular wall, may experience dysfunction and contribute to increased plaque and blood pressure. Certain stimuli can trigger the dysfunction of the endothelial cells, deeming these stimuli proatherogenic. Diabetes and oxidative stress are amongst the major players that contribute to the eventual loss of functionality of the endothelial cells, along with dyslipidemia, abnormally elevated cholesterol or fats (lipids) in the blood (Chistiakov, et. al. 2015).

Glucosamine, often used in the management of osteoarthritis, has anti-inflammatory effects by contributing to a redox state in an oxidative environment. In an attempt to take advantage of its properties, glucosamine supplements consisting of 300 mg were given to 20 individuals daily for 30 days and arteriosclerosis and CVD were compared to 19 controls. Flow-mediated vasodilation was monitored in all participants at the start and for the duration of the four-week trial. At the end of four weeks, parameters such as blood pressure, blood glucose, and lipid levels were measured. No significant changes were found in these clinical markers prior to or following the study between the two groups. However, glucosamine supplementation significantly improved flow-mediated vasodilation ($P = 0.02$) highlighting the role that oxidative stress independently plays in atherosclerosis and cardiovascular disease (Katoh, et. al. 2017).

This is similar to what is seen in the strong correlation between diabetes, specifically type 2, and incidence of CVD. The American Heart Association considers diabetes to be one of the seven top causes of cardiovascular disease. More than 68% of people over the age of 65 with diabetes will die from a form of heart disease. Adults with diabetes are twice to four times as likely to die of heart disease than adults of the same age without diabetes (American Heart Association, 2018).

Oxidative stress has been shown to contribute to atherosclerotic buildup in animal models. Elevated blood glucose in type 2 diabetic patients allows for glucose persisting in the bloodstream to become damaged, increases the amount and rate at which reactive oxygen species (ROS) accumulate. ROS are potent oxidants that, without adequate antioxidant activity, can proliferate and cause oxidative damage in individuals leading to the dysfunction and apoptosis of endothelial cells, worsening atherosclerosis and hypertension leading to worsening cardiovascular complications (Jay, et. al. 2006).

A double-blinded crossover study looked at the role that antioxidants could play in lowering levels of ROS and oxidative stress and mortality risk due to CVD in diabetes over the age of 55. Exclusion criteria included uncontrolled hypertension and history of a myocardial infarction and/or stroke within the last month. Participants were randomized to receive either a vitamin E (a potent Anti-oxidant) supplement or a placebo pill. At the end of eight weeks, researchers measured forearm blood flow and found that vitamin E supplementation significantly improved blood flow ($P < 0.01$), demonstrating improved cardiovascular profiles in diabetic patients receiving antioxidant supplementation (Alshiek, et. al. 2017).

Implications

The Lifestyle Heart Trial suggests a correlation between a lifestyle change in patients with atherosclerosis and regression in their artery lesions. Forty-one participants with atherosclerosis were randomized to an experimental group (n=22) and control group (n=19). Angiographies were conducted at baseline and again at a one-year follow-up. During the year the experimental group was given a low-fat, vegetarian diet with less than 10% of calories coming from fat and a ratio of PUFAs to saturated

fat of less than 1. The experimental group was also required to participate in aerobic exercise and attend weekly support group meetings. Following one year, blood serum was collected and analyzed from all 22 participants. The results showed an average percentage diameter stenosis decrease from 16.9% to 16.5% amongst the experimental group. The control group who continued with their usual diet and lifestyle showed stenosis progression from 15.5% to 18.5%. The average percentage regression in the diameter stenosis of the control group was 82% (18 out of 22). In the experimental group, total cholesterol was lowered by 24.3% and LDL concentration by 37.4%. This study shows that a lifestyle change in patients with coronary heart disease can lead to significant regression in arterial stenosis (Ornish, et. al. 1990). Changes in lipid profiles and angina pectoris are displayed in the tables (ref., tables 1 and 2).

Conclusions and Recommendations

The Dietary Guidelines for Americans published every five years is a set of evidence-based guidelines to promote healthy nutrition habits and prevent nutrition-related disease in the American population. These guidelines are constantly evolving based on the latest research findings and available science. Despite its regular updates, the DGA has remained relatively consistent in its recommendation for the dietary consumption of fats.

Recent National Health and Nutrition Examination Survey data shows Americans are consuming 33% of their calories in the form of fat. This paper reviewed the literature on types of fats and their individual relationship to the progression of cardiovascular disease, the cause for one-third of all deaths in this country.

Indeed, the type of fat a person is consuming impacts his or her risk of cardiovascular disease. Higher amounts of saturated fat in the diet have been found to be associated with increased

	Mean (SD) at Baseline		Mean (SD) at 12 Months	
	Experimental Group (n=20-22)	Control Group (n=17-19)	Experimental Group (n=20-22)	Control Group (n=17-19)
Serum Lipids (mmol/l)				
- Total chol	5.88 (1.29)	6.34 (1.02)	4.45 (1.15)	6.00 (1.55)
- LDL	3.92 (1.25)	4.32 (0.77)	2.46 (1.55)	4.07 (1.17)
- HDL	1.00 (0.26)	1.35 (0.52)	0.97 (0.40)	1.31 (0.38)
- Triglycerides	2.38 (1.26)	2.45 (2.47)	2.91 (1.47)	2.24 (1.79)
Lipid Ratios				
- Total/HDL	6.33 (2.14)	5.32 (1.89)	5.15 (2.23)	4.93 (1.59)
- LDL/HDL	4.18 (1.53)	3.59 (1.37)	2.89 (1.92)	3.33 (1.42)
Blood Pressure (mm Hg)				
- Systolic	134 (13)	140 (26)	127 (13)	131 (20)
- Diastolic	83 (8)	82 (13)	79 (7)	77 (11)
Weight (kg)	91.1 (15.5)	80.4 (22.8)	81.0 (11.4)	81.8 (25.0)

Table 1: Changes in Risk Factors Source: Ornish, D. 1990

Chest pain	Mean (SD) at baseline		Mean (SD) at 12 months	
	Experimental Group (n=20)	Control Group (n=17)	Experimental Group (n=20)	Control Group (n=17)
Frequency	5-10 (14-1)	2-35 (3-77)	0.45 (0.76)	6-24 (12-9)
Duration (min)	2-73 (4-69)	3-47 (7-95)	1-58 (4-48)	6-97 (14-5)
Severity	2-3 (1-6)	1-8 (1-1)	1-7 (1-2)	2-5 (1-2)

Table 2: Changes in Angina Pectoris Source: Ornish, D. 1990

risk of CVD. Replacing saturated fat with carbohydrates has not been found to improve lipid profiles or CVD risk factor biomarkers. On the other hand, replacing saturated fat with unsaturated fats, specifically PUFAs, have been associated with lowering an individual's risk of developing CVD. The data on MUFAs remains inconclusive at this time.

The research suggests that health care professionals should encourage their patients and clients, especially those at risk for cardiovascular disease, to consume a diet lower in saturated fat and to mindfully replace those items with wholesome, real foods, and fats higher in PUFA content while remaining wary of processed carbohydrates. As part of a healthy diet, consumers should also consume adequate foods high in antioxidants to provide a buffer for the potentially oxidizing effect of a diet higher in PUFAs.

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What Are the Mechanisms and Effects of Age-Related Shortening of the Spine?

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Abstract

Osteoporosis and Degenerative Disc Disease can cause a loss of height in the spine. This paper focuses on the mechanisms that cause age-related loss of height and its effects. Possible relationships between receptor activator nuclear factor kappa-B ligand (RANKL) and estrogen with osteoporosis are discussed, as well as possible genetic causes. Exercise may prevent osteoporosis. Osteoporosis may cause a loss of height, vertebral fracture, a decrease in maximum lung capacity, and hip fracture. Degenerative disc disease may cause shortening of the spine. Disc height loss can result in a reduction in the range of motion and stiffness at the affected spinal segments. Degenerative disc disease may cause a small increase in vertebral height which can lead to complications in treatments.

Introduction

As early as the age of 30, people may begin to become shorter. Studies show that from the age of 40, people begin to lose about half an inch per decade. Some health risks associated with loss of height are spinal fracture, back pain, hip fracture (Hannan et al., 2012), and a decrease in lung capacity (Krege et al., 2015). Total health care costs in the United States of osteoporotic hip fractures is estimated at 18 billion dollars annually (Hannan et al., 2012).

There are three main reasons why people become shorter as they age, and they are all related to the spine. One reason is osteoporosis, a decrease in bone density. As the skeleton loses density, it compresses, making the body shorter. Osteoporosis is the most common bone disease, affecting 200 million people worldwide, and leading to over 9 million fractures annually (Yu & Wang, 2016). Giving the aging population, the predicted annual healthcare costs of an osteoporotic compression fracture in the USA alone is 25.3 billion dollars. Loss of water in the intervertebral discs due to aging also accounts for shortening of the spine. The discs become compressed, because of the weight placed on them by the upper body, resulting in a loss of height (Jarman et al., 2015). Another possible cause for shrinking is the abnormal bending of the spine. However, the body isn't really shortening, rather bending, and just giving the appearance of loss of height. Flattening of the arch of the foot may also give a person the appearance of a shorter stature; however, most of a person's height loss can be attributed to changes in the lumbar spine (Videman et al., 2014). This paper discusses the possible causes and effects of osteoporosis and degenerative disc disease, and how they result in a loss of height.

Methods

Data was found by using ProQuest and PubMed databases through Touro college's online library. Keywords used were loss of height, osteoporosis, and degenerative disc disease.

Physiology of Osteoporosis

Bone is 30% collagen fibers and 70% non-organic minerals such as calcium. Osteoblasts are cells that make bone. Osteoclasts break down the bone and resorb the minerals into the blood, but osteoblasts keep making new bone to counter the loss; this is known as bone remodeling. Osteoporosis is a condition where osteolysis overshadows osteogenesis (Yu & Wang, 2016). Osteoporosis can begin at the age of 30, but it is most pronounced in older people, especially in postmenopausal women because estrogen suppresses the receptor activator of nuclear factor kappa-B ligand (RANKL), a molecule that promotes osteoclast differentiation and formation (Eghbali-Fatourechi et al., 2003). Estrogen also increases the expression of osteoprotegerin (OPG), which functions as a decoy receptor for RANKL (Hofbauer et al., 1999). During menopause, when there is a decrease in estrogen, there is an increase in RANKL and decrease in OPG, resulting in increased osteoclast activity, and a net loss of bone.

Studies show that men also experience bone loss due to a decrease in estradiol concentrations (Falahati-Nini et al., 2000). However, in both men and women, a decrease in growth hormone secretion due to aging is also responsible for a decrease in bone formation (Drake et al., 2015).

Trabecular bone loss begins in the third decade of life while cortical loss typically begins in the sixth decade of life (Drake et al., 2015). Trabecular loss, which occurs before gonadal sex steroid deficiency, may be caused by secondary osteoporosis. Secondary osteoporosis is bone loss caused by factors other than aging or postmenopausal status. Glucocorticoid excess can cause decreased production of osteoblast precursors and increased apoptosis of mature osteoblasts. Primary hyperthyroidism causes cortical bone loss. Vitamin D deficiency causes demineralization of bone and is associated with lower bone density. Type 1 diabetes mellitus causes osteoporosis because of an inability to reach peak bone mass (Emkey & Epstein, 2014). There are many other causes of secondary osteoporosis, but this paper focuses on age-related causes.

Since both genders experience trabecular bone loss with aging, age-related factors other than sex steroid deficiency may be involved. Cytokines accumulate due to aging factors, including menopause, and make the bone marrow prone to inflammation (Yu & Wang, 2016). Inflammation activates transcription factor nuclear factor kB, which causes osteoclast differentiation and inhibits osteoblasts (Chang et al., 2009). Also, oxidative stress, characterized by the presence of too many reactive free radicals in the body, increases with age. Oxidative stress activates nuclear factor-kB which in turn causes bone loss (Khosla et al., 2011). It is unclear if osteoporosis causes further inflammation in the bone marrow which would then activate more nuclear factor-kB, exacerbating the osteoporosis (Yu & Wang, 2016).

Osteoporosis may indirectly cause further bone loss.

Osteoblasts and adipocytes both come from a mesenchymal stem cell. In osteoporosis, since osteoblasts production is inhibited, there are more adipocytes forming instead. Adipocytes secrete the hormone leptin which has been found in mice to promote adipogenesis and inhibit osteogenesis (Yue et al., 2016).

Osteoporosis is also caused by gene expression. Histone Demethylases KDM4B and KDM6B remove gene silencing histones from the osteogenic master regulator gene (Ye et al., 2012). Mice that had KDM6B knocked out had impaired osteogenesis (Zhang et al., 2015). Aged mice and female mice that had their ovaries removed had elevated levels of the osteogenic gene silencing histones, suggesting an epigenetic link between aging and low estrogen levels with impaired osteogenesis (Ye, et al., 2012).

Studies of astronauts have shown that they lose bone from bones that are normally weight bearing, such as the femur and tibia. Astronauts returning to Earth have extensive bone resorption, which shows that osteoclasts were breaking down the bone (Shigematsu et al., 1997). Normal bone density was not restored after reambulation (Vico et al., 2000). Prolonged bed rest is also associated with decreased bone density in weight bearing. Interestingly, a study of 24 bedridden women showed that high load resistive exercise had no significant impact on bone loss, although it did prevent muscle atrophy (Beller et al., 2011). These studies show that the force of gravity is necessary for maintaining normal rates in bone remodeling, and a lack of this force may result in osteoporosis.

Predisposing risks for osteoporosis occur during childhood and adolescence because maximizing peak bone mass is important for its prevention. However, exercise during maturation can mitigate the effects of osteoporosis. Weight-bearing exercises are found to be effective in generating bone anabolism (Santos et al., 2017). Exercise may be a form of prevention, but once osteoporosis develops, exercise may lead to osteoporotic fracture due to the stress put on the weak bones.

Osteoporotic Vertebral Fracture and Height Loss

The decrease in bone density caused by osteoporosis can cause vertebral fractures. Vertebral fractures can be measured using the spinal deformity index (SDI). Using the SDI, a mild compression fracture (20-25% compression) in a vertebra is given a value of one, moderate fractures (25-40% compression) are two units, a severe fracture (more than 40% compression) is given a value of 3 (fig. 1). The SDI is the sum of the units of each vertebra from T4 to L4. Height loss was calculated by subtracting the current height from the arm span. The arm span length was considered the person's peak height. In a study of women aged 70 years and older with osteoporosis and a history of at least one moderate or severe vertebral fracture, for each unit increase in SDI, height decreased by about 0.5 cm (Krege et al., 2015). Osteoporosis causes a loss of bone in the vertebrae, resulting originally in a decrease in density of the vertebrae. The weight placed on the spine may then cause the vertebrae to compress, increasing the bone density of the vertebrae (the density may be elevated due to compression; however, there is a decrease in bone mass and height). Therefore, height loss is a marker for vertebral fractures.

	FRACTURE STATUS	FRACTURE GRADE
T4	none	0
T5	none	0
T6	mild	1
T7	moderate	2
T8	none	0
T9	none	0
T10	none	0
T11	mild	1
T12	none	0
L1	mild	1
L2	none	0
L3	none	0
L4	none	0
SDI = 1 + 2 + 1 + 1 = 5		

Fig. 1. Spinal deformity index is the sum of fracture grades for T4 to L4 vertebrae. In the example shown, the patient has three mild and one moderate vertebral fracture, for an SDI of 5. SDI, spinal deformity index. Modified from Krege et al., 2015

Osteoporosis and Lung Capacity

SDI is also negatively correlated with pulmonary function. For each unit increase in SDI, forced inspiratory vital capacity, which is the maximum volume of air inhaled, decreased by 1.62% and inspiratory time, the time it takes for the maximum volume of air to be inhaled, decreased by 2.39% (Krege, et al., 2015). There was no significant correlation between the flow rate, which is a measurement of the volume of air inhaled per an amount of time,

and the SDI. This result indicates that vertebral fracture is linked with restrictive, not obstructive, lung disease. Restrictive lung disease occurs when the lungs aren't compliant, and they become stiff, limiting the lungs' expansion, and causing a volume decrease. An obstructive disease does not affect the lung volume; rather it causes a decrease in flow due to the resistance of the obstruction. Therefore, since people with vertebral fractures experience a decrease in lung capacity but not in flow rate, osteoporosis can be classified as a restrictive lung disease. The reduction in inspiratory time is caused by a decreased lung volume, so there isn't as much air going into the lungs, and therefore it requires less time for inhalation. Perhaps the decrease in inspiratory vital capacity is due to a loss of volume in the thoracic cavity because of compression of the spine. This decrease in volume may limit the compliance of the lungs. No evidence was found that would suggest an effect of height loss on the heart.

Other studies were performed using expiratory measurements. One study found that for each thoracic vertebral fracture forced vital capacity declined 9% (Leech et al., 1990). Others suggested that there is no relationship between vertebral fractures and pulmonary function. However, the analysis performed by Krege et. al. is unique in its use of SDI to quantify the vertebral fracture. Another possible reason for different results found in the studies is because the other studies used expiratory measurements while Krege et al. used inspiratory measurements. Also, the measurement done by Leech et. al. was only considering thoracic fractures while the study done by Krege et al. considered both thoracic and lumbar segments. A limitation to the Krege et al. study is that it combined both thoracic and lumbar segments rather than assessing them separately.

Osteoporotic Hip Fracture

Height loss is also associated with osteoporotic hip fracture, defined as incident fractures of the proximal femur occurring either after age 50 or in postmenopausal women. A study that followed 3081 adults over 50 years revealed a positive correlation between height loss and risk of hip fracture. For this study, hip fracture was defined as a first-time fracture occurring in the absence of overwhelming trauma. The study recorded height loss of male and female participants over 24 years. Most participants were middle-aged during these 24 years. The study also considered recent height loss, height loss occurring during the two years prior to the hip fracture, as a possible correlation to the risk of hip fracture. Most participants were elderly during the two years of their recent height loss.

The study used Cox proportional hazards regression (HR) to calculate hazard ratios and 95% confidence intervals for height loss and risk of hip fracture. For each inch of height loss occurring during the 24 years, the HR was 1.4 in men and 1.04 in women. These results show that height loss was significantly associated with hip fracture in men but not in women. Men with long-term height loss of two inches or more had about twice the risk of fracture than men with less height loss. Recent height loss occurring two years prior to hip fracture increased the risk of hip fracture by 54% for men and 21% for women. Recent height loss was more of an indicator of fracture risk compared to long-term height loss most probably because recent height loss occurred during elderly ages while long-term height loss occurred during the participants' middle-aged years.

That study proved a relationship between height loss and hip fracture; however, height loss can be caused by many factors, postural changes, osteoporosis, disc degeneration, and kyphosis which may contribute to fracture risk. No information was given about posture for the participants, which is a limitation of the study. The exact cause is unknown, but height loss is an indicator of an increased risk of hip fracture. Another limitation of the study is that participants were all from one town and were primarily Caucasian (Hannan, et al., 2012).

Physiology of Degenerative Disc Disease

The intervertebral disc has a hard-outer portion called the annulus fibrosus and a gelatinous inner portion called the nucleus pulposus. The annulus fibrosis can further be divided into 2 portions. The outer zone is made of type 1 collagen fibers and the inner zone is comprised of type 2 collagen fibers. The nucleus pulposus is 85% water. The intervertebral discs act as a water cushion and distribute pressure uniformly over the endplates of the vertebrae. While a person is in an upright position, the weight placed on the intervertebral discs causes the discs to lose water. The water escapes to blood vessels in the bone marrow space in the endplate of the vertebrae. These blood vessels are also responsible for giving the intervertebral discs nutrition. When the pressure on the intervertebral discs decreases, like when a person lays down or in a zero-gravity environment, fluid reenters the intervertebral disc. The difference in height between a person in a vertical and horizontal position can be 1% of his height (Schuenke et al., 2011).

As people age, they may get what is known as degenerative disc disease. However, there are those who view degenerative disc disease different than normal disc ageing, maintaining that degenerative disc disease is "an accelerated ageing process including structural failure", while there are those who use the two terms synonymously (Galbusera et al., 2014). In this paper, degenerative disc disease refers to disc degeneration associated with normal ageing.

Over time, the annulus fibrosus weakens and loses elasticity. Also, proteoglycans, water retaining molecules, diminish within the degenerating discs. With the decrease in water, the discs lose their ability to resist compression and torque. The discs shrink because of the weight placed on them, and less water is present to counter this force (Jarman et al., 2015). This compression of the intervertebral discs results in a decrease in a

person's overall height. Studies show that a decrease in estrogen can cause disc degeneration (Lou & Chen, 2014).

Disc Height Loss

A study of 37 men and 33 women, with an average age of 48 showed that degenerative disc disease is positively correlated with disc height loss. The 70 participants were asymptomatic for back pain to exclude pathologic degeneration. Additionally, participants were not diagnosed with osteoporosis and didn't have any spinal fractures. T2-weighted MR images were used to determine if the patient had degenerative disc disease. T2-weighted MRI signals water content; therefore, an MR image showing a white disc indicated a healthy disc that contained water, while images of a dark disc indicated degenerative disc disease since the dark color in the image indicated that the disc lacked water. Another indicator of degenerative disc disease is an absence of the differentiation of the nucleus pulposus and the annulus fibrosis. The study used MR imaging of the lumbar spine to assess the height and shape of the intervertebral discs. The height of the disc was measured as the mean of the anterior and posterior disc height. Disc convexity was calculated as the ratio of the central disc height and the mean of the anterior and posterior height.

The study showed that disc degeneration resulted in a decrease in disc height. Also, the lower intervertebral discs were shorter than discs higher in the spine (for example, the disc between L2 and L3 was shorter than the disc between L1 and L2), most probably because the greater weight placed on the lower discs cause them to be more compressed. Degenerative effects on disc height were found to be more pronounced with increasing age. Weight, overall height, and gender did not correlate with disc height. Discs were found to be less convex in the presence of disc degeneration. Also, the lower level discs were less convex. One drawback of this study is that participants didn't have osteoporosis, so the effects of degenerative disc disease together with osteoporosis can't be determined (Pfirrmann et al., 2006).

Height Changes in Intervertebral Discs and Vertebrae

Another study was done using 232 monozygotic twin men. The fact that they are twins is not relevant to the findings of this study. Intervertebral disc and lumbar vertebrae heights were measured from MRIs and compared with the measurements of one group after five years and the remainder after 16 years. After five years, the vertebrae did not significantly increase in height. However, after 15 years, the vertebrae significantly increased by an average of 3.1% which was an average of 0.8 mm. The lower vertebrae increased in height more than the upper vertebrae did. The disc heights had a mean decrease of 3.45% or 0.4 mm after five years and an average decrease of 10% or 1.2 mm after 15 years. Disc height loss was greater than the height increase of the vertebrae, resulting in a net loss of height. The axial disc areas significantly increased by 4.7% over 5 years and 14.2% over 15 years. Disc volume was calculated by multiplying the mean disc height by the axial disc area. Over 5 years, the disc volume did not significantly change, but after 15 years, the volume significantly increased by 2.3% in the discs between L2 and L4, and significantly increased by 3.7% in the discs between L4 and S1.

This study shows that disc shortening is associated with an increase in vertebral height. A 1 mm decrease in the height of either the superior or inferior discs was associated with an increase of .09 mm in vertebral height. The effects of the superior and inferior discs are additive. Age was not a confounder on the relationship between the discs and the vertebrae. Since the vertebrae increase as the discs decrease, the lumbar spine only minimally decreases. The reason for this vertebra-disc relationship may be because the increased tension between the vertebra and annulus fibrosus causes ossification to strengthen the connection between the disc and the vertebra. This reason would help explain why vertebral height increases occur more at the anterior and posterior sections of the endplates, because it attaches to the annulus. Another possible explanation for the relationship between disc height and vertebral height is that endplate lesions can expose the vertebrae to disc substances, which may lead to inflammation and bone growth on the endplate. Support to this hypothesis is that studies have found an association between disc degeneration and lesions of the bony endplate.

Over the 15-year study, the lumbar spine decreased by an average of 1.4% or 2.0 mm. The mean decrease in total height of participants was 3 mm. Most of the overall height loss experienced by participants of this study occurred in the lumbar spine (Videman et al., 2014).

Degenerative Disc Disease and Spinal Flexibility

Intervertebral disc height loss may result in a decrease in the range of motion and stiffness of the affected spinal segment. It is unclear however if disc height loss is the cause, or possibly due to damages of annular tissue or ligaments. One possible way to restore spinal flexibility is to inject a hydrogel to compensate for the loss of height of the discs (Balkovec et al., 2016).

A possible concern with simply using a hydrogel to add height to the discs is that sometimes the surrounding vertebra increases in size as was found in the Videman et al. study, so there may no longer be sufficient space for a larger disc in the spine.

Conclusion

Osteoporosis may cause a loss of height, vertebral fracture, a decrease in lung capacity, and hip fracture. Osteoporosis is caused by activation of RANKL and a reduction in osteoprotegerin. Histone demethylase KDM4B is also linked to osteoporosis. A

decrease in Estrogen can cause osteoporosis. Weight-bearing exercise may reduce the progression of osteoporosis.

Degenerative disc disease may cause shortening of the spine. Discs lower in the spine experience a greater loss of height, presumably because the greater weight placed on them causes them to condense more. Disc height loss may result in a decrease in the range of motion and stiffness of the affected spinal segments.

Further studies should be done to determine the effects of the enlargement of the vertebral bodies in response to degenerative disc disease. Using a hydrogel to restore height in the intervertebral discs may not be a sufficient method of treatment since the intervertebral space may be partially occupied by newly formed vertebral bone. Also, further studies are needed to examine if degenerative disc disease can cause an increase in bone density of the lumbar spine. Bone density scans are taken of the lumbar spine to diagnose a patient with osteoporosis, but if degenerative disc disease is increasing the bone density, then the diagnosis may be incorrect. The bone density measurements would appear to be within normal limits even though the patent may have osteoporosis.

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Xenotransplantation: The Science, the Advantages, the Ethics

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Abstract

Xenotransplantation is a breakthrough medicinal technology that is an attempt to change the lives of millions of people. The problems in the current organ transplant system risk the lives of patients each and every day. Intense waiting periods and extremely costly procedures exemplify the stress and pressure that these patients face as an attempt to save their own lives. Xenotransplantation is the idea of growing human organs in a different species, using incredible stem-cell and CRISPR technology. This can introduce an answer to some of the issues in the current transplant system. Many technical and ethical issues are becoming relevant with the introduction of this new medical phenomenon. If these barriers can be overcome, xenotransplantation can offer a quicker, cheaper, and more effective option for patients in need of an organ transplant.

Introduction

Thousands of sick patients are in desperate need of an organ transplant, often having their entire lives depending on this one vital procedure. Before they can undergo treatment, a proper organ donor must be found, and the process is harder than ever imagined. Numerous factors affect the long waiting time, a period that can frequently become over a year. The main slowdown is the simple shortage of organs ready for transplant. In response to the overwhelming need for organ donations, new medical research is being explored as an attempt to alleviate the high demand. Xenotransplantation poses as a possible solution to this problem and has the capabilities to be very helpful in the medical world; releasing the great amount of stress and anxiety involved in the classic organ transplant system that is now in place. However, this new medical process introduces new biomedical and ethical issues. Scientists and doctors are currently exploring the possibilities of this new technology, weighing the costs against the benefits; an important attempt to improve the medical world of organ transplantation.

History of Organ Transplants

The concept of organ transfer in a procedural sense has been around for centuries, in-fact cases of organ transplantation dates back to ancient times. There is even written proof and documentation from archeological records that suggest that organ and tissue transplantation is thousands of years old. However, modern-day organ transplantation is a relatively new medical phenomenon that has saved the lives of millions of people from end-stage organ failure.

In the early 1900's, Dr. Alexis Carrel experimented with kidney transplantations in cats, becoming the first surgeon to explore vascular surgery (Howard et.al., 2012). Carrel'journey to organ transplantations started with success in vascular surgery and suturing of vessels. These experiments also advanced the knowledge of organ preservation, showing that human tissue could be stored in either saline solution, the patient's serum, or defibrinated blood, until it is needed. Carrel then went on to organ transplantations, experimenting with kidney transplantations between dogs, and from dogs to cats. He realized that the animals only lived with the transplant for a short period of time and he knew that there was some sort of rejection going on in the host's body. Alexis Carrel made extraordinary advancements in the medical exploration of organ transplants and created the possibility for more research in this area (Rothwell, 2011). In 1923, Dr. Harold Neuhof wrote a book called The Transplantation of Tissues, which revealed the work of many different tissue transplants including skin, cornea, muscle, pancreas and nerve tissue (Howard et.al., 2012).

Soon after, the first human kidney transplant was recorded using a kidney from a donor that had died six hours prior. The blood type of the donor was type B and the recipient was type O, which prevented the kidney from functioning, leading to the patient's death. However, this may not have been the only cause for the disfunction of the kidney as other trials have also been proven unsuccessful. In 1953, a kidney transplant was done from mother to son which only lasted three weeks before the recipient rejected the donor organ. In the beginning years of the 1950's, Dr. David Hume performed almost ten kidney transplantations which are now considered the first of their kind in the modern era of transplantation. However, none of the recipients had a long-term survival after their kidney transplant.

These and other trials revealed that such organ transplants would not be possible without immunosuppressant drugs. With this development, organ transplant became a real option for patients with organ failure. Dr. Joseph Murray performed the first successful kidney transplant in 1962, leading into many other victorious cases around the world. The '60's were very progressive years for this specific research and medication, as the first lung and heart transplants were also done during this time (Howard et. al., 2012).

Problems With the Current Organ Transplant System

As immunosuppressant drugs allowed organ transplantation to start becoming a viable option for victims of organ failure, the need for transplantable organs became overwhelming. In the past, kidneys from living donors were being used, but this supply is limited and not nearly enough to provide for all in need. Even with deceased donors for transplants, the available donor organs, unfortunately, do not cover the demand (United Network for Organ Sharing, Data, 2018).

Although most technical problems involved in the process have been solved, organ transplantation is not seen as a long-term cure for patients as there are still biological issues and financial problems.

The biggest and most complicated problem is the patient's rejection of the new organ. When a patient undergoes an organ transplant surgery, the body's immune system becomes stimulated against the "foreign" organ and tries to kill it. Patients are usually given strong medication to suppress their entire immune system, but that can become counterproductive as the patient is now vulnerable to other diseases. Different drugs, such as Cyclosporin, have been approved to control much of the rejection, but transplantations can still have those dangerous effects (MTF Biologics, 2017). Although they have been shown to improve symptoms, these drugs can have many negative side effects including tremors and seizures.

Another problem is the long waiting period that patients wait in order to receive a proper match for donation. There are simply not enough donors to fill the demands, causing many victims to die in the 'waiting room'. Although the shortage of organs is the main cause for the waiting time, the length that the patient waits can vary, depending on a number of factors. The blood or tissue type, the size of the organ, and the medical urgency of the situation are some factors that will fluctuate the waiting time. In any case, the patient almost always needs to wait for a proper donor before proceeding with the transplant. This is not practical in many cases when the organ transplant is extremely urgent. On average, twenty people die every day while waiting for an organ donor (United Network for Organ Sharing, Data, 2018).

Also, organ transplants are extremely costly and many people simply cannot afford such medical procedures. Kidney transplants cost over four hundred thousand dollars, and a heart transplant goes up to over one million (Rapp & Vandermey, 2017). This cost does not even include the health maintenance of such a procedure, nor the cost of immunosuppressant drugs that cost as much as ten thousand dollars per year, for life (Gordon, et. al. 2008).

Methodology of Xenotransplantation

Looking for a solution to this unmet medical need, researchers have come up with a new method known as Xenotransplantation, which is the process of "grafting or transplanting organs or tissues between members of different species." Xenotransplantation is using stem-cell and CRISPR technology to grow human organs in a host of a different species. This idea comes from chimerism, the ability to create a "living thing that is composed of cells from two or more organisms" (Dunlap, 2017). The basic procedure is to use the pig as a host by cutting out the HOX genes that code for growing certain organs and the genes that code for porcine microorganisms from their genome. HOX genes are the sequence of genes in the genome that directs the body organization in an embryo (KhanAcademy, Khanacademy.org, 2018). Using CRISPR-Cas9 technology, described below, genetic engineers can knock out the genes in the pig that code for the development of specific organs, and replace them with specialized human stem cells that will grow human organs instead. The hypothesis is that infusing differentiated human stem-cells into this embryo will result in the pig growing a human organ instead of its own.

CRISPR-Cas9 Technology

CRISPR-Cas9 stands for Clustered Regularly Interspaced Short Palindromic Repeats, and is breakthrough technology in genome editing. CRISPR is a special region in DNA that is characterized by having repeated nucleotides and spacers. This unique DNA region was first recognized in bacterial genomes and has since been adapted in the laboratory as the CRISPR Cas9 complex (Vidyasagar, 2018). Cas9 is a protein that acts as the molecular scissors that can cut the DNA in a double-strand break. When combined with a guide RNA (sgRNA), it forms the Cas9 Complex. The Cas9 first binds to a sequence in the genome and the guide RNA unwinds the double helix. The guide RNA is precisely designed to match up with the specific strand of DNA that needs to be edited. Then, the Cas9, with the help of its nuclease domains, cuts the DNA, creating a double-strand break in the double-helix. The DNA tries to repair itself but the reparations are usually flawed which inevitably shuts off that particular gene. This is why CRISPR is "a great tool for knocking out specific genes" (NatureVideoChannel, 2017).

Stem-Cell Research

Another biomedical technique is somatic cell nuclear transfer, known as SCNT, or cloning. Conceptually, the nucleus from a somatic (body) cell replaces the nucleus in a fertilized oocyte of another individual. This hybrid blastocyst is capable of generating a stem cell line" (Columbia.edu, 2018). The created stem cells will be identical to the donor and have many uses in the medical field. One such example would be to use these cloned cells to create human organs in pigs a step in the xenotransplantation process. The idea is to fuse the stem cells from the patient into a porcine embryo, hoping that the pig will grow human organs that can then be used as the transplant for the intended patient. In a few short months, the patient could potentially be on his/her way to a complete recovery. This will only be a possibility, however, if the barriers of Xenotransplantation are overcome.

Brief History of Xenotransplantation

Aside from cross-species blood transfusions, which were around since the 17th century, skin grafts are considered the first attempted trial in xenotransplantation. Skin grafts from animals to human patients were performed as early as the 19th century. Frog skin was the most popular species to was the most frequent source of the donor material to the skin grafts. Different attempts at xenotransplantation were tested, mostly using primates as the donor animal. Out of thirteen trials, only one kidney transplant from a chimpanzee to a human patient

was successful with the patient living for an entire nine months before dying as a result of rejection of the kidney. In 1964, the first heart xenograft from a chimpanzee to a human was performed. The patient died within two hours of the operation. Later, in 1992, a doctor named Starzl performed a baboon to human liver transplant, with the patient surviving for seventy days. Along with the numerous failures, some of the early xenotransplants resulted in temporary patient survival. But, in general, the organs were rejected in an even more serious case than with a same-species transplant. The rejections of this kind are so severe that immunosuppressive drugs are not strong enough to stop the rejective response to the xenografts (Cooper, 2015)

The Advantages of Porcine Xenotransplantation

Recently, the focus has been on the use of organs from the pig and hog family, porcine, as they are comparable to human organs in size, anatomy and physiology, compared to other species (Niu, Wei, & Lin, 2017). Pigs, compared to other species such as baboons, would be a valuable candidate for organ xenotransplantation. Pigs can be bred in large herds and with a relatively short maternity period, making them a more available option. In addition, they are not a costly animal to breed (Cooper, 2015).

The advantages of xenotransplantation from pigs would be enormous, starting with the obvious increase in availability of organs for transplantation. Right now, there is a shortage of human organs for transplants, but using organs from another species will solve this problem, it will also make immediate transplant surgery possible for patients with an urgent situation. In addition, organs that are retrieved from human cadavers have already experienced the trauma of brain death which may lead to other issues later on. With animal donor organs, the organs will be removed from a healthy pig that is under anesthesia, avoiding the problem altogether. Another important solution that xenotransplantation brings is the decrease of the risk of a pathogen being transferred from the donor to the patient. Diseases such as the West Nile Virus and rabies have been passed through organ transplant surgery resulting in a number of deaths. The pigs intended to be used for xenotransplantation are being raised in the best conditions and are being supervised closely which reduces the likelihood of any illness to be transferred from donor to patient (Cooper, 2015).

Zoonoses and Porcine Endogenous Retrovirus (PERV)

CRISPR-Cas9 can be used in xenotransplantation research by knocking out specific genes in the pig that cause rejection when transplanted into human patients. Zoonoses are diseases that are transferred between humans and animals, caused by bacteria, parasites, and viruses. These diseases pose the major barrier preventing xenotransplantation from becoming proto-call in the medical world. The potential transfer of porcine related infections into the human genome is a major risk of xenotransplantation. The pig herds intended to be used for medical reasons have been raised under the most sanitary conditions and have been screened for most pathogens and viruses that can possibly harm the recipient. However, porcine endogenous retroviruses that lie in the porcine genome have the capability to hurt human health. PERV are viral elements in the porcine genome, and being that they are innately a part of the DNA, the retroviruses cannot be eliminated by means of selective breeding or drugs. PERVs have the ability to recombine with other genetic elements of the recipient's genome. Similar to pigs, humans have their own set of endogenous retroviruses, known as human endogenous retroviruses, or HERVs. If the PERV were to come in contact with the human endogenous retroviruses, the former genes can recombine and disturb the human genome. Naturally, the HERVs are attempted to be halted by some sort of stop codons or deletions, but some bypass the stop codons and actually "play an important role in human physiology as well as in pathogenesis" (Machnik, et al., 2014).

Studies of HERVs have concluded that class I of HERVS group together with γ- and ε-retroviruses, and during a xenotransplantation it is these genes that can recombine with PERVs that also belong to the γ-retroviral group (Machnik, et al., 2014). Studies have recently confirmed that two out of three PERV types, namely PERV-A and B, have been seen to replicate in human cells during in-vitro research, meaning in a clinical setting. To date, no PERV infection of human cells have been documented in-vivo, but the possibility of this occurring needs to be taken into real account as new pathogens can be created at the point of interaction, and these new pathogens can cause unpredictable damage (Prabha & Verghese, 2012).

Recent discoveries, however, have shown major advancements in this area. Scientists have used CRISPR technology to successfully cut out sixty-two PERV sequences from a porcine genome. The nuclei of these cells were then transferred into enucleated pig oocytes by somatic cell nuclear transfer, and then implanted into surrogate female pigs. The results were astonishing with thirty-seven piglets being born, all with inactivated PERVs in the genome. This research greatly expands the possibilities in the realm of xenotransplantation, although other issues are yet to be resolved (Denner, 2017).

The Issue of Rejection

With all the prementioned advantages, there are still barriers preventing pig organ xenotransplantation from becoming the standard medical procedure. Scientifically speaking, the biggest barrier to overcome is the problem of rejection; the patient's natural response to an alien in the body. Firstly, within seconds or minutes of the transplant, the possibility of hyperacute rejection can happen. This type of rejection occurred when pig organs were transplanted into nonhuman primates. The

preformed antibodies of the receiver attached themselves to the pig's vascular endothelium and as protocol reaction to a pathogen, the cascade system was initiated. This caused the endothelial cells to convert into procoagulant phenotypical cells, resulting in hyperacute rejection. Attempts have been made to prevent this by depleting the antipig antigens, or the complement system from the recipient's serum, however, there are still other forms of rejection that may occur. Acute humoral xenograft rejection may occur during the first few days after the transplant, and cellular rejection can become a problem even weeks after the operation. This rejection is acute-cell mediated, meaning that different cells in the patient's body recognize the foreign organ as unfamiliar and begins to attack it. The cells involved are usually the natural killer cells, macrophages, and cytotoxic T cells. It is believed that even immunosuppressant drugs cannot prevent this rejection from being triggered. If the recipient is able to survive with the graft for a longer period of time, chronic rejection may occur. The exact cause of this type of rejection is not completely understood. However, the main point regarding all types of rejections is that the antibodies or killer-cells in the recipient attack the donated graft, resulting in a slow, but ultimately fatal destruction of that organ (Esker & Cooper, 2010). The information regarding the rejection or acceptance of a pig organ in a human patient is extremely limited, as only a few procedures have been tested so far. The scientists and doctors cannot study this further until a patient survives the procedure for a longer time (McLean & Williamson, 2004).

Rejection of the organ can occur in any transplant, regardless of whether the graft is from the same species or not. The difference is that in a xenotransplant rejection, the immunosuppressant drugs are not strong enough nor sophisticated enough to significantly extend the patients survival time.

Potential Solution for Xenograft Reject

Genetic Engineering of the pigs is the newest and most successful method to date to prevent or mitigate the rejection of the organ graft. One major advancement is the creation of a genetically engineered pig that expresses a human complement-regulatory protein. This protein is found in the pig's vascular endothelial cells and protects the tissue of this donated organ from being attacked by the pre-formed antibodies during hyperacute rejection (Bloom, Moulton, McCoy, Chapman, & Patterson, 1999). Genetic engineers have also produced pigs which have had the gene for α1,3-galactosyltransferase removed. The gene for α1,3-galactosyltransferase codes for the production of the enzyme that adds Galα1,3Gal oligosaccharides to different basic glycoproteins and glycolipids in pigs. In general, Gal is a major target for the human antibodies, as it is seen as a major invader. The removal of this gene, and therefore the halt of the Gal production in the pigs, has significantly reduced the hyperacute rejections that usually occur during the pig to nonhuman primate xenograft trials (Esker & Cooper, 2010).

The results of this research have been positive; the length of survival time increasing by the use of the genetically engineered pig organs. A pig heart of this type lasted for 3-6 months, kidneys for close to three months, and livers for days. The extension of the survival period is proof of the success of the genetic research (Esker & Cooper, 2010).

There are also genetic solutions being studied that will disable the natural killer cells and the macrophages from attacking the xenograft, although they have yet to be tested in pig to non-human-primate transplant. Genetic engineers are working on producing pigs that are HLA-E or HLA-G transgenic as these immunoregulatory molecules are expected to stop natural killer cell cytotoxicity (Esker & Cooper, 2010).

The Issue of Coagulation Dysregulation

Another barrier that has arisen during the xenotransplantation trials is the development of thrombotic coagulopathy and consumptive coagulopathy, different types of coagulation dysregulation. Coagulation dysregulation is irregular blood clotting in areas of the circulatory system. The xenograft recipient will usually develop one of these, or possibly both, which poses a big limitation to the survival time of the graft. In heart transplants from genetically modified pigs to baboons, thrombotic coagulopathy is the predominant symptom, while in the kidney graft, the baboon developed consumptive coagulopathy. It is thought that physiological differences between pigs and primates are the causes for these problems, although the exact reason for the coagulation dysregulation development remains unclear. It is known, however, that activated endothelial cells of the donated organ are the cause for inflammation and coagulation in the recipient. Ischemia, or inflammation, is inevitable in most types of organ transplants, but various types of intertwined factors can be the cause of graft endothelial cell activation (Cowan, Robson, & d'Apice, 2011). One proposition suggests that the endothelial cells activated by either the antibodies or the start of the complement, increase the activity of TF, tissue factor. The introduction of the TF into the portal vein activates the instant blood-mediated inflammatory reaction, known as IBMIR. Characteristics of IBMIR are platelet binding, complement activation, and thrombosis (Esker & Cooper, 2010).

Ethical Problems With Xenotransplantation

In addition to the biological barriers of Xenotransplantation, problems regarding ethical and moral topics come into question when researching the possibilities for the future. Transplantation in general has a public risk associated with the administration of immunosuppressant drugs. These drugs are known to lower the patient's ability to fight off infections, and therefore increase the risk of contamination. For the patient, the risks involved need to be weighed by their own physician; however there are public

concerns involved as well. The new genetic engineering, plus the previously used immunosuppressant drugs, have the potential to "open the way for the emergence of new viral mosaics into the general population" (Anderson, 2006).

The opposing side of the ethical debate brings up valid points as well. With thousands of people in desperate need of organ donations, is it ethically fair to deprive them of the possible solution that can potentially save so many lives? The introduction of xenotransplantation can greatly reduce the waiting period for an organ donation, and additionally provide the patient with a long-term graft. The immunosuppressant drugs have saved so many people from death; is it morally okay to ban them on the slight chance that they can cause an infection outbreak? These questions are some of the many that have come up in discussion over the last couple of years, as the medical world is coming closer to introducing this new phenomenon to regular medical protocol.

Another ethical problem involved in xenotransplantation is the issue of personal privacy and confidentiality. This question arises due to the necessity of monitoring of the patient after an organ transplant, going as far as to institute a lifetime surveillance of the patient. This surveillance has become a part of the system as an attempt to prevent pathogenic diseases from spreading through organ transplant surgery. As previously discussed, the pathogenic risks involved in xenotransplantation are very great and have caused medical outbreaks in the past. In fact, in the year 2000, a hepatitis C breakout was reported due to an infected donor that was mistaken to be healthy. Since then, tremendous precautions have been taken while looking for organ donors, as the transfer of pathogens through transplants can lead to numerous fatalities. Opponents of the monitoring system believe that this provision is an invasion of the patients' privacy, as well as the privacy of their family and friends. Here lies the question whether or not the invasion of privacy is considered to be ethical for the safety of the patien and the "problem of maintaining ethical standards in situations such as this is vexing" (Anderson, 2006).

The only possible way to effectively protect society from infectious diseases via xenotransplantation is if the national and international powers decide on a logical system, keeping the ethical debates in mind. The World Health Organization has previously come together to discuss this exact topic, although no agreement was yet to be decided. To date, the only guidelines put in place are those of the Food and Drug Administration's Recombinant DNA Advisory Council. The RAC approved gene recombination studies to be done at Harvard and MIT, advancing the medical research of this field. The RAC's permission was granted with the consent of the citizens of Massachusetts, justifying the ethical questions involved in such research (Anderson, 2006).

The American Society of Transplant Physicians voice their concern of infectious diseases being spread internationally. They feel that United States regulations are not strong enough in comparison to the severity of the risk involved. It is presently legal for patients in need of an organ donation to receive organs from live donors living in impoverished countries, where infections are known to be rampant. For example, there is a website named Liver4you.org which arranges liver donations from people in the Philippine Islands. Bringing these diseases across to the United States through the organ transplant poses as a major threat to the wellbeing of society (Anderson, 2006).

Often, doctors and surgeons are also researchers of new xenotransplantation medication, which poses additional ethical questions regarding the split between their medical responsibilities and their personal studies. The physicians can frequently be confronted with "the well-being of the patient in direct opposition to the advancement of academic medicine" (Anderson, 2006). An example of what might result from such a situation is a doctor convincing a patient to participate in a clinical trial that he/she would otherwise not join. An infamous case of this sort, known as the Baby Fae case, took place in 1984 when a young child was used in a medical trial of a xenotransplantation using a baboon heart. The parents were encouraged by optimistic doctors who hoped to provide the baby with "immediate and long-term survival" but she only survived for four weeks following the graft. Medical researchers have become cautious getting involved in xenotransplantation studies in fear of this sort of issue cropping up, in addition to the history of medical science misconduct (Anderson, 2006).

Additionally, with the introduction of stem cells into the pig embryo, the possibility of creating a pig with human elements presents an ethical issue. The ethical debate discusses the possibility of human gametes to be created and an embryo to be formed as an interspecies chimera. Although it is agreed upon that the embryo would not be able to develop, the mere creation of it poses as an ethical question. Also, the differentiation of human stem cells needs to be studied in depth as the question arises of what is stopping the stem cells from entering the pig's brain, or other areas, which will blur the margin of what is considered human and what is considered animal? Is it morally permissible to perform a procedure that can potentially give a pig, human, high-level brain functions? These ideas are unlikely in the scheme of things, but pose concerns in the biomedical world nonetheless (Masaski & Nakauchi, 2017).

Conclusion

Scientific researchers are constantly trying to better the medical world; looking for solutions and applying their incredible knowledge to helping humanity. The biomedical field is exploring the possibilities of genetic engineering combined with stem-cell research, hoping for xenotransplantation to one day become a standard medical procedure for patients with organ failure. When it comes to genetic engineering, the possibilities are endless; however, xenotransplantation has many barriers to

overcome before it can become the standard medical procedure. However, the latest research may explore the best course of action regarding this dynamic research. Researchers have proposed for xenografts to be used for patients in the waiting period that are waiting for an organ donor. This way, although xenotransplantation is not yet a final solution, it will at least minimize the number of deaths of patients, and the health risks for waiting for a proper transplant. This change in proto-call has the tremendous potential to save millions of lives, giving the patient the ability to live the few months until they can receive a proper transplant. Researchers are only beginning to uncover the technology's tremendous potential and long-term survival of xenografts may be a few years away. Even so, it is possible that for now, temporary xenotransplantation can be the life-saving procedure that patients are hoping for (Servick, 2017).

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Can Somatic Cell Nuclear Transfer Produce Human Pluripotent Stem Cells for Regenerative Medicine?

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Abstract

In the last half a century, researchers and scientists discovered the application of somatic cell nuclear transfer (SCNT) to clone mammalian embryos to produce a line of pluripotent stem cells for medical and laboratory use. This is a breakthrough technology that is applied to stem cell research, regenerative medicine, and cloning. Somatic cells are non-germ cells that are differentiated but provide the nuclei that are transferred to enucleated oocytes. The replacement of the nuclei results in a developing embryo that contains the genetic information of the donated nucleus, which can either be transplanted into a surrogate mother to produce a genetically similar offspring or grow in-vitro to extract embryonic stem cells (ESC). This process has made it possible for the cloning of numerous mammalian species, such as pigs, cattle, mice, and, recently, primates. Although success has been evident in mammals, human derivation of pluripotent embryonic stem cells has been difficult to obtain. The difficulty stems from the premature activation of the oocyte and the improper reprogramming of the donated nucleus. This paper focuses on the development of human nuclear transfer embryonic stem cells (ntESC) and its application in regenerative medicine. Studies done on primates provide information on the barriers of this procedure on humans and the proper modifications on regular SCNT protocol. The use of deacetylase inhibitor TSA, phosphate inhibitor caffeine, and HVJ-E for proper membrane fusion are only some of the recent methods found for proper nuclear reprogramming and embryonic development. Breakthroughs in the methylation of DNA and histones in mice provided insight to a barrier in human embryo development. As a result, derivation of embryonic stem cells was successful and tested for pluripotency. Insulin beta cells and cardiomyocytes have been produced using this modified SCNT protocol and hold great potential for the future of science. The use of nuclear transfer embryonic stem cells is important in the development of stem cells that can differentiate into specialized cells, such as neurons, that can potentially be used to cure disease, like Parkinson's. Even more so, these cells will retain the genome of the patient and reduce immune incompatibility. The paper goes on to discuss the ethical issues that impede researchers from advancing in this area.

Introduction

Somatic cell nuclear transfer (SCNT) is a laboratory technique in which the somatic cell nucleus is transferred into an enucleated oocyte. The egg is developed into a blastocyst, an early embryonic stage where the opening of a cavity in the morula between the inner cell mass and the enveloping layer is filled with fluid. This procedure was first theorized in 1938 by Hans Spemann, a German embryologist, in his book Embryonic Development and Induction. He proposed an experiment to replace the nucleus of an unfertilized egg with a differentiated embryo nucleus. He pondered on whether such a transplant of a differentiated nucleus can give rise to an organism (Wellner, 2010). This was a start to the idea of cloning that was initially attempted in 1952. Scientists Robert Briggs and Thomas King transplanted nuclei from an advanced blastula cell into enucleated eggs of Rana pipiens, also known as leopard frogs. They saw a normal development of embryo resulting in the first cloned organism. Their study provided a basis for the SCNT protocol. As well, they determined that success is attributed to the transfer of a nucleus from the same species of frog. Transplanting the nucleus of a bullfrog led to an arrest at the blastula stage and inevitably died (Briggs et. al, 1952). Alluding to the core principle in SCNT mechanics of donor nucleus and oocyte recipient compatibility.

In 1996, the first cloned mammal using a fully differentiated adult cell was executed by Campbell et al. (1996) in Scotland. Dolly, the cloned sheep born in 1997, was a breakthrough in determining the nuances of SCNT. They figured out that the development of embryos reconstructed by nuclear transfer is highly dependent on the interaction of the donor nucleus and recipient cytoplasm (Campbell et al., 1996). Their findings aided in further successes of cloned organisms and nuclear transfer embryonic stem cells (ntESC) derivation. Such findings include the effects of cytoplasmic kinase activity and maturation promoting factor (MPF) on chromosomal damage. The expression of cytokeratin and lamin A/C, markers associated with differentiation, was also reported indicating the development of ESC. Research in this area after this incident paved the road to develop human ntESC and the extensive studies done on primates contributed to a modified SCNT approach.

The important application of this process is to produce a culture of embryonic stem cells that are created from the inner cell mass of the blastocyst. Stem cells are undifferentiated cells that can either produce cells that continue as stem cells or are differentiated into specialized cells. The implications of this procedure of generating personalized embryonic stem cells is useful for disease mechanism, and development of therapies (Tachibana et al, 2013), i.e. regenerative medicine or replacing damaged cells in patients, like cardiomyocytes to replace damaged heart tissue or insulin-producing beta cells for diabetic patients, by eliminating the prospect of immune incompatibility (Lanza et al, 1999). However, therapeutic cloning refers to the derivation of nuclear transfer embryonic stem cells without uterine transplantation that has been successful in mice and cattle, derived from ntESC lines in cloned blastocysts (Yang et. al, 2007). However, human ntESC has been notoriously difficult due to its failure to progress past the eight-cell stage because of inactivation of critical embryonic genes. Due to ethical and legal restrictions, further research in this area is moving at a slower pace. Therefore the question arises, can SCNT be used to produce human pluripotent stem cells for regenerative medicine?

Methods

The information gathered in this paper was collected from several sources such as Touro College Online Library and NCBI. The searches for "Somatic cell nuclear transfer", "Cloning", and "Therapeutic Cloning" led to the majority of articles used obtained from PubMed and Nature. The articles discuss the experimental studies done at various times and an analysis of these studies provided material for this discussion. Some review articles were necessary in the development of this analysis.

Discussion: Nuclear Reprogramming

For successful embryonic development the proper nuclear and cytoplast-mediated reprogramming is necessary. Campbell and colleagues (1993) specified morphological events that occur in nuclear transfer at the merging of a nucleated blastomere or karyoplast into the cytoplast of an enucleated metaphase II arrested oocyte (MII). Oocytes in MII state have a higher success rate in nuclear reprogramming in the cytoplasm due to certain factors. In mammals, the fusion of the two specimens is induced by an electric pulse to promote fusion and activation of the enucleated egg. Studies in mice, pigs, and cattle display that certain morphological events occur at the point of fusion, including nuclear envelop breakdown (NEBD) followed by premature chromosome condensation (PCC), dispersal of nucleoli, reformation of the nuclear envelope, and nuclear swelling (Campbell et. al, 1993). The induction of NEBD and PCC are important for the reprogramming of gene expression and increased embryo development. These events vary depending on both the species and nuclei at different cycle stages. In rabbits, blastocyst development was found to be greater when the blastomeres were in G1 or early S phase than in late S phase or G2 (Collas et. al, 1992). A possible explanation for such observations was found in the cell cycle regulation with an emphasis on cellular DNA replication by maturation promoting factor (MPF).

A study showed the necessary implication of MPF activity at G1/S and G2 stages. MPF is a complex of two proteins, cyclins and p34cdc2. P34cdc2 is a protein kinase that is regulated by phosphorylation changes and interaction with cyclins. P34cdc2 does not change during the cell division cycle while cyclin does. P34cdc2 kinase triggers the entry of the cell into the M phase that produces NEBD, chromatin condensation, and other morphological changes in the cell. Therefore, MPF primary function is to promote nuclear breakdown, spindle formation, and chromatin condensation. Mammalian oocytes at the MII phase contain high levels of MPF. Upon fertilization or activation, MPF levels decline resulting in the decondensation of chromatin and formation of pronuclei. Therefore, NEBD and PCC occurrence in the donor nucleus suggests a correlation between MPF with NEBD and PCC activity. NEBD and PCC probably remove the nuclear membrane to allow ooplasmic remodeling factors access to the donor cell chromatin for the re-replication of previously replicated DNA and the synthesis of DNA in the donor nucleus at any cell stage. As well as, contributes to the reformation of the nuclear membrane. A problem arises when MPF activity is low at the time of fusion (Campbell et al., 1993).

When MPF is low at the time of fusion, from premature activation of the enucleated egg, the nuclear membrane is maintained. G1 donor nuclei will replicate their DNA, however, G2 nuclei will not rereplicate and a low frequency of development arises. However, interestingly enough, this suggests that, at slow MPF decline at the time of fusion, in any other phase other than G1, the DNA will rereplicate resulting in aneuploidy. This correlates with the results of the study done by Collas et. al (1992) regarding the development of rabbit blastocyst. In rabbits, at G1, early S, and late S phases, metaphase plates, and spindles were detected but abnormalities, like incomplete spindle formation and incomplete chromosome condensation, was present in late S phases (Campbell et. al, 1993).

A study was done by Mitalipov et. al (2007) built on the aspect of MPF regulation in primates and nuclear remodeling through lamin A/C staining. Lamins, an intermediate filament superfamily of proteins, are part of the nuclear lamina found on the inner layer of the nuclear envelope. Lamin types A and C are expressed in many differentiated somatic cells. Lamins A/C are essential in size, shape, and strength determination of the nuclear envelop as well as maintaining lamina structure. (Hutchison and Worman, 2004). These proteins tend to depolymerize during late prophase and become undetectable in the MI or MII oocytes. At fertilization, cytoplasmic lamins are gathered into the forming pronucleus (Prather et al., 1989). Lamin A/C has been used to assess the extent of nuclear remodeling following SCNT because of the changes in the nuclear lamina, as shown in this 1study, in monkeys.

MPF levels, as mentioned before, result from premature oocyte activation and a failure to induce nuclear remodeling. In studies with mice and cattle, the fusion of a donor cell with an oocyte was done through electroporation. Electroporation in a calcium ion fusion medium increases the calcium levels that trigger a rapid decline of histone H1 kinase and even MPF activity. Using electrofusion with calcium ion or magnesium ion free buffers or performing all manipulations free of these ions can minimize premature activation of the oocyte (Mitalipov, 2007). An improvement in the in-vitro development of pig (Boquest et al., 2002) and primate blastocysts (Mitalipov et al., 2007), which was shown from lamin A/C profile under these modifications was similar to those detected in sperm-fertilized controls. To supplement electroporation as a fusing agent in human SCNT, HVJ-E virus, hemagglutinating virus of Japan-envelope, a non-infectious vesicle used as an agent for cell fusion, showed a high rate of fusion. However, embryonic development past the compact morula failed, even though ionomycin and 6-Dimethylaminopyridine (DMAP) were used to activate the cell at the appropriate time.

Therefore, implementing electroporation, which is not necessary for cell fusion but rather for timely cell activation, increased blastocyst development. It is necessary as an activation stimulus in conjunction with ionomycin and DMAP (Tachibana et. al, 2013).

Extended exposure of the chromatin to the non-activated MII cytoplast enhances embryonic development. An MII oocyte has high levels of MPF but enucleation, which also removes cytoplasm from the oocyte, reduces MPF levels (Lee & Campbell, 2006). The disassembly of the nuclear lamina with the chromosome condensation is attributed to the phosphorylation of MPF. To prolong the presence of MPF, use of caffeine, a protein phosphatase inhibitor, or MG-132, a proteasome inhibitor, increased nuclear remodeling in the donor nucleus. MG-132 prevents spontaneous oocyte activation, as seen by the weak/partial lamin A/C signal in C/C1 (figure 1). More exposure to MG-132 reduces the lamin A/C signal and increases the chromatin condensation, as seen by DAPI blue stain (Mitalipov et. al, 2007). However, proteasome activity impacts development events, in contrast to caffeine that has high cleavage rates and regular blastocyst formation. However, caffeine does not affect the frequency of blastocyst formation (Lee & Campbell, 2006; Mitalipov et. al, 2007). Embryos usually do not develop past day-12. These conclusions suggest there is more than merely nuclear remodeling that affects the success of SCNT.

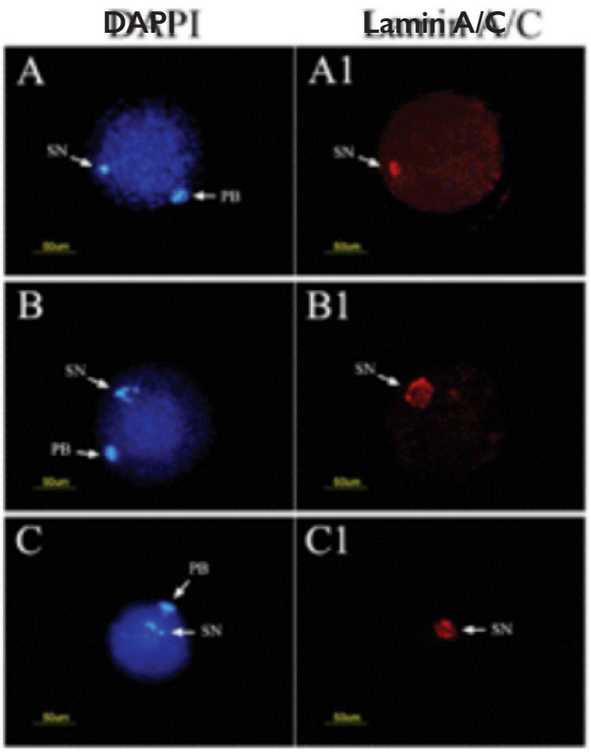

Figure 1: Lamin A/C signaling and DAPI staining for exposure of MG-132 (Mitalipov et al, 2007)

Premature activation of the MII oocyte is a likely occurrence, especially in aged or overstimulated oocytes. Therapeutic cloning or ESC derivation from discarded, aged, or failure-to-fertilize oocytes are unlikely to succeed (Mitalipov et al., 2007). Ovarian stimulation can also affect the quality of the oocyte, thus, tampering with the success of the procedure. A study was conducted to compare three groups of oocyte donation cycles, <10 oocytes per cycle, 11-15 oocytes per cycle, and >16 oocytes per cycle. Survival of the egg after spindle removal until embryonic cleavage was similar amongst the three groups. However, more >16 oocyte/cycle groups arrested after the eighth cell stage. Additionally, the quality of the blastocysts correlated inversely with the number of oocytes collected per cycle. ntESC derived from these oocytes were mainly from the <10 oocyte/cycle group and none from the >16 oocyte/cycle group (Tachibana et al., 2013). This shows that both the donated nucleus and recipient oocyte contribute to the success of ntESC derivation and development.

Another challenge to SCNT embryonic development is the consequence of microtubule and centrosomal protein depletion. This is a result of enucleation of the oocyte that extracts the spindle with defective reformation of the spindle by the donor nucleus. However, when the somatic cell nucleus is transferred to an oocyte containing its own chromosomes it results in a functional polyploid blastocyst that can develop into ESC. This can mean that certain reprogramming factors, associated with the spindle apparatus, are present in the MII oocyte (Tachibana et al., 2013) and are removed upon enucleation. Modification in the SCNT protocol of spindle removal is necessary to successfully execute nuclear reprogramming and blastocyst formation. The standard procedure of enucleation and spindle extraction is by adding bisbenzimide staining and UV exposure in mammals, such was executed in pigs (Polejaeva et al., 2000). However, if meiosis-specific factors are retained during spindle removal then only spontaneous activation of the cell causes its decline. By using the methods above to protect against premature activation, SCNT embryo development increases and spindle-like structures are formed. This method increased ESC derivation in primates that displayed similarities to human IVF-derived blastocysts than the manipulated spindle transfer embryos (Tachibana et al., 2013).

Epigenetic Regulation

Nuclear reprogramming is a crucial step for the success of embryonic development. However, it is not limited to the first steps of activation. Reprogramming gene expression in the inner cell mass (ICM) is a necessary component for the continuation of embryonic development and especially for derivation of pluripotent ntESCs. Normal development requires epigenetic modifications, which includes DNA methylation and histone modification. The ICM and trophectoderm are distinguished in the blastocyst with a predominant increase of DNA and histone

methylation in the ICM, affecting genomic imprinting and X chromosome activation in females (Yang et al., 2007).

In normal embryonic development, methylation occurs preferentially in the ICM, a process in which a methyl group is added to the 5th carbon of the cytosine ring next to a guanine base, through DNA methyltransferases (DNMT). In female mice, for example, demethylation occurs due to DNMT during subsequent cleavages. In male mice, it is demethylated in the pronucleus after fertilization. Chromatin configuration establishes the 'histone code,' a cellular memory responsible for maintaining the identity of differentiated cells. Histones are proteins that contribute to the make-up of chromatin. They have an exposed N-terminus end, which is either modified through methylation, acetylation, ubiquitinylation, or phosphorylation. For example, the acetylation of histone 3 Lys9 (H3K9) induces an open chromatin configuration giving transcription factors access while the methylation or demethylation of H3K9 inactivates it (Yang et al., 2007).

Trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, has shown a significant impact on embryonic development in mammals and primates (Sawai et al., 2012; Mitalipov et al., 2007). A study was conducted on bovine animals using 5nM and 50nM of TSA. The group that was treated with 50nM of TSA showed significant blastocyst development. TSA increased histone H4K8 and histone H3K9K14 acetylation, which activates gene tran-

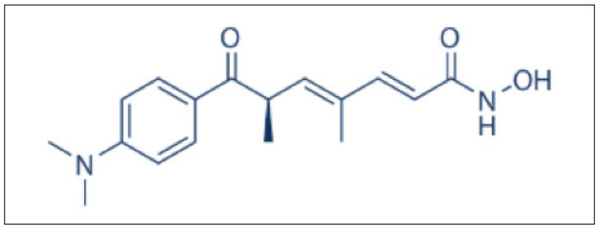

Figure 3:TSA structure. Selleckchem.com

scripts. However, FGF4 in bovine, required for ICM formation and trophectoderm differentiation, was still considerably low and did not change through the treatment of TSA. It is mainly affected by DNA methylation signifying that DNA methylation is an important factor for nuclear reprogramming (Sawai et al., 2012). Therefore, it is proper to conclude that TSA is necessary, as Mitalipov's study on primates and Tachibana's study on human ntESC both come to the same conclusion, yet DNA methylation is another component to embryonic development.

DNA and histone methylation is pertinent to improve the ntESC derivation. The first developmental defects of SCNT appears at the time of zygotic genome activation (ZGA) which occurs at the 4 to 8 cell stage in humans and bovine and at the 2-cell stage in mice. Difficulties in ZGA are due to epigenetic barriers existing in the genome. Certain genomic domains resistant to ZGA in SCNT embryos, known as reprogramming resistant regions (RRRs), have been identified. Enrichment of mouse RRRs with histone H3, lysine 9 methylation (H3K9me3) in the donor genome results in a barrier to transcriptional reprogramming. They must be removed by specific demethylase or by ridding it of H3K9 methyltransferases. Kdm4d, H3K9me3-specific histone demethylase, greatly reduces H3K9me3 levels. The injection of Kdm4d mRNA increased the blastocyst rate up to 88.6% (Matoba et al., 2014).

In the study done by Chung et al. (2015), it was found that H3K9me3 also serves as a barrier in human SCNT. Using the same oocytes that failed to develop into blastocysts, they demonstrated that Kdm4a overexpression significantly improved embryonic development. Compared to the IVF derived embryos, of 707 genomic regions in SCNT embryos, 308 were termed as RRRs with the same enrichment of H3K9me3 as seen in mice. This study also showed that the injection of Kdm4a wild-type, not Kdm4d catalytic mutant used in the original study on mice, had a greater blastocyst rate, 90.3%, in SCNT embryos. To test the efficiency in this demethylase on humans, Kdm4a mRNA was injected at different stages of SCNT embryonic develop-

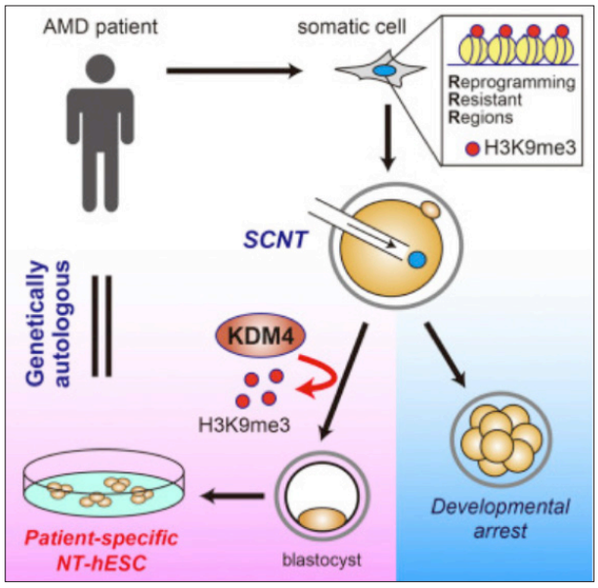

Figure 4 (Chung et al., 2015)

ment. Injecting it upon activation, after fusion by HVJ-E, showed no effect prior to ZGA completion. However, the effect was noticeable at the end when 8 blastocysts were developed, with no development of the control group. In a conventional ESC derivation medium, seven attached to the MEF feeder cell and four stable ntESC lines were derived. As shown in Figure 5, immunostaining revealed that NANOG, OCT4, SOX2, SSEA-4, and TRA1-60, transcription factors that maintain ESC differentiation, were all expressed similar to human ESC line derived by IVF. Likewise, these ntESC's expressed pluripotency marker genes that were indistinguishable from IVF derived ESC. Additionally, these ntESCs underwent immunostaining of embryoid bodies (EB), aggregates of ESC, for two weeks in vitro giving rise to the

differentiation into all three germ layers. Proving its differentiation ability. Karyotyping, the process of chromosome pairing, demonstrated normal chromosomal number and the same sex chromosome of the donor nucleus, and Short Tandem Repeat (STR) analysis showed all 16 repeat markers on the genome is perfectly matched to the donor somatic cell. A sequence analysis of mitochondrial DNA (mtDNA) demonstrated that the single-nucleotide polymorphism of ntESCs matched the mtDNA of the donor oocyte and not the donor nucleus. A necessary analysis that demonstrates the idea that mtDNA is transmitted by the fertilized oocyte and not from the DNA in the cell's nucleus (Chung et al., 2015).

Although blastocyst development in SCNT embryos was improved, the success of humans, merely 27%, compared to the success of mice, 90%, suggests that all modifications to SCNT protocol must be utilized, especially proper oocyte quality. That is a major factor for ntESC derivation. Human oocyte varies tremendously even from a single ovulation. Therefore, even some blastocysts formed through IVF were not supported (Chung et al., 2015).

Fetal vs. Adult Somatic Cell

In order for SCNT to be regarded as having practical medical significance there must be a way to generate ntESC from adult somatic cells. Most experiments use various fetal fibroblasts as somatic nuclei donors. However, patients that are in need of this procedure are mainly aged. Therefore, a recent study done by Chung et al. (2014) determined whether dermal fibroblasts from 35 and 75- year-old males can undergo successful SCNT procedure. The debate on adult vs. fetal cells is regarding the age-related changes, such as shortened telomeres and oxidative DNA damage that can potentially obstruct nuclear reprogramming. Even though fetal fibroblasts were used in numerous researches, there were no clear claims of age impedance on SCNT. Using the non-modified, standard SCNT protocol, no aneuploidy was produced in the resultant human ESC. Therefore, the modified SCNT protocol established by Mitalipov et al. (2007) and Tachibana et al. (2013) on fetal fibroblasts was used, with two groups forming. One group was activated 2 hr after implantation and the other group was activated after 30 min. However, only two blastocysts hatched from the 2 hr group, one from each age subtype, leading to the generation of ESC lines. The difference in time duration can be explained by the possibility for the necessary extra time needed for reprogramming that is dependent on the exposure of the donor nucleus to the recipient oocyte cytoplast. Both ntESC lines displayed the same sixteen STR markers located on the human autosomal and allosomal loci as the donor somatic cell and differed from the oocyte donor. The mtDNA was verified being inherited by the oocyte donor in both ntESC lines and expression of transcription factors OCT-4, SSEA-4, TRA-1-60, and TRA-1-81 was present. Pluripotency was confirmed through spontaneous differentiation into the three germ layers by immunostaining of embryoid bodies. Further confirmation of pluripotency was through teratoma formation assays that demonstrated outgrowth of tissue from the germ layers. Despite the low success rate, this study portrayed the efficiency of the modified SCNT protocol and greatly implies the possibility of using SCNT to generate patient-specific ESC for medical use (Chung et al., 2014).

Application of Therapeutic Cloning on Regenerative Medicine

Therapeutic cloning holds great promise in regenerative medicine and gene therapy. It has numerous research and clinical applications, such as producing a vector for gene delivery, creating animal models of human diseases, and cell replacement therapy in regenerative medicine. It can permanently cure Parkinson's disease or diabetes mellitus. Barberi et al. (2003) conducted a study on mice using SCNT with nuclei from cumulus and tail-tip cells. Two ntESC lines were differentiated into motor, GABAminergic, serotonergic, and dopaminergic neurons, that displayed synapse formation and normal electrophysiological properties in vitro. Mice with Parkinson-like lesion were injected with dopaminergic neurons into the cortical striatum induced by 6-hydroxydopamine. An 80% survival rate 8 weeks post-transplantation was observed from ntESC contrary to 40% for stem-cell derived neurons (Kfoury, 2007).

Furthermore, SCNT can allow for organogenesis and patient-specific rescue of genetic mutations. In cell replacement therapy, therapeutic cloning has the potential to create various types of tissues such as osteoblasts to counteract osteoporosis. Deshpande et al. (2006) transferred motor neurons derived from ESC to rats with a severed spinal cord showing SCNT application for spinal cord regeneration. A potential cure for paralysis in humans. Therapeutic cloning eliminates the issue of immune rejection and organ shortage by engineering tissues and organs. Patient-specific cardiomyocytes, blood vessels, and skin can treat infarctus, atherosclerosis, and severe burns (Kfoury, 2007). With the use of the modified SCNT protocols, diploid pluripotent stem cells that were derived from a patient with type 1 diabetes were produced (Yamada et al, 2014). Such advancements in medicine hold hope for cures of various diseases.

iPSC vs. ntESC

IPSCs, induced pluripotent stem cells, is a laboratory technique that reprograms differentiated cells back to pluripotency using specific transcriptional factors. The medical application of human iPSCs has opened the door for rapid stem cell therapy. A set of essential transcription factors, called reprogramming factors, trigger the destruction of the existing state of somatic cells by changing their epigenetic status, leading to alterations in their gene expression. The changes in the gene expression induce secondary epigenetic changes, including DNA methylation and

alterations of the nuclear structure. However, there are roadblocks to reprogramming that need to be removed because cellular identity is stabilized. However, transcription factors such as Oct4, Sox2, Klf4, and c-Myc are sufficient to destabilize the existing order in the original cells and re-construct a new order. Therefore, transcription factors associated with development may undertake important actions during the reprogramming process. This produces stem cells that are similar to embryonic stem cells but has a few disadvantages when compared to ntESCs (Takahashi & Yamanaka, 2013).

ESC is considered the ground state which is believed to have a greater chance of successful differentiation than iPSC. During reprogramming, iPSCs-specific methylation and transcriptional abnormalities in imprinted regions and X chromosomes were observed with such abnormalities less frequent in ntESCs (Nazor et al.,2012). In some instances, a number of highly proliferative colonies appear that are not pluripotent and can form into tumors. Furthermore, iPSCs do not efficiently silence the expression pattern from where they are derived from and fail to induce some ESC specific genes (Bilic & Belmonte, 2011).

A major advantage of SCNT-based ntESCs is the fact that it contains mtDNA originating from the oocyte. Irrespective of the nuclear donor cell mtDNA, ntESCs have the potential to produce functional cells and tissues for cell therapies. Thus, SCNT offers a strategy for correcting mtDNA mutations and retaining the metabolic function of pluripotent cells from patients with inherited mtDNA diseases.

MtDNA that is specific to the oocyte recipient of cloned embryos was first determined in the study done by Evans et al. (1999), with co-researcher Dr. John Loike. They showed nuclear transfer-derived sheep were homoplasmic. The random partitioning of mtDNAs does not occur. This may be due to the failure of the donor mitochondria to enter the ooplasm following electrofusion. It can be hypothesized that an active mechanism operates to destroy the donor mitochondria in the recipient ooplasm, similarly to what is thought to happen to sperm-derived mitochondria in fertilized ova in human reproduction. These results have implications for future attempts to correct maternally inherited mitochondrial genetic disorders by nuclear transfer involving a somatic or germline cell from a woman containing a pathogenic mtDNA mutation but normal nuclear DNA and a recipient enucleated oocyte.

Ethical and Legal Issues

Research in this field has been held back due to controversial ethical issues. Legal constraints and lack of funding results in the impediment of therapeutic cloning (Lo & Parham, 2009). A major objection to SCNT is the belief that creating embryos for research, with the intention of mutilating these embryos, violates human respect and integrity. Sandel (2004) argued in his article on embryo ethics, "Although every oak tree was once an acorn, it does not follow that acorns are oak trees, or that I should treat the loss of an acorn . . . as the same kind of loss as the death of an oak tree." This is an opinion based question whether an embryo varies in essence from a developed human being. Evaluation whether the use of embryos for medical research justifies its destruction. Meanwhile, legislative actions, especially in Europe, hinders SCNT advancement that contributes to its slow progression.

Conclusion

Therapeutic cloning is feasible through modified SCNT protocol. Research in mammals and primates aided in the success of human ntESC derivation by establishing the correlation between oocyte and donor nucleus quality, nuclear reprogramming, and proper gene expression. All steps led to the derivation of pluripotent ESC that were differentiated into various specialized cells, such as cardiomyocytes and insulin beta cells. The developed embryo harboring mtDNA from the recipient oocyte holds an advantage over iPSC cells by constituting metabolically functional cells from mitochondrial diseased cells. More research is needed to expand and apply this technique in medical practice; however, ethical and legislative actions repress the advancement in this area.

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Relaxin as a Cure for Fibrosis

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Abstract

Until recently, relaxin has been known as a hormone that pertained to the female reproductive system. Its ability to remodel the extracellular matrix is responsible for its known reproductive effects. Current research has indicated that it may be useful as a drug to combat fibrosis. Relaxin has been proposed as an antifibrotic drug to target a variety of organs, including the skin, lung, kidney, liver, and heart. Studies done using the relaxin null knockout mouse have given scientists insight into the workings of this hormone. Human studies have also been done to test the efficacy of relaxin in its reversal of fibrosis. With more research, perhaps relaxin can be used as a drug in the future.

Introduction

Fibrosis is a hardening or scarring of tissue that results from the repair of injuries in the body. It is estimated that 45-50% of deaths in the Western world are caused wholly or in part by fibrosis in various organs in the body. Currently, there is no available cure for damage caused by fibrosis (Summers, 2016).

Relaxin is a peptide hormone usually associated with the reproductive system. Relaxin has been found to be pleiotropic, meaning that it is active in many varying roles in the body. It is produced in both pregnant and non-pregnant females as well as in males (Pallardy, 2016). It appears that relaxin can be manipulated to target injured organs in order to reverse fibrosis and prevent its further progression. There have been studies conducted that point to relaxin as a possible cure for fibrosis in various organs, including the skin, lung, kidney and heart (Tan, et. al. 2016). Fibrosis is a part of the pathology of many different human diseases and thus is said to account for 45-50% of deaths in the western world (Summers, 2016).

Methods

Information in this paper is based primarily on research accessed through the Touro Library Databases. The peer reviewed, scholarly journal articles were retrieved through the Proquest or Pubmed databases.

Fibrosis and its Causes

Fibrosis is an exaggeration of the scarring and hardening of tissue usually caused by the body's normal response to injury. It is characterized by a build-up of extracellular matrix (ECM) components, or scar tissue, in place of normal tissue. Particularly, there is a buildup of fibrillar collagens, such as types I and III. Fibrogenesis is the body's mechanism of healing and repairing wounds. What occurs in fibrogenesis is that myofibroblasts rush to the site of the injury and release cytokines. This causes fibroblasts to proliferate and build up the ECM producing fibrillar collagen creating a scar in place of the damaged tissue (Baum, et. al. 2011). Excessive build-up of connective tissue can be harmful to underlying tissue and organs. This is because the ECM is the framework for the cells and extracellular structures in the body. Therefore, if the normal form of the ECM is disrupted, the tissue in which it is present will not continue to function as it should (Bathgate, et. al. 2003). Fibrosis is often caused by chronic inflammatory reactions that can be a result of various stimuli among which are persistent bacterial and viral infections, autoimmune and allergic reactions, and tissue injury (Wynn, 2008).

The body has a system that works to clear accumulated extracellular matrix materials. Matrix Metalloproteinases (MMP's) are a group of enzymes that break down extracellular matrix proteins, such as collagen. Part of the body's normal way of repairing itself after tissue injury is to inhibit the production of these enzymes and to increase the secretion of Matrix Metalloproteinase Inhibitors (MMPIs), and more specifically, Tissue Inhibitors of Metalloproteinases (TIMPs). Thus, scar tissue replaces the injured tissue, and the body can heal itself. This too is favorable in a normal situation, but if this process continues unceasingly, it can result in disruption of normal organ function as the matrix builds up without anything present to break it down. An imbalance between collagen degrading enzymes will also cause fibrosis (Bennett, et. al. 2009).

What is Relaxin?

Relaxin was first discovered in 1926 by Frederick Hisaw as a hormone present in pregnant guinea pigs. The function of relaxin observed at the time, was that it relaxed the pelvic ligaments and the cervix of the uterus to make it possible to carry the fetus and to prepare the area for parturition. Later, it was discovered that it was through collagen remodeling that relaxin was able to play this important role in pregnancy and birth (Pallardy, 2016). Upon closer inspection of the relaxed ligaments it was seen that the collagen was remodeled from dense bundles to looser, less structured fibers (Bennett, et. al. 2009).

Relaxin is produced by various structures in the female's reproductive tract including the placenta, corpus luteum and uterus, though this varies by species. During the first trimester of pregnancy, relaxin rises from practically undetectable levels to 1ng/mL and then slowly wanes as delivery draws near. Relaxin is also present in non-pregnant human females and males (Pallardy, 2016). In males, relaxin is produced by the prostate gland and can be found in the seminal fluid, though it is not usually detected in circulation (Bennet, 2009).

Relaxin is a peptide hormone whose two-chain structure is similar to that of insulin. In humans and higher primates, three genes for relaxin have been discovered, which include encoding proteins known as H1, H2 and H3 relaxin. In rodents, only two relaxin genes have been discovered: relaxin-1 and relaxin-3, which are equivalent to the H2 and H3 in humans, respectively. The H3 relaxin gene was discovered in 2002 and is known to act mainly in the brain. H2 relaxin in humans- the product of the RLN2 gene, and relaxin-1 in rodents- the product of the RLN1 gene, are the major circulating forms of relaxin and are the ones

that are thought to have an effect on fibrosis (reference to relaxin for the duration of the paper will refer to these forms of relaxin) (Samuel, et. al. 2007).

There has been a lot of research in the past few years that has tried to find more uses for relaxin in the body. There have been identified relaxin-binding sites in various places in the body in both males in females, including in the heart and brain. This suggests that relaxin may also influence nonreproductive areas of the body and leads scientists to do further research into these possible uses (Samuel, et. al. 2016).

Knockout Mouse Studies

To aid scientists in the understanding of relaxin, a relaxin-null nockout mouse was created by The Howard Florey Institute in Melbourne, Australia. A knockout mouse is a mouse that was genetically modified in order to remove or "knockout" a specific gene. Using such a mouse helps give scientists insight into the biology of a particular gene (Austin, et. al. 2004). The mouse established to research relaxin is the Rln1-KO mouse, meaning that the Rln1 gene, that codes for the major circulating form of relaxin in animals, was removed. However, the Rln3 gene was not removed. Monitoring the development of these mice showed that the reproductive organs, in both males and females, were underdeveloped. In pregnant females, the mammary glands, nipples, and pubic symphysis were underdeveloped, and lactation was prevented. In the male mice, the testis, epididymis and prostate did not mature properly. Later, it was discovered that abnormal development in knock-out mice was due to excess collagen. In addition to this, as the mice aged, there was a buildup of interstitial collagen in the heart, lung, kidney, and skin. This eventually caused malfunction in these organs, appearing more prominently in the male mice.

Scientists then administered recombinant human (H2) relaxin to the knockout mice and saw that it was helpful in reversing the fibrosis and restoring organ function. The relaxin was helpful in both early and late stages of the fibrosis, but worked to different extents depending on the organ. These findings point to a use of relaxin as a drug to reverse and prevent fibrosis in human pathological situations (Bathgate, et. al. 2003 and Samuel, et. al. 2005b).

In 2009 a study was done on relaxin null knockout mice to test the efficacy of using recombinant relaxin as a treatment for the fibrosis that developed in them. The study also aimed to test if it was significant when in the progression of the fibrosis the relaxin was administered. There were two groups of mice, in one group, relaxin was given to 9 month-old mice, during early stages of fibrosis. The other group was given relaxin when their fibrosis was more progressed, at 12 months of age. There was less fibrosis in the 9-month group, and no difference in the 12-month group when compared to the untreated controls. This points to the fact that administered relaxin will be more effective in earlier stages of fibrosis (Giannakis, et. al. 2009).

Relaxin null knockout mice were used to test the effects of relaxin on scleroderma, a form of dermal fibrosis. It is a connective tissue disease that causes fibrosis or various internal organs in addition to skin thickening. Untreated, scleroderma can cause irreversible damage. The study indicated that relaxin is more effective in treating dermal fibrosis in its early stages. (Samuel, et. al. 2005a).

How does Relaxin Help Reverse Fibrosis?

Relaxin binds with its endogenous receptor, relaxin family peptide receptor 1 (RXFP1), which is also known as LGR7. This inhibits the actions of major profibrotic factors such as transforming growth factor beta 1 (TGFb1), and angiotensin II in several organs. As a result of this, there will be a decreased expression of types I, III, and V collagens, interstitial collagens and type IV basement membrane collagen. There will also be an increase in the breakdown of collagen via the activation of MMP's and an inhibition of the TIMPs (Samuel, et. al. 2016, fig 1)

Mechanisms

The mechanisms involved in the antifibrotic effects of relaxin are not currently well known by the scientific community. The primary receptor for relaxin is LGR7, or RXFP1, and was only recently discovered in 2002. Before this was known, the LGR7 and LGR8 relaxin receptors were known as orphan G-protein receptors. (Bennet, 2009). It is interesting to note that these receptors exist in organs outside the reproductive tract, pointing to the fact that relaxin has other functions than it was historically thought. Perhaps as the understanding of the pathway used by relaxin is enhanced, we will better be able to use relaxin as a drug for the treatment of fibrosis (Samuel, et. al. 2005a).

Discussion: Clinical Trial for Relaxin

In the 1950's, there was the emergence of the idea that relaxin could be used to treat fibrosis, and it was clinically tested then and again in the 1990's. These studies failed to reach the levels of effectiveness that were required for it to pass as a drug, but shed a lot of light onto the antifibrotic actions of relaxin (Samuel, et. al. 2005a).

A randomized, double-blind, placebo-controlled study was conducted using recombinant human relaxin in the treatment of systemic sclerosis. This was a phase III trial for testing relaxin as a possible drug to reverse fibrosis. Systemic sclerosis, also called systemic scleroderma, is a disease in which there is a buildup of collagen in various organs in the body. This is an ideal disease with which to test for the efficacy of relaxin, as the hallmark of this disease is ongoing fibrosis. The patients were divided into 2 random groups, one of which was given the actual drug, and the other group was given a placebo. In addition to this, neither those being treated, nor the researchers administering the medication knew who belonged to which group.

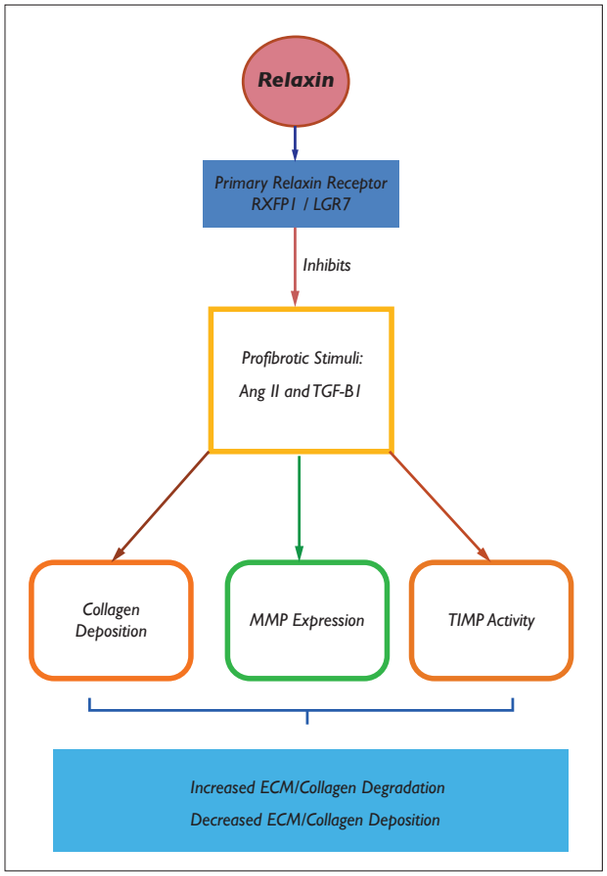

Fig.1 Mechanism for relaxin's role in inhibiting fibrogenesis. (Modified from Samuel, et. al.2016.)

There was not a significant difference in the levels of fibrosis between the two groups. In addition, it seems that there were negative side effects to those that were administered the relaxin drug. The forced vital capacity in those patients given relaxin was decreased, and there were adverse renal effects primarily after they stopped giving them the relaxin. The study concluded that if relaxin is ever to be used in the reversal of fibrosis, there must be intensive monitoring of blood pressure and of renal function (Khanna, et. al. 2009).

One proposed reason for why relaxin was not effective in this study was because the degree of scleroderma in the patients in this trial was quite advanced. In the relaxin-null mouse model it was also seen that relaxin was less effective in more advanced stages of fibrosis (Bennet, 2009). Although this study is less optimistic about the use of relaxin as a drug in the near future, it may be possible that as research continues, and we learn more about relaxin and how it works in the body, it will seem more plausible.

Other Human Relaxin Studies

There was a study done to test the safety of administering recombinant human relaxin to people. The scleroderma patients were administered 200mcg/kg/day for 28 days. This is approximately 50 times the relaxin present in a normal pregnant woman. The relaxin was unhelpful in reducing the fibrosis, but it was concluded that in these doses relaxin was safe and well tolerated. Some adverse side effects included; Development of a rash and pain at the site of infusion, minor bleeding in some cases, and decreased concentration of blood hemoglobin (Seibold, et. al. 1998).

It appears that the body itself uses relaxin as a mechanism to combat fibrosis. This was shown in a study of 50 patients with systemic sclerosis, an autoimmune disorder that causes fibrosis of the skin and internal organs, and 50 healthy patients in which normal relaxin levels were measured and the results were statistically analyzed. In the diseased patients, the measured levels of relaxin in the bloodstream was significantly higher than in the healthy subjects. It was hypothesized that this was the body's response and effort to reverse the fibrosis (Giordano, et. al. 2005). Thus, using relaxin as a drug for fibrotic patients is in accordance with the way nature would tackle the problem, and therefore worth further research.

Pulmonary Fibrosis

IPF or Idiopathic Pulmonary Fibrosis, is a lung disease in which there is fibrosis of the lungs for an unknown reason. It was found that the gene expression of the main relaxin receptor, RXFP1, is 2.9-fold less in lung samples taken from patients with IPF compared with lungs of normal control subjects. There was also a study done in an in vitro model, in which IPF fibroblasts were grown in media that was treated with transforming growth factor TGFb1. It was found that the expression of the RXFP1 protein was decreased in immunoblots. It was thus hypothesized that relaxin treatments will not be too effective in patients with IPF. The authors also speculate that the loss of RXFP1 expression is a common factor in fibrotic diseases and therefore may have been part of the reason that the clinical trials to treat scleroderma with relaxin failed (Tan, et. al. 2016). Royce et. al. point out that these findings are surprising because relaxin has proved to be effective in reducing fibrosis in many human fibroblast culture models in which TGFb1 is used to stimulate collagen synthesis. They argue that the functional activity of the relaxin receptor cannot be determined using gene expression studies alone and point out that there may have been other factors involved that caused these results. Therefore, they conclude, more research must be done before relaxin-based therapies for fibrosis can be either ruled out or implemented clinically (Royce, at al, 2016).

Hepatic fibrosis

Fibrosis of the liver, like forms of fibrosis in other organs, is categorized by increased collagen deposition and decreased ECM degradation. To test the effects of relaxin on hepatic fibrosis in

vivo, scientists established fibrosis in mice using carbon tetrachloride. The mice were administered the carbon tetrachloride for 4 weeks, followed by 4 weeks of administration of relaxin in addition to carbon tetrachloride. Relaxin decreased the hepatic collagen expression, increased the expression of the MMPs, and decreased the TIMPs. This suggests that relaxin might be a possible treatment for established hepatic fibrosis (Bennet, et. al. 2014).

The common endpoint of many liver diseases caused by chronic liver injury is fibrosis. The major profibrogenic cell type that is activated in cases of chronic liver injury is the hepatic stellate cell-myofibroblast (HSC-MF). This cell produces scar tissue and contributes to portal hypertension (PHT) by increasing the resistance within the hepatic vascular system. It was seen in past studies that the expression on RXFP1 was increased in human and rat HSC-MFs and in various studies on parts of the liver that were affected by fibrosis. In the model of rat fibrosis it was seen that the administering relaxin helped to reduce the fibrogenesis and reduce the PHT (McBride, et. al. 2017).

Relaxin has a short in vivo half-life. This is an obstacle to using it as a drug for treatment, especially in models of disease that require long-term administration of the drug, such as liver disease. In an effort to find a new drug to combat liver fibrosis, there was an attempt to find small molecules that are similar to relaxin and that will act as agonists for the RXFP1 receptor. There are currently high throughput screening (HTS) technologies which allow scientists to explore vast libraries of compounds in a quick and efficient manner. This helps to identify molecules that can be used as starting points for creating new drugs. One compound, ML290, was found to be most promising. It binds with RXFP1 and was therefore tested extensively. Although ML290 did exhibit some relaxin-like activity, there were many differences in the way it bound to RXFP1 and its actions. Its effectiveness in combating liver fibrosis has not yet been tested and for now it does not seem that it will be a feasible replacement compound for the treatment of fibrosis. Also, being as the actions of RXFP1 differ in various organs of the body, and are not well understood in general, more research must be done before such a drug can be produced (McBride, et. al. 2017).

Cardiac Fibrosis

Fibrosis is a hallmark of hypertensive cardiac disease. The ECM has a great impact on the function of the heart, including regulating ventricular diastolic and systolic function. It also provides the framework for the cardiomyocytes and coronary vessels. There are many cardiac pathologies that result in fibrosis. Among these are ischaemic injury, myocardial infarction, hypertension, and many others. This results in a vicious cycle, as the accumulation of extracellular matrix via fibrosis leads to an increase in the amount of those negative cardiac events, which can in turn lead to more fibrosis. It was shown in relaxin knockout mouse studies that relaxin deficient mice have an elevated collagen content in their cardiovascular system which leads to diastolic dysfunction of the left ventricle (LV).

Spontaneously hypertensive rats (SHR), in which fibrosis was not induced by having a lack of relaxin, rather it was the natural result of hypertension, were studied. When the rats were administered relaxin over a 14 day period the initially elevated collagen levels in the LV were now reduced. Fibroblast proliferation was inhibited, and MMP-2 expression increased. This means that there was a decrease in collagen synthesis as well as an increase in its breakdown. The study concluded that relaxin is a potent drug that can be used to combat hypertensive diseases. In addition to this, relaxin was compared to other hypertensive drugs such as angiotensin converting enzyme inhibitors and aldosterone inhibitors. These drugs have many side effects and only begin to help after prolonged periods of treatment. Relaxin, on the other hand, managed to work at a much faster rate to reduce fibrosis and did not have any notable side effects in this study (Lekgabe, et. al. 2005).

Renal Fibrosis

Renal fibrosis is the accumulation of collagen in the kidney. Specifically, in the renal resistance vessels, glomeruli and interstitial space. There is a very strong relation between cardiovascular diseases and renal diseases, as both diseases include high blood pressure. The SHR were also used to study renal involvement. The experimental rats exhibited renal as well as cardiac fibrosis. Administration of relaxin over a 14-day period showed to reduce the fibrosis in the kidney cortex, thus halting renal failure. (Lekgabe, et. al. 2005).

Another study treated rats with bromoethylamine, or BEA, which causes severe renal interstitial fibrosis, one week later, the rats were administered relaxin via an osmotic pump for a period of 28 days. The aim was to see whether the relaxin would inhibit the fibrosis that was caused by the BEA. The structure of the renal tubules had been affected by much collagen deposition in the rats treated with BEA alone. The rats treated with BEA in conjunction with relaxin exhibited a 75% decrease in collagen deposition and tubular structure was almost completely maintained. In addition to this, it was seen that renal function was largely restored in the relaxin-treated animals. The levels of creatinine clearance were 75% of those of the control mice. It is thus hypothesized that the reduction of the fibrosis by relaxin is the cause for the restoration of renal function (Garber, et. al. 2001).

Conclusion

Fibrosis accounts for nearly half the deaths in the western world and yet there are no known cures. Relaxin's natural antifibrotic properties make it a viable candidate for the treatment of fibrosis. Although much research has already been done, relaxin must be proven to be both safe and effective before it can begin to be implemented clinically, and thus there is a need for further research.

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Music and the Brain

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Abstract

Music is an important part of cultures worldwide. It has been used throughout the ages as a method of conveying emotions to the listener. However, there is some confusion about exactly which areas of the brain are affected by music. This study shows the general areas of the brain stimulated by music, explaining how these sections influence emotion and learning capability. In addition, this paper demonstrates that music therapy may be helpful in relieving stress or some neurological disorders, based on the areas activated by music. Training in music has cognitive and motor coordination benefits, as well, because this training causes structural changes in brains of musicians.

Introduction

According to the Merriam-Webster dictionary, music is the "vocal, instrumental, or mechanical sounds having rhythm, melody, or harmony (Merriam-Webster, 2018)." However, the word "music" suggests a much deeper meaning than the formal dictionary definition implies. Music is known as the "language of the soul." It speaks to its listener, conveying a message of emotions, such as a calming sensation of relaxation or intense feeling of elation or sadness. The language is universal; even people who have lost their ability to verbally communicate can understand the empathy inherent in music (Ridder, et al., 2009). When a musician plays his instrument, he is forming an emotional connection with his listeners. But music has effects beyond emotions. Learning to play music facilitates cognitive reasoning, motor coordination, and sensory perception. Because music has this unique combination of emotional connection and cognitive benefits, music therapy has been developed to alleviate emotional and physical hardship in a number of neurological illnesses. This analysis will discuss the varying effects of music on the brain, including the physical changes that manifest and the mechanisms through which music leads to enhanced learning and emotion. Additionally, through this study, the possible benefits of music therapy are revealed.

Methods

Research for this paper was gathered from Touro's online library from databases including ProQuest and EBSCO. Articles found discuss the different areas of the brain affected by music, the usefulness of music therapy, differences in anatomy and processing of stimuli in the brains of musicians and non-musicians, whether music induces emotion, and the potential learning benefits to be gained through musical training. Review articles, case studies and experimental studies were used in forming the analysis.

Parts of the Brain Affected by Listening to Music

It is unclear, as of the research to date, exactly which parts of the brain are stimulated by music. The general mechanism for music that was heard travelling to the brain is as follows; music enters the ear and is received by the cochlea. It is then transferred to the thalamus, and then to the auditory cortex for processing (Boso, et al., 2006). If the person listening to the music is deciding whether to buy that music, there is a further association between the auditory cortex and the ventral striatum, which processes enjoyment (Koelsch, 2014).

Although most reward stimuli do not activate the hippocampal region, music does. The hippocampus's main roles are the conversion of short-term memory into long term memory and spatial relations. It is also responsible for encoding information from the amygdala, which controls emotion, with the long-term memories. In other words, emotional memory becomes combined and remembered with long-term memory through the hippocampus. This supports a growing theory that the hippocampus plays a role in emotional recollection that occurs when listening to music. Because music affects emotions in a way unlike money, food or other reward stimuli, it couples emotion and the memory of a specific piece of music. This activates the hippocampus, leading to feelings of peacefulness, tenderness, joy, or even sadness when the piece is later recalled (Koelsch, 2014).

People can have varying parts of their brain stimulated, depending on how they think of the song. For example, remembering a song in one's head can stimulate the visual cortex, while hearing the song stimulates the auditory cortex. Also, different parts of music affect different areas of the brain; pitch, melody, harmony and beat are usually processed in the right hemisphere of the temporal lobe, while lyrics, pacing, frequency, and intensity are analyzed in the left temporal lobe. The connection between music and thoughts, which will stimulate emotions or previous memories, is formed by the frontal lobe. The stimulation of the frontal lobe is activated by the limbic system which provides the emotions felt (Bennet, Bennet, 2008).

Although both speech and music utilize pitch, pitch seems to be used differently in the different circumstances. While speech cannot be "off-tune," music can. It is possible, therefore, that coarse contour in speech and music are processed in one area, while a more finely-tuned pitch perception is analyzed in a different area. This argument is strengthened by the fact that there were cases of people who lost the ability to understand language prosody but retained musical pitch acuity. However, people who lost basic musical contour perception also lost their speech prosody awareness (Zatorre, Baum, 2012).

Music is also processed in different areas depending on whether the music is perceived as pleasant. Music that has dissonance and disharmony is more likely to be processed in the temporal lobe, while music that is pleasant and has harmony will be processed in the frontal lobe (Boso, et al., 2006).

Pleasant music is processed in the same way that primary and secondary pleasure stimuli are generally handled. Music activates the nucleus accumbens, ventral tegmentum area, hypothalamus, orbitofrontal cortex, and the insula-frontal cortex. The nucleus accumbens is the center which processes pleasant stimuli, and then releases dopamine into the ventral tegmentum

area. In the brains of both musicians and non-musicians, listening to music activates the mesolimbic dopaminergic reward system (Herholz, Zatorre, 2012, Schlaug, et al., 2010, Menon, Levitin, 2005). Pleasant music, such as music which gives the listener chills, or goosebumps, causes the release of dopamine both in the ventral tegmentum and in the ventral striatum. The anticipation of chills, itself, releases dopamine, as well. However, the exact area where dopamine is released when expecting such music differs from when the expectation is fulfilled. Expected chills activate dopamine in the ventral striatum. When the music causes the chills to actually happen, the dorsal striatum becomes activated with dopamine (Koelsch, 2014, Boso, et al., 2006). The hypothalamus, too, gets activated, which lowers the heart rate and breathing rate, leading people to feel calmed and relaxed by music (Menon, Levitin, 2005).

Music Therapy as a Useful Tool

Music therapy is unique in that listening to music sends signals to the visual, auditory and motor cortices simultaneously (Schlaug, et al., 2010, Walworth, et al., 2008). The fronto-temporo-parietal connection formed by music is a component of mirror neurons. Because mirror neurons are important for the coupling of perceptional events and their motor response, listening to music, and thereby activating these areas, may help link cognition and perception, heightening concentration, memory and executive control (Schlaug, et al., 2010).

Connected to the general cognitive and perceptive benefits is the effect of music on reducing cognitive stress. This idea was proven through a study conducted on eighty-nine college students. These students were given a cognitive stressor; they were told that they would have to present a complicated speech. Then, the control group worked on preparing their speech without background music, while the experimental group was exposed to music. The groups were given surveys to fill out before and after the stressor was given. Also, salivary IgA levels were measured to check if biologically, music can help boost the immune system. The IgA test and the stress surveys indicated that the group listening to music experienced less stress and had a more effective, boosted, immune system than the control group (Knight, Rickard, 2001). A study of patients who were waiting to undergo surgery showed that music can return heart-rate, cardiac output and blood pressure values to normal, non-stress levels. Additionally, blood glucose levels and skin temperature will be overcompensated in the return to normal status when listening to music. Not only will the brain restore blood glucose levels and skin temperature to the normal levels in response to listening to music, it will establish levels corresponding to a state of relaxation. Music, therefore, can relieve its listener of feelings of stress and induce calmness in its place (Miluk-Kolasa, et al., 2002).

The stress-relieving benefits of music are helpful clinically as well. Music can destress a patient before a difficult surgery. Research done by Walworth et al. demonstrated that music therapy can have psychological effects on patients undergoing brain surgery. In their experiment, twenty-seven subjects who were scheduled for a surgery were divided into two groups. The control group did not receive any music therapy prior to surgery, while the experimental group received twenty to fifty minutes of therapy. Surveys taken by the subjects before and after the surgery analyzed mood level, perception of hospitalization, and to what extent each subject felt anxious, stressed, or pain. The results indicated that subjects who had received the music therapy felt less anxiety and stress and were more relaxed before and during the surgery. They generally had a better perception of their hospitalization. This is important since studies have shown that pessimism and anxiety correlate with worse outcomes in brain surgeries (Walworth, et al., 2008). Another study which focused on spinal epidurals, as opposed to brain surgery, concurs with Walworth et al. that music therapy can decrease stress in patients undergoing surgery. The patients having spinal anesthesia who listened to music throughout the surgery had a shorter induction time and required less medication than the control group. Interestingly, the patients listening to music did not recall hearing the music, suggesting that the effects of music occur subconsciously. A possible explanation for the reduction of necessary anesthesia is that the auditory and pain pathways inhibit each other. This can be an important method for decreasing the amount of anesthesia necessary non-pharmaceutically (Zhang, et al., 2005).

Music therapy can increase the quality of life for a patient with fronto-temporal dementia. Listening to familiar music can allow these patients to express themselves nonverbally, and thus allows for the building of social interactions and communication. After four weeks of music therapy, patients had less agitated behavior and were less distressed. Whether the patient actively participated in the therapy sessions did not diminish the benefit of the therapy, as both active and inactive participants clearly showed less agitation than before the sessions (Ridder, et al., 2015).

In addition to helping patients psychologically by reducing stress, music, and specifically musical training can help patients physically. In one study, stroke patients learned to play an electronic drum and MIDI keyboard. The results showed that the drum training improved gross motor skills, and the piano lessons honed fine motor skills. These patients began to recover some of their motor coordination. Additionally, brain tests demonstrated that these patients had more success with motor planning and neuronal coherence with music therapy than other therapies. Interestingly, these skills were not limited to the musical instruments played. The coordination had improved overall, in every activity attempted (Schneider, et al., 2007).

Different Music Affecting the Brain Differently

Music is a very broad term. It includes many genres, styles and

pieces. Just as the varying aspects of music are processed in different areas of the brain, the various genres within music affect the brain in unique ways.

A network analysis was conducted, based on imaging from 21 subjects, on different genres of music. Network imaging shows the connections of the brain, along with the important functioning parts at a given point. The analysis illustrated that different genres of music created "hubs" of activity in varying areas of the brain, depending on the music. Specifically, classical music made the largest hub in the auditory cortex. It is possible that the size of the hub is related to the complexity of the music being heard, and because classical music is often complex, its hub is the largest (Wilkins, et al., 2012).

In addition to the different genres affecting the brain, preferred music, as opposed to disliked music, shows different patterns in the brain. When comparing a subject's favorite song with songs that the subject liked and disliked, all songs stimulated the default mode network, and particularly the precuneus. The precuneus is in the parietal lobe, anterior to the occipital lobe. This area is important in the processes of self-reflection and episodic memory, along with cognitive thinking and creativity. Although the precuneus was activated by every music heard, there was a strong correlation between the favorite song and liked song in activation of the lateral parietal and medial prefrontal cortices. The disliked song, however, had excitement that remained isolated in the precuneus. Another difference between the favorite song and the liked and disliked songs is the involvement of the hippocampi. While all the songs showed some stimulation of the hippocampus, both the liked and disliked songs had auditory cortex "hubs" that included the hippocampi, while the favorite song had two separate hubs: one for the auditory cortex, and a second for the hippocampi. The activation of the hippocampi stimulates the encoding or retrieval of emotional memory. It is possible that the separation of hubs when listening to the favorite song leads to emotional memory retrieval, rather than inscription. The interconnection of the precuneus and the other areas contributes to the default mode network and causes the feelings of self-reflection and creativity, along with memory encoding, specifically of emotional memories (Wilkins, et al., 2014).

Although liked-music stimulates the default mode network and hippocampi leading to feelings of self-reflection and introspection, if the liked music is unfamiliar, it may not necessarily lead to the mesolimbic reward. Fourteen participants in a study listened to music to determine familiarity and preferences of songs. These participants listened to twelve songs in each category previously determined: familiar liked, familiar disliked, unfamiliar liked and unfamiliar disliked. Familiar music activated the anterior cingulate cortex, amygdala, thalamus, putamen, right nucleus accumbens, left hippocampus, temporal pole and orbitofrontal cortex. Unfamiliar music either did not activate these regions altogether or activated them only slightly. There was an increased blood oxygen level dependence, indicative of a lot of activation, in the emotion-related areas, including the putamen, amygdala and nucleus accumbens. The high amount of stimulation in the limbic and reward systems for familiar songs suggests that familiarity with a song will increase the liking of the song. The only difference in activation between the liked and disliked songs was in the right anterior cingulate cortex and the inferior frontal gyrus. This stimulation during liked songs correlates with the known functions of these areas, namely, judging beautiful stimuli (Pereira, et al., 2011).

Musicians vs. Non-Musicians

There are numerous differences between the way that musicians and non-musicians process music, and therefore, differences in their brain structures. Firstly, as mentioned previously, music affects both hemispheres concurrently. However, while the right hemisphere usually processes the long-term patterns, such as pitch and melody, the left, usually dominant hemisphere, evaluates the short-term patterns, including changes in rhythm and frequency. Generally, while a musician is listening to music, he is examining the form. Therefore, his left hemisphere would be stimulated predominantly. On the other hand, a non-musician, who listens to music to enjoy the melody would be mainly stimulating his right hemisphere (Bennet, Bennet, 2008).

Although in each case there is largely one hemisphere that is excited, both hemispheres are stimulated each time music is heard. Besides for hearing the music, the musician often performs complex hand sequences with his instrument, also exciting both hemispheres with his actions. The information is transmitted across both hemispheres through the corpus callosum. Neurons stimulated most often get the most strengthened by myelin sheath to allow for more efficient processing and faster reactions (Iusca, 2011). Because musicians listen to and practice playing music on a consistent basis, their neurons routinely fire across the anterior corpus callosum, leading to a larger anterior corpus callosum with more myelinated axons and a greater number of neuronal fibers than that the non-musician (Iusca, 2011, Herholz, Zatorre, 2012).

Another outcome of the need for musicians to use both hands is that musicians lose dominance in a single hand and become more ambidextrous. This is a necessary adaptation for the musical profession, which has underlying neuronal reasoning. Because the musicians use both hands to play complex musical sequences, the part of the motor cortex which stimulates both hands, rather than just the dominant hand, becomes strengthened with more myelin sheath and a greater number of synapses. This phenomenon is especially true for a keyboard player (Iusca, 2011).

Musicians have an enhanced short-term plasticity in the motor cortex, resulting in higher motor performance and synchronization in intricate manual tasks. For example, pianists were able to learn a nonmusical finger tapping sequence quicker

than non-musicians, which correlated to an increased activity in their motor cortex. This, too, correlates with the noticeably larger finger area in a musician's motor cortex (Herholz, Zatorre, 2012).

Besides for music affecting the cerebrum, musical training also affects the cerebellum. The cerebellum is used for fine motor skills. Playing an instrument requires these skills, and, therefore, learning to play them leads to more synapse connections, bigger capillaries to supply blood to these cells, and more glial cells to create a more effective support for the frequently used neurons in the cerebellum. Musicians' cerebellums are larger than those of non-musicians because of the extra stimulation (Iusca, 2011).

Musicians can process simultaneous information more accurately than non-musicians can. When fifteen non-musicians were told to report their touch sensations while simultaneously listening to two sounds, they had a hard time distinguishing between the auditory and sensory information and generally reported feeling two touches rather than one. However, musicians who underwent the same test were more than twice as accurate at differentiating between the two stimuli. One reason for increased accuracy in musicians is that musicians are accustomed to reading sheet music, feeling their instruments and responding to the sounds produced. This balance of a combination of different stimuli enables the musician to better ignore one stimulus in favor of another (Sparks, 2013). In another study, musicians and non-musicians were presented with audio-visually incongruent stimuli. The non-musicians tended to react more to the visual stimulus, while the musicians relied on their auditory perception. The likelihood of musicians relying on audio information is dependent on the length of time the musician has trained in his craft. (Abel, et al., 2016). Although the results of this experiment seem incongruent with first study, in which musicians relied on touch rather than sound to determine how many stimuli were before them, the studies are demonstrating different aspects of musicians' skills. The first experiment aimed at establishing the accuracy of musicians' sensory perception when coupled with incongruent auditory input. The musicians were successful in distinguishing between the two stimuli and, therefore, were able to accurately state the number of stimuli they touched. The second study, on the other hand, was not attempting to determine accuracy. Rather, the goal was to discover which stimulus the musician would rely on when presented with two incongruent stimuli. Because the musician was not attempting to determine a specific stimulus, he was able to respond to whichever stimulus he perceived. This study proved that, given the option of two conflicting stimuli, musicians are more prone to relying on auditory perception than visual perception.

One reason that musicians rely more on auditory than visual stimuli when the two are conflicting is that the musical training process provides auditory feedback, strengthening the sensorimotor associations between the auditory and motor systems. Because of this continual strengthening, when the musician encounters inconsistent visual information, he has an easier time blocking it out and relying solely on his auditory perception. A non-musician, who never had such training, has poorer auditory perception, and therefore compensates with his visual perception (Abel, et al., 2016). Although this argument is compelling, the possibility remains that music does not, in fact, change perception preferences from visual to auditory in musicians. Perhaps people who naturally are inclined towards using auditory information, rather than visual, are inherently more disposed to becoming musicians, and that is the reason for the trend observed (Abel, et al., 2016, Banai, Ahissar, 2013).

Effects of Beginning Musical Training Young

Although musical training can affect the brain at any age, it has an extra impact when started at a young age. String instrument musicians who began training early have a greater representation of their left fingers in their motor cortex (Herholz, Zatorre, 2012). Another neuroanatomical change found in musicians who began training before the age of six or seven is found in the corpus callosum. Because these children are practicing complex sequences daily, their brains need to accommodate a large transfer of information efficiently. This continual stimulation leads to an enlarged anterior corpus callosum (Iusca, 2011).

When studying the brains of forty-eight young adults who had at least one year of musical training in their youth, a researcher found that those who had begun training before the age seven had more developed brain areas connected to language and executive function (Sparks, 2013).

Starting musical training young seems to lead to improved visuomotor and auditory-motor synchrony, (Herholz, Zatorre, 2012). It is possible, however, that the children had exemplary visuomotor or auditory-motor coordination, which led them to begin music training young. If this is the case, the auditory-motor synchrony is the cause for early music training, rather than the reverse.

Music Facilitates Learning

Music can facilitate learning in numerous ways. Firstly, music affects the limbic system and subcortical regions, the areas that control long-term memory. Areas such as the hippocampus and cingulate cortex are stimulated through music. These areas convert short-term memory into long-term memory. When learning while listening to music, the long-term memory areas are already stimulated, and the learning will become part of long-term memory as an association of the music heard (Koelsch, 2014, Wilkins, et al., 2014).

Listening to music seems to improve other cognitive tasks as well. A music director in North Carolina arranged for a woodwind quintet to play music three times a week to first grade, then first and second, and finally, first, second and third. Although the average IQ score for this school's first through

fifth graders had been below the national grade level, after the three years of listening to music the third-grade's academic performance increased tremendously. Eighty-five percent of the students were above grade level for reading and 89% for math. The improved math skills received from music is understandable. Music is inherently related to math because music measures beat and rhythm which are mathematical concepts (Bennet, Bennet, 2008).

Although relatively little musical training can improve basic perceptional skills, especially among the originally poor performers, the larger population of musicians do not overall show improved verbal and cognitive skills. When children were given one year of after school music lessons, they did not show improved non-auditory skills. In other words, the students had better pitch discrimination, but the training did not generalize into verbal memory. The learning did not extend past the auditory perception the students gained directly from their music training. Possibly, with more time, other skills, such as verbal and cognitive skills, could emerge. Perhaps had the children had three years of music training, like the students from the previous study, the boost in reading and math skills would be evident as well.

Word decoding and reading comprehension were studied in six to nine-year-old musicians. The evidence showed no association between length of training and word decoding, however, there was a close association between reading comprehension and musical training. Possibly, the reason that there was no significant difference between those with musical training and those without it with respect to word decoding is that the children in the control group were old enough that they cognitively caught up on word decoding. If the children had been studied at a younger age, evidence may have shown a difference in word decoding ability. If musical training does in fact facilitate word decoding in younger children, perhaps the reason that the musical children tested had superior reading comprehension was because they had earlier and more proficient reading comprehension. According to this theory, the children did have a superior word decoding ability when they were younger, which led to an earlier reading comprehension. Because the musical children have been reading and effectively decoding, they show a higher reading comprehension than the non-musical children (Corrigall, Trainor, 2011).

In addition to reading comprehension, musical training facilitates improved reading skills in general, as well as the discrimination of small pitch variations in speech. Because pitch differentiation is an important part of music, learning to play an instrument will improve pitch discrimination for both the instrument being learned and speech in general (Moreno, et al., 2009).

Since music training affects the motor cortex, which enables the musician to play more complex pieces with greater dexterity, learning that involves the motor cortex is easier for musicians. Long-term musical training, specifically, augments short-term plasticity in the motor cortex. This is the reason that musicians have an easier time learning a nonsensical finger tapping sequence. Overall, motor performance and coordination are improved and, therefore, any learning that involves the motor cortex will be facilitated by musical training (Herholz, Zatorre, 2012).

Besides for the anatomical changes music effects, listening to music produces psychological benefits. Listening to music puts one in a good mood. When in a good mood, people are more productive and generate a higher quality of work. A study of employees listening to music in the workplace demonstrated that the weeks that the employees listened to music, higher quality of work was reported. However, it is possible that the higher review was an effect of the employees' improved mood, and the quality of work was unchanged (Lesiuk, 2010).

The Mozart Effect

In the first study that correlated music and learning ability, participants were placed into three groups and told to work out a spatial reasoning problem. The first group listened to Mozart's music, the second practiced various relaxation techniques, and the third, the control group, did no preparation. The results showed that the group listening to Mozart performed the best. The phenomenon was coined the "Mozart Effect." However, the benefit from the music lasted for a very short time, ten to fifteen minutes. Also, similar studies later showed that other music can achieve the same results. Therefore, it is possible that the result was not exclusively related to Mozart's music but that music with an even tempo or rhythm may temporarily improve spatial relations (Bennet, Bennet, 2008).

Length of Training Needed to Effect a Change

Music training affects the brain's structure. Some learning changes are evident almost immediately, while others take longer. Temporarily improved auditory-motor coordination is evident after twenty minutes of instrument practice. However, the effects do not last long. More permanent improvement in this area happens after five weeks of training (Herholz, Zatorre, 2012).

Besides for length of training, the age that the musician begins his lessons determines the extent of his brain's structural changes. For example, string instrument musicians who began training young have a greater representation of their motor cortex dedicated to their fingers (Herholz, Zatorre, 2012). Also, musicians who began training before the age of seven had a larger corpua callosum than musicians who began later in life. For example, the change in the corpus collosum of a six-year-old child would begin after about fifteen months of training (Iusca, 2011, Herholz, Zatorre, 2012). The phenomenon is logical; the brain goes through the most changes during childhood, discovering which areas require more synapses based on frequency of use. The younger the child begins music training, the earlier the

brain learns to accommodate the varying complexities needed, and the more prominent the resulting structural changes of the brain will be (Iusca, 2011).

Music Affecting Emotions

Although there is some controversy about whether music can induce emotions, there are some indications that music does play a role in stimulating emotion. There are six mechanisms which can explain the emotions generated by listening to music. The first is a brain stem reflex. The brain stem reacts to sounds that are loud and discordant with an arousal mechanism designed to protect the person from possible danger. Listeners who enjoy arousal will be stimulated by jarring music.

A second mechanism is conditioned response learning. When music is heard for the first time in an emotional environment the emotions become connected to the music heard. Later, when the music is replayed, the emotional feeling is regenerated.

Another mechanism is emotional contagion. The mirror neurons in the brain react by imitating stimuli in the environment. The tone, frequency or pitch of music will be mirrored by the listener by eliciting either calm or excitement.

A fourth mechanism through which music stimulates emotions is visual imagery. Often, while the listener is hearing the music, his brain conjures up images associated with the sounds he hears. It is possible for those images to lead to emotions. The images recalled may or may not be from memory. However, when the images are from the listener's episodic memory, a different mechanism provides the emotions associated with the memory.

Finally, momentary surprise or confusion will occur when the listener anticipates a particular sequence of chords. For example, if a listener hears a C sharp, D sharp, and then E sharp, he naturally expects the next note to be an F sharp. If the music breaks the rhythm, the listener is temporarily put off balance and may be confused. Although this will not lead to emotions such as joy or sadness, it can cause brief surprise or confusion (Juslin, Västfjäll, 2008).

Besides for these mechanisms, specific areas of the brain related to emotion are stimulated by music. One area activated by music is the amygdala. Because the amygdala controls the processing of emotions, stimulation to that area leads to feeling emotion. Joyful music stimulates the superficial amygdala, leading to happiness. Both happy and sad music stimulate the latero-basal amygdala, which is associated with positive and negative reinforcement. This reinforcement can activate the mesolimbic reward system in both musicians and non-musicians (Koelsch, 2014).

Pleasant music activates the nucleus accumbens and ventral tegmental area, which controls motivation through reward. The "reward" feeling is in the form of dopamine being released into the brain. Performing an action that leads to reward is enjoyable. Because listening to music stimulates the nucleus accumbens and ventral tegmentum which send the dopamine reward as a response, the listener appreciates the music (Koelsch, 2014, Schlaug, et al., 2010, Boso, et al., 2006, Menon, Levitin, 2005, Pereira, et al., 2011). Reward feelings are enjoyable, and therefore, improve the listener's mood and affect his emotions. Besides for activating the dopaminergic reward system, listening to music causes the release of endorphins into the blood stream. Endorphins reduce pain and stress and can lead to feelings of happiness (Koelsch, 2014, Herholz, Zatorre, 2012, Boso, et al., 2006).

Conclusion

Music affects a variety of areas in the brain, including areas that control cognitive learning, coordination and emotion. Not all music affects the brain in the same way. Different genres of music cause different size "hubs" of activation in the auditory cortex. Similarly, music that the listener likes is processed in a different way from music that he dislikes. Based on areas of activation in the brain, music therapy can be a useful tool for treating neurological disorders, such as stroke, cerebral palsy, and fronto-temporal dementia, as well as calming stress. Because musicians practice playing their instrument, and in general have more exposure to music, there are distinct differences in the structural anatomy of a musician's brain, as opposed to that of a non-musician. Musicians tend to have a larger anterior corpus callosum, as well as greater representation of their fingers in the motor cortex, especially when musical training had been started at a young age and continued for at least fifteen months. Because playing an instrument requires complex multitasking, musicians develop superior multitasking skills, such as the ability to distinguish between conflicting auditory and visual stimuli. Additionally, since music affects areas of the brain connected to learning, people who listen to music or train in music show higher reading comprehension and math ability compared to non-listeners and non-musicians. Listening to music can be an emotional experience, in addition to the learning benefits, because of specific mechanisms associated with emotion which are stimulated by listening to music, as well as activation in areas which control underlying emotions, such as the amygdala and the mesolimbic reward system. Music is more than just sounds strung together. The effects of music are broad and should continue to be studied to determine the full extent of the benefits.

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UVB Induced Mutation of p53 in Non Melanoma Skin Cancer

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Abstract

There is a clear correlation between excessive sun exposure and the development of skin cancer. UVB radiation from the sun is potent, and as the ozone layer gets depleted, more UVB can reach Earth and cause cell damage. UV radiation causes DNA lesions, such as 6-4 photoproducts and cyclobutane pyrimidine dimers. Cyclobutane pyrimidine dimers are more abundant and take longer to be repaired and therefore are responsible for most of the mutation and DNA damage. These DNA lesions lead to mutation of the p53 gene. The signature mutation on p53 from UV radiation is a CC to TT mutation, which generally occurs at the binding site of p53. As a result, of the mutation, p53 is inactivated and can no longer perform its tumor suppressive functions. As a result, cancerous or damaged cells in the skin can proliferate and form tumors. P53 is an early step in skin carcinogenesis, and p53 mutation is found in abundance in actinic keratosis, a precancerous lesion of Squamous Cell Carcinoma. Experiments conducted on mice prove the effectiveness of sunscreen. Mice treated with UVB blocking sunscreen had significantly decreased percentage of mutation, compared to mice without the sunscreen.

Introduction

When summer comes around, it is quite challenging to find a place to sit at the beach. The sands are covered with people sunbathing and basking in the sunlight. Although it is widely known that excessive sun exposure directly correlates to the onset of skin cancer, many people feel that they are immune to the sun's harmful rays and therefore will not develop skin cancer. However, this is not the case, and as they lie there, in denial of the harmful effects of the sun's radiation, they are hurting their bodies. Tanning may cause a more appealing appearance , but in just a few years the harmful effects can emerge and cause a quite dangerous condition, long after the beauty wears away. Sitting directly in the sun, especially without sunscreen, is understandably harmful, but what many people do not realize is that even in cloudy weather eighty percent of the sun's rays can penetrate through the clouds and can still affect the skin (Bowes, 2012). No matter the weather, spending too much time outdoors without the proper protection is detrimental to a person's skin.

In the United States alone there are approximately 500,000 cases of basal cell carcinoma (BCC) and 100,00 to 150,000 cases if squamous cell carcinoma (SCC) reported every year (Kanjilal et al., 1995). Sun exposure proves to be the major factor in the formation of these cancers. Melanoma generally is caused by short incidents of high intensity ultraviolet (UV) radiation. On the other hand, BCC and SCC, known as the non-melanoma skin cancers (NMSC) are generally caused by incidents of exposure to UV radiation which gradually build up. Since NMSC is a result of the collective UV radiation absorption, it is more commonly found in older people (Armstrong and Cust, 2017). Generally skin cancer is more prevalent among fair skinned people, who have less melanin, pigment, which acts as a barrier to UV radiation. Moreover, people who live close to the equator are also at a greater risk for solar UV induced skin cancer, as the sun's rays hit Earth most directly at equator.

No matter the skin tone, too much sun exposure can lead to various issues, such as inflammation, photoaging, erythema, sunburn, eye damage, DNA damage and skin cancer (Benjamin et al., 2007). UV radiation causes damage to DNA, specifically on the p53 gene, a gene with tumor suppressive functions. Once this gene is mutated, it can no longer act as an anti-oncogene, and cancerous skin cells can metastasize.

Methods

The research discussed in this paper was obtained from various published articles from the Touro Library and Google Scholar. Most of the articles present original information from studies and experiments of tissue and DNA

Discussion: Ultraviolet Radiation

The electromagnetic spectrum is a range of all forms of light, based in their frequency and wavelength. Visible light has wavelengths between 400 and 750 nanometers. UV radiation has shorter wavelengths and therefore higher frequency than visible light. Wavelengths of UV rays are 100 to 400 nanometers (US EPA, 2018). UV radiation is divided into 3 categories, UVA, UVB, and UVC. UVA has wavelengths between 320 to 400 nanometers, and can penetrate through the ozone layer, a protective layer of gas that surrounds earth and protects from harmful UV rays. UVB ranges from 290 to 320 nanometers, and is mostly blocked by the ozone layer, but some rays do come through the ozone and reach Earth. UVC ranges from 100 to 290 nanometers, and is almost entirely blocked by the ozone (Chouinard et al., 2001).

Although UVB is mostly blocked by the ozone, as the stratospheric ozone layer is depleted, UV radiation is more potent and can raise the instances of skin cancer (Nakazawa et al., 1994). UVB has been proven to be the major factor to induce DNA damage, because that specific wavelength can be absorbed by the nucleotide bases that make up DNA (Ichihashi et al., 2003). Wavelengths below 290 nanometers usually get absorbed on the surface of the epidermis, and thus do not cause BCC and SCC (Radosevich, 2014).

Only ten percent of sunlight is UV radiation, as the rest comes from visible and infrared light. Of the ten percent, ninety five percent of it is UVA radiation, and only five percent is UVB (Bowes, 2012). A little bit of UVB is essential for the production of Vitamin D, however, excessive exposure to it can cause erythema, cataracts, or skin cancer (Radosevich, 2014). Even small amounts of UVB exposure can be dangerous if there are

repeated incidents, as it will affect the cell formation in the skin by attenuating the basement membrane structure (Chouinard et al., 2001). The weakened membrane allows the UV radiation to reach a person's DNA, specifically that of the p53 gene, a gene with tumor suppressive functions. Once this gene is mutated, it can no longer act as an anti-oncogene.

Function of p53

The p53 protein is made from a 20 Kb gene on the short arm of chromosome 17, which codes for a phosphoprotein involved in transcription and regulation in the cell cycle (Benjamin and Ananthaswamy, 2007). It has been dubbed the name "the guardian of the genome" and "the cellular gatekeeper" (Zilfou and Lowe, 2009). P53 is known this way because of its tumor suppressive functions. P53 gets activated via phosphorylation at a serine residue, at either the amino or carboxyl terminus (Benjamin et al., 2007). Then, it can inhibit the growth of cancerous causing cell cycle arrest, apoptosis, and DNA repair (Ouhtit et al., 1998). Cell cycle arrest gives the cell the opportunity to correct DNA complications before the DNA gets replicated. Since the cell is not continuing mitosis at that point, no new cells will be formed with the incorrect DNA. On the other hand, when p53 causes apoptosis, the cancerous cell gets killed and destroyed, and therefore cannot proliferate and become a cancerous growth(Benjamin et al., 2007). Moreover, it can preserve the correct form of the DNA (Einspahr et al., 1999). This is accomplished by "negative growth regulation," which essentially means that p53 stops cell division and proliferation of cells with abnormal DNA (Benjamin and Ananthaswamy, 2007).

Mutation of p53 is usually a missense mutation, a mutation which switches nucleotide in the DNA sequence. Therefore, the codon in the DNA will code for a different amino acid. Such a change affects the protein's function if the mutation results in an amino acid change in the binding site of the protein, which is the case in the p53 missense mutations (Einspahr et al., 1999).

When p53 is mutated it loses its ability to act as an anti-oncogene and suppress tumors, so growth of tumors are then able to grow and form. Tumorigenesis gets inhibited when p53 binds DNA and triggers a different gene to make p21. Once p21 is made, it reacts with a cell division stimulating protein(cdk2), and forms a complex with it. As a result, cdk2 is inactivated and the cell cannot continue with cell division. By stopping cell division, the cells cannot multiply and grow to form a tumor. However, when p53 gets mutated, it can no longer interact with DNA the same way, so p21 is not produced, cdk2 is not inhibited and cancerous cells can continue multiplying and form a tumor (National Center for Biotechnology Information (US), 1998).

UV Damage Adducts: Cyclobutane Pyrimidine dimers and 6-4 Photoproducts

BCC and SCC are mainly caused by UV radiation(You et al., 2001). Studies have shown that 58% of SCC has a mutation on the p53 gene(Benjamin et al., 2007). UVB specifically causes bulky linkages between pyrimidine dimers(Sarasin and Giglia-Mari, 2002). DNA is made up of nucleic acid, which is a linear polymer of nucleotides. Each nucleotide contains a ribose sugar, phosphate group and a nitrogen base. There are four different nitrogen bases found in DNA: adenine, guanine, cytosine and thymine. Adenine and guanine have similar ring structures and are classified as purines. Cytosine (C) and thymine (T) have a similar ring structure (different than that of the purines) and are classified as pyrimidines. A pyrimidine dimer can form when two pyrimidine nucleotides are adjacent to each other on a DNA strand.

These pyrimidine dimer linkages can mainly be corrected by nucleotide excision repair. (Sarasin and Giglia-Mari, 2002). Nucleotide excision repair can repair UV induced pyrimidine dimer linkages before DNA replication, or after replication by using a specific DNA polymerase which corrects DNA. The repair process begins with recognizing the damage on the DNA .The DNA is then cut on either side of the lesion on a single strand of DNA, and the damaged area is extracted. Next, the removed segment is replaced with new nucleotides to recreate the DNA properly. The process is complete when ligation occurs, and the newly formed DNA segment is linked to rest of the strand. Two approaches to nucleotide excision repair are transcription coupled repair, which is done on DNA strands that are actively transcribed, and global genome repair which occurs on the non-transcribed strand of DNA. Transcription coupled repair requires is generally faster than global genome repair in DNA repair (de Gruijl and Rebel, 2008). Global genome repair deals with damage recognition and correction of pyrimidine dimer linkages. The damage recognition stage takes some time, depending on the effect of the damage on the structure of the DNA helix, and is the rate limiting aspect in repair, which makes global genome repair slower than transcription coupled repair (Ichihashi et al., 2003). The significance of nucleotide excision repair can be understood by studying patients with Xeroderma Pigmentosum, a genetic disease in which DNA repair from UV radiation, nucleotide excision repair, is impaired. Individuals with this disorder have a much higher rate of developing skin cancer, since they have more uncorrected pyrimidine dimer linkages that lead to mutation (de Gruijl and Rebel, 2008). Early growth of skin cancer is noted in Xeroderma Pigmentosum patients with a frequency of approximately 4000 more than regular people (Sarasin and Giglia-Mari, 2002).

UVB can cause two types of DNA lesions on the p53 protein, cyclobutane pyrimidine dimers (CPD) and pyrimidine 6-4 pyrimidone photoproducts (6-4PP). Both of these linkages lead to C to T or CC to TT mutations, known as "signature mutations," on gene p53 in DNA (You et al., 2001). In a study of tissue with different levels of sun exposure and sun damage, it was noted that 74% of the mutation of p53 occurred at dipyrimidine sites, and

54% were C to T, or CC to TT mutations (Einspahr et al., 1999).

CPDs are the cause of 80% of the DNA mutations, since 6-4 PP can be repaired more efficiently (You et al., 2001). The repair of 6-4PP can occur within less than 24 hours of the UV exposure (de Gruijl and Rebel, 2008). In fact, 56% of keratinocytes corrected the 6-4 PP within 24 hours. Conversely, only 11% of CPDs were remedied within the first 24 hours after UVB exposure (Chouinard et al., 2001). CPDs are less effectively repaired and can lead to mutation of p53 and then skin cancer. Moreover, there are generally CPDs are 3 to 4 times more commonly formed than 6-4 PP (de Gruijl and Rebel, 2008). Experimentally it has been observed that 6-4 PP were formed significantly less often than CPD overall, and even more rare in experiments with solar UV simulation. Therefore, in natural sunlight, 6-4 PP are found at a much lower percentage than CPDs (You et al., 2001).

UVB from sunlight will cause CPD formation, especially at pyrimidine dimers which have methylated cytosine nucleotides (You, 2000). A CCG sequence with a methylated cytosine is a common place for mutation, since a methylated cytosine is less stable than regular cytosine and can be deaminated easily. The deamination of a methyl cytosine yields a thymine nucleotide, a DNA mutation (Sarasin and Giglia-Mari, 2002). CPDs only can cause mutations to develop after the deamination of a methylcytosine in adduct (You et al., 2001). The CCG sequence codes for the amino acid arginine, and when the cytosine nucleotide gets converted to thymine, the sequence now codes for tryptophan. This switch of amino acids is extremely important to the p53 gene since that arginine is found at p53's binding site (Sarasin and Giglia-Mari, 2002). Since arginine and tryptophan have different side chains they react differently and thus will affect the binding of p53 to the target DNA. Arginine contains a second amino group on its side chain, which can act as a base and get protonated. Tryptophan side chain is two fused aromatic rings, and the nitrogen in its side chain is an extremely weak base, as it loses its aromaticity when protonated. Therefore, mutation and transition of the nucleotide at this sequence greatly affects the function and capabilities of p53.

Mutation of p53 and Carcinogenesis

Genetic mutations of p53 have clear correlations with the growth of cancer. When p53 is inactivated or mutated, the skin becomes more vulnerable to carcinogenesis (de Gruijl and Rebel, 2008). UV radiation provokes the growth of skin tumors, mostly SCC and BCC(You et al., 2001). Basal cells are on the bottom layer of the epidermis and rise slowly as new layers grow beneath them. As a result, these cells stay in the epidermis the longest, until the rise from the bottom layer until they eventually reach the top and die, Therefore UV damage to these cells has a long term capability to cause cancer, since they are in the skin for the longest time (de Gruijl, 2002).

P53 mutation is found in about 50% of all types of cancer. UV radiation causes specific mutations in p53 which can result in skin cancer. It causes cancerous growths by stimulating different growth factors and activating their receptors. Moreover, each instance of UV exposure helps mutated cells reproduce and take over healthy epidermal cells, even if it does not induce new mutations. Although the cell has protective mechanisms against DNA mutation, UV sometimes can still cause DNA damage. Buildup of mutations on critical genes leads to carcinogenesis of the skin. Many mutations of p53 are even noted in normal looking skin which has been exposed to the sun (Benjamin and Ananthaswamy, 2007). Most of the mutations found in p53 in people with skin cancer, prove to have UV radiation as the cause. Once p53 is mutated, it affects the availability for apoptosis to occur in keratinocytes, as a response to UV radiation. Apoptosis is a programmed cell death. In tissues that are constantly renewing, apoptosis is involved in homeostasis , as more cells are reproducing, others are destroyed through apoptosis. When a gene which deals with apoptosis is mutated, it can result in too much growth, since cells are not dying as new cells are being formed. Moreover, p53 maintains "genomic integrity" by repairing the DNA or causing apoptosis in a cell with mutant DNA. However, when p53 is mutated, it can no longer function in this way. In cancer, generally the mutation of p53 occurs further into the tumorigenic process. In contrast, in NMSC the mutation in p53 is an early step in carcinogenesis (Einspahr et al., 1999).

Mutated cells have overexpression of the p53 protein and form "p53 patches." Most p53 patches had signature UVB damage. The mutation spectrum observed is quite similar to the mutation spectrum of SCC. As a result of the damage, the functions of p53, like apoptosis and cell cycle arrest, are interrupted. Since these processes generally occur to prevent cancerous growth, UV induced damage which impacts them likely occurs early in cancer development. Although all p53 have the potential to become SCC, only a few do actually become cancerous. Approximately 8000 to 40000 p53 patches are around by the time the first tumorous growth begins (de Gruijl and Rebel, 2008).

UV induced mutations in p53 most likely occur as an early step in the process of skin carcinogenesis. However in other cancers, like colon cancer p53 mutations generally occur later in the development of the cancer, particularly related to the change from an adenoma to a carcinoma (Benjamin and Ananthaswamy, 2007). This phase of cancer is much later in the development as cancer progresses from normal epithelium, to abnormal epithelium, to a small adenoma, to larger adenoma, and finally to a carcinoma.

Actinic keratosis (AK) is a precancerous lesion which can lead to SCC. The frequency of a p53 mutation has been studied with different conditions of skin. In a particular study, the frequency of a p53 mutation in SCC was 53.8%, in AK it was 62.5%, in sun damaged skin it was 38.5%, and in normal skin it was 14.3%. The mutation is observed to have a higher frequency in skin

from an AK lesion. This could be because the tissue samples obtained from AK lesions are closer to the surface of the skin, and the tissue samples of SCC are deeper in the skin and could be contaminated from the stromal tissue(Einspahr et al., 1999).

Approximately 89% of the mutations found in AK, were UV signature mutations. Therefore, it is believed that AK is the growth of mutated, cancerous cells. Moreover, the UV does not only cause great increase in reproduction of the mutated cells, but also causes apoptosis of normal cells in the area of hyperproliferation (Benjamin and Ananthaswamy, 2007). Various studies of skin have found a lot of p53 mutations in BCC and SCC. Samples of 342 tissues from patients with aggressive and nonaggressive cases of SCC and BCC were studied for p53 changes. In BCC, 66% of the aggressive cases and 38% of the non-aggressive cases had p53 changes. In SCC, p53 mutations were observed in 35% of the aggressive cases and 50% of non-aggressive cases (Benjamin and Ananthaswamy, 2007).

In a study of eight samples of NMSC, from the head and neck, 7 of the samples (88%) had p53 mutation. Six of the tissues had at least two amino acid changes as a result of the mutation, which were mainly at the binding site of p53 (Kanjilal et al., 1995).

In Approximately 38-50% of patients with NMSC, a second tumor grows within five years of the first one. A patient with skin cancer needs a lot of medical intervention, surgical procedures, and sometimes radiotherapy (Kanjilal et al., 1995).

Prevention

Since NMSC can be caused by UVB from sunlight, protection from the sun's powerful rays would be a great means of prevention. DNA damage and mutation of p53 are the beginning steps of the development of skin cancer, by preventing them skin cancer can be prevented as well (El-Deiry, 2007). Sunscreen can effectively protect the skin from UV induced DNA damage and p53 mutation, since it can absorb or reflect the harmful UV rays so they do not penetrate the cells (Radosevich, 2014). Sunscreens have been proven to protect against DNA damage, skin aging, sunburn, immunosuppression, and skin carcinogenesis. Although it can be obviously observed that sunscreen prevents erythema and sunburn, experiments and studies must be conducted to see if it is useful in preventing deeper damage, like p53 mutation (Benjamin et al., 2007).

The effectiveness of sunscreen in preventing mutations, can be proven based on a murine experiment. After conducting experiments to prove that UV radiation causes p53 mutation, another experiment was conducted to understand if sunscreen can prevent the p53 mutations from happening. Both sunscreens used in this experiment had an SPF (sunburn protection factor) of 15. One sunscreen had only UVB protection, and the other had protection for UVB as well as protection from UVA. The mice who had the UVB sunscreen, developed p53 mutations 88% less than mice without sun protection, and those with UVB +UVA sunscreen had 92% less mutations. Therefore, this experiment clearly displayed the capability of sunscreen to inhibit p53 mutation. Since p53 mutation is an integral step in carcinogenesis, if mutation is prevented, skin cancer is less likely to develop (Ananthaswamy, Ullrich and Kripke, 2002).

Another strategic tactic to avoid or delay carcinogenesis is to minimize sun exposure. Some UV exposure is necessary for the body, as it plays an important role in the synthesis of vitamin D , excess exposure can cause skin cancer (Radosevich, 2014). Studies have proven that p53 patches grow more when exposed to additional UV exposure. Although reducing UV exposure cannot eliminate the possibility of skin cancer, it can delay its progression and development(Benjamin et al., 2007).

The Environmental Protection Agency (EPA) notes that at noon the sun's rays are most powerful as they travel the least distance to reach Earth. Therefore, it would be wise to avoid unnecessary UV exposure at that time. The EPA also recommends applying sunscreen, seeking shade when possible, keeping aware the UV index, and being exceptionally cautious around areas that can reflect the sun's rays, such as water, snow, and sand (US EPA, 2018).

Conclusion

UVB induced mutation of p53 leads to the development of skin cancer. UV radiation causes the formation of pyrimidine dimers on the p53 protein. P53 is an anti-oncogene, which prevents tumorigenesis by causing cell cycle arrest or apoptosis. When p53 is damaged by UV mutation it can longer function and cancerous basal and squamous cells can proliferate and grow. Cancerous areas show overexpression of p53 and are referred to as "p53 patches." These patches will grow more when exposed to more UV radiation, even if the radiation will not cause a new mutation to form in that instance. Sunscreen usage and minimization of sun exposure are affective approaches to prevent UV damage of p53 and skin carcinogenesis.

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