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Sickle Cell Anemia: Current Treatments and Potential Advancements

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SICKLE CELL ANEMIA: CURRENT TREATMENTS AND POTENTIAL ADVANCEMENTS

by

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Table of Contents

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Abstract

Sickle cell anemia is a disease that affects red blood cells, specifically the hemoglobin protein. An amino acid mutation in the gene that encodes β-globin leads to malformation of the β subunit of hemoglobin.[3,] Valine becomes glutamic acid in the mutated β-globin gene.[9] The mutation malformation leads to the red blood cells becoming sickle shaped, or crescent shaped.[3,4] The sickle shape of red blood cells in individuals with sickle cell disease leads to vaso-occlusive crisis.[9] Vaso-occlusive crisis includes complications such as blood clotting, chronic pain, organ failure, organ death, and possibly early mortality.[9] The Centers for Disease Control and Prevention reported that there are approximately 100,000 Americans who have sickle cell disease as of their August 2016 records.[21] One of every 365 African-Americans, and one in every 16,300 Hispanic-Americans have sickle cell anemia.[21] There are therapies and treatments that have already been developed based on the knowledge gained regarding the disease and its complications. Currently, hydroxyurea therapy is the most widely accepted and used treatment for individuals with sickle cell disease with proven relief of vaso-occlusive crisis complications. However, with hydroxyurea therapy there are several unwanted effects that have an impact on the patients, such as jaundice, blood clotting, and usual bleeding just to name a few.[44] Studies have noted an upregulation of the glycolytic enzyme, fructose-bisphosphate aldolase (aldolase) in patients who have undergone hydroxyurea therapy. Aldolase is an important enzyme that plays a role in energy conversion. With this in mind, I propose that sickle cell disease can be treated with an increase in aldolase by myoblast treatment and/or higher fructose consumptions thereby reducing the complications that come with the disease, as well as, drastically eliminating the unwanted effects that come with hydroxyurea therapy.

Keywords: *Sickle Cell Anemia, SCD, hemoglobin, VOC complications, aldolase, hydroxyurea therapy, treatments, therapy*

CHAPTER ONE: SICKLE CELL ANEMIA

Introduction

Sickle cell anemia (SCA), also known as sickle cell disease (SCD), is a disease that alters the hemoglobin in red blood cells (RBC).[1] Red blood cells are commonly known to have a circular shape. The fact that RBCs have this shape, with other unique features, make it possible for them to travel throughout the body with ease.[2] This flow of RBCs throughout the body is what allows for oxygen, iron, chemical ions, and other important nutrients to move from one location to another and reach their designated destination.[2] In SCD, the RBC is sickled, or crescent shaped.[3, 4] These sickled cells can lead to complications known as vaso-occlusive crisis (VOC), such blood clots, which can furthermore lead to tissue and/or organ failure. It was first brought to the attention of the United States in the early 1900s by Dr. Herrick while he was viewing Walter Clement Noel's red blood cells.[3, 4] Since then, there have been many contributors to our knowledge and understanding of SCD, including Hahn and Gillespie, Dr. Neel, Nobel prize winner Dr. Pauling, and Dr. Itano, just to name a few.[3]

Background

Discovery. Prior to the discovery of sickle cell anemia in the 1900s, there was not enough knowledge to explain the manifestation of sickled cells. In 1910, Dr. Herrick described unusual findings in the blood of a young black man, of which he had never come across before.[3] Dr. Herrick was very thorough writing his observations, describing things such as the overall physical features including chest deformity, redness in the throat, enlarged glands, and several scars up to 3 cm in diameter on his legs and thighs; enlarged left side of the heart with a

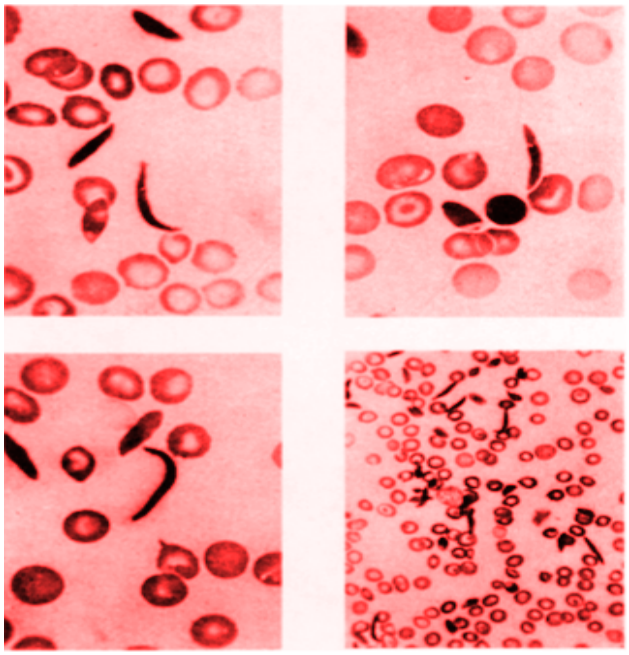

Figure 1: Dr. Herrick's view of sickle cells[4] with added contrast for enhanced visualization

murmur and heart rate between 64 and 100; rattling in the lungs heard throughout his chest and back; fever that fluctuated from 99°F to 101°F; increased urine output; feces output that contained bloody mucus; and blood descriptions, such as the shape of the red blood cells he saw (figure 1).[4] Herrick included the history of the young man named Walter Clement Noel including that he formerly lived in Grenada, West Indies[4]; and addressing important associated symptoms such as shortness of breath and palpatations[3], which are defined as feeling a pounding or fluttering heart.[4] These are common features of sickle cell anemia, however, this information was not known at that time. Also, Herrick noticed the distinct sickle shape of the red blood cells, which is the reason for the name sickle cell anemia. A vast number of Noel's red blood cells were crescent-shaped, elongated, and thin.[4] Dr. Herrick recognized the abnormality of the red blood cells remained the same in all of the blood samples, regardless of how the blood was smeared or treated, even after exposing the cells to heat, placing them in alcohol or ether solutions, and staining them with Ehrlic stain.[4] Dr. Herrick, after comparison to other healthy individuals and looking through various aspects of Noel's condition, could not come to a conclusion about what the diagnosis of the patient could be. He suggested syphilis because some of the symptoms seemed to be similar, however, no conclusion was made. Dr. Herrick ended his article with a statement about leaving the case with an open diagnosis until more cases arose with similar features as the young man he observed.[4] All this to say, during that time period not too much was known about what sickle cell anemia really was and what caused it.

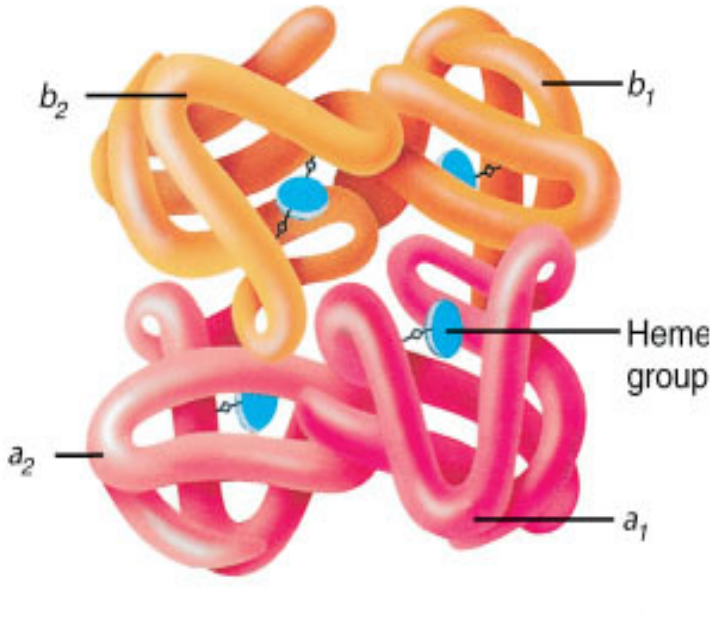

Figure 2: Red blood cell hemoglobin protein depicted α (bottom, pink) and β (top, orange) subunits[6]

Development. Hahn and Gillespie in 1927 discovered what causes a red blood cell to sickle. The red blood cells are the oxygen transport of the body and when they are not carrying oxygen they form the crescent shape that is known as sickle.[3] Dr. James V. Neel suspected

that sickle cell anemia was a hereditary disease in 1949. Following that suspicion, in 1951, Nobel prize-winning chemist Dr. Linus Pauling and Dr. Harvey Itano, together made the discovery of the chemical structure of the protein hemoglobin.[3] Hemoglobin in red blood cells is a four-part protein. These four parts consist of two pairs that encode the two protein subunits. Those two pairs are the α-globin and the β-globin. Hemoglobin with its α-globin and β-globin subunits are shown in Figure 2.[6] Dr. Pauling and Dr. Itano discovered that hemoglobin was quite different in those with sickle cell anemia than in healthy individuals, leading to sickle cell anemia being the very first disease considered a molecular disease.[3] In 1956, not long after the two noted the chemical structure difference, Dr. Vernon Ingram determined the specific amino acid that led to the restructuring of hemoglobin.

Then & Now. Due to the sickling of the red blood cells, the flow of blood within the vascular system is very poor and can often lead to vascular restriction. Before further discoveries and investigations on the cause of organ tissue death, vaso-occlusion seemed to be the only reason these tissues died in patients with sickle cell disease. In more current findings, an overall interaction between red blood cells and endothelial cells, which are cells that form the lining of blood vessels[7], plays a role in leading to dysfunction of organs. Mohandas, Hebbel, and Kaul were the three who prompted this concept in the 20th century. As vaso-occlusion continued to be studied by people such as Duits and Schnog, it became more evident that these vascular blocking episodes, which occurred in people with sickle cell anemia, caused the dysfunction in the organs differently than in healthy individuals.[8, 9] Further investigation has shown a connection between red blood cells and blood vessel walls. It is not just the shape of the red blood cell (sickle-shape), but also the vessel wall adhesion, the way neutrophils are recruited and adhere to the cell wall causing blockage of sickled red blood cells.[8, 10]

Genetics

Sickled red blood cells are noted to have only one amino acid different than normal blood cells.[3] Figure 3 shows the genes for the α-globin and β-globin subunits of hemoglobin (discussed in background) and their locations on chromosomes 11 and 16, respectively.[10, 11] The single amino acid that gets substituted is Valine, which becomes glutamic acid.[10] The year Dr. Pauling designated sickle cell anemia as a molecular disease was the same year that it was found to

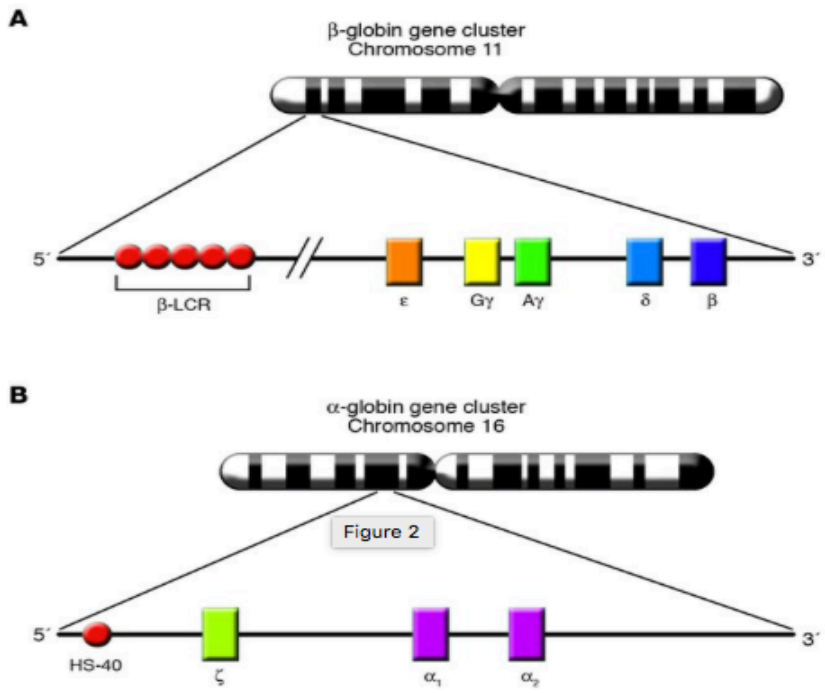

Figure 3: Location of genes encoding α- globin and β-globin subunits of RBC hemoglobin on chromosomes 11 and 16, respectively.[10, 11]

specifically be an autosomal recessive disease. The severity of sickle cell anemia is dependent on the interaction between the α-globin and β-globin subunits.[10] When the genes are homozygous recessive, sickle cell anemia is at its most severe and most common form. However, there are still variations of the disease that can occur based on how the genes interact. Usually it is the β-globin mutation that leads to abnormal interactions and various degrees of sickle cell disease. When a β-globin is not produced normally, or is produced in small quantities, the gene is known as β-thalassemia.[10] The interaction between α-globin and mutated β-globin (or β-thalassemia) subunits determines the severity of the disease. A normal α-globin and a mild mutation in β-thalassemia can result in mild expression of the disease.[10] Even with normal α-globin, β-thalassemia in its inactive state resembles a recessive mutated β-globin and the severity of the disease is very high, similar to an individual who has have sickle cell anemia.[10]

Fetal hemoglobin (HbF), with 2 γ-globin subunits, is found during fetal development and is replaced by adult hemoglobin (HbA), with 2 β-globin subunits, as an individual gradually develops into adulthood (figure 4).[10, 11] Fetal hemoglobin becomes adult hemoglobin due to hypomethylation by the enzyme methyltransferase, which does as its name implies, transfers methyl groups ($-CH_3$).[12] It is not until the development of HbA and replacement of HbF is complete that any mutations in β-globin become more phenotypically present.[11] Among some Eastern areas of Saudi Arabia and in some locations of India, lower rates of sickle cell disease were noticed coupled with higher levels of fetal hemoglobin in adults.[13, 14] In another study, low levels of fetal hemoglobin were closely associated with increased death rates at an early age,

50% of the individuals died before their fifth decade.[15] These findings led to future studies in HbF and its potential in treating sickle cell anemia.

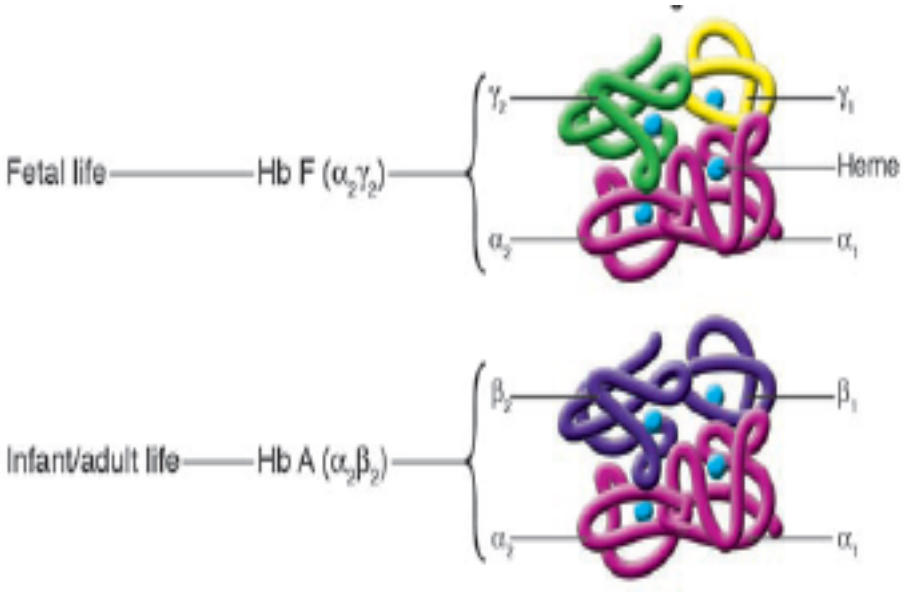

Figure 4: Fetal hemoglobin with 2 γ-globin subunits (top hemoglobin) before development and replacement to 2 β-globin subunits in adult hemoglobin (bottom hemoglobin)[10, 11]

Demographics

Adults. As studies look at sickle cell disease more closely, new findings lead to increased knowledge and understanding. Specifically, more is known about the way proteins interact within the body on a molecular level. Glutathione has an important part in antioxidant defense mechanisms in adults.[16] Quastel, Stewart, and Tunnicliffe confirmed glutathione was a dipeptide of cysteine and glutamic acid[17] after the structure was originally proposed to be just that by Hopkins in 1921.[18] This defense system is very important because it helps control and buffer the effects of reactive oxygen species (discussed later in chapter).[19] Glutathione is now more specifically known to be composed of glycine, glutamate, and cysteine that come together under the catalyst glutamate cysteine ligase enzyme to form glutathione (figure 5).[16, 20] The intermediate of this reaction is γ-

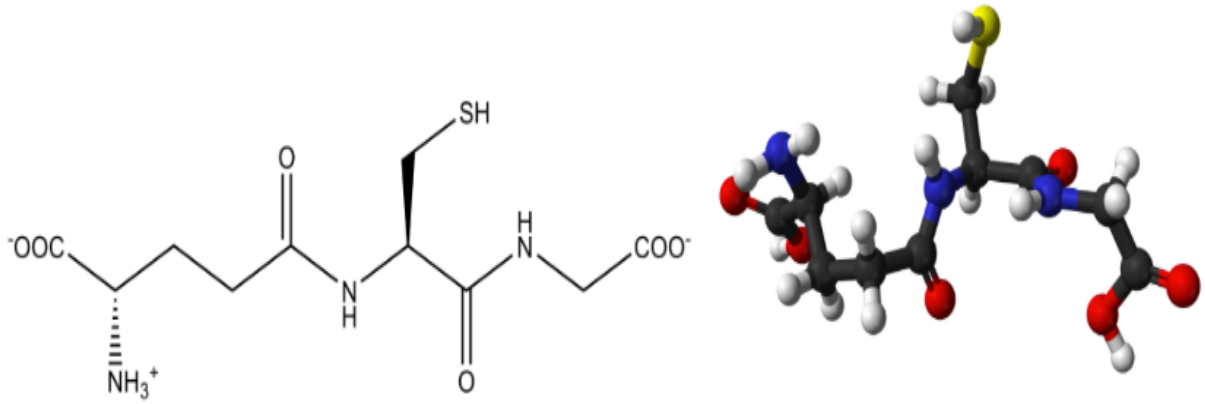

Figure 5: Structural formula (left) and ball-and-stick model (right) of glutathione[20]

L-glutamyl-L-cysteine, which is also the rate-limiting-step in the synthesis of glutathione. On the other hand, another reaction that can lead to the synthesis of glutathione is from a glutathione disulfide by glutathione reductase enzyme. Glutathione is considered to be considerably low in those who have sickle cell anemia, but the reason as to why or how the deficiency occurs is not fully known. There seem to be factors, based on studies, that lack of sulfur amino acids consumed in one's diet can have an influence on the lack of glutathione that is produced.[16] Another possible explanation is because individuals with sickle cell anemia have such high oxidative stress within their bodies, then glutathione produced gets used by the body so quickly that red blood cell count decreases because of the consumption. The latter seems to be what is occurring on the molecular lever.[16]

African Decent. In 1910 when sickle cell anemia was first discovered in the United States, the first view of sickle cells was in a young black man from the Grenada Islands in the West Indies.[5] Furthermore, sickle cell anemia has been known by other names in Africa for over five thousand years.[4] Yet, it was not until the early 1900s when the disease started to become more clearly understood. There is commonality of the disease mostly in those from African descent. In Jamaica, there are increased occurrences of homozygous β^s disease, which is a type of sickle hemoglobin disease.[21] If the homozygous mutation of β-globin is not present, an individual can still be considered a carrier of sickle cell disease due to the presence of mild mutation in the gene. This particular presence of a mild mutation that does not lead to sickle cell disease is known as having sickle cell trait.[22] In many areas of Africa where malaria disease is highly prevalent, individuals with sickle cell trait were also more prevalent and seemed to strive.[22] Malaria is a disease that causes rupturing of red blood cells due to infection of parasites.[23] Individuals with sickle cell trait, who do not have sickle cell disease, have lower susceptibility to contracting malaria, however, the mechanism by which this occurs is not understood.[24] Although being a carrier of sickle cell trait can be beneficial against malaria, when individuals who carry the sickle cell trait in these areas reproduce, their children are more likely to be born with the homozygous recessive sickle cell disease and all of its complications.

World Health Organization published data regarding the prevalence of sickle cell anemia among various regions of the world in 2003 (Table 1).[25] At that time, it was once again confirmed that individuals of African decent have higher percentage of having sickle cell anemia.[21] The table shown also indicates that those of African descent also have higher amount of births affected by sickle cell anemia that those in any other region of the world.[25]

Table 1. Estimated prevalences of carriers of haemoglobin gene variants and affected conceptions

	Demography2003				% of the populationcarrying			Affected conceptions(per 1000)			
WHO region	Population (millions)	CrudeBirthrate	Annual births (1000s)	Under-5 mortality rate	Significant variant[a]	α+ thalassaemia[b]	Any variant[c]	Sickle-cell disorders[d]	Thalassaemias[e]	Total	Affected births (% of under-5 mortality)
African	586	39.0	22 895	168	18.2	41.2	44.4	10.68	0.07	10.74	6.4
American	853	19.5	16 609	27	3.0	4.8	7.5	0.49	0.06	0.54	2.0
Eastern Mediterranean	573	29.3	16 798	108	4.4	19.0	21.7	0.84	0.70	1.54	1.4
European	879	11.9	10 459	25	1.1	2.3	3.3	0.07	0.13	0.20	0.8
South-east Asian	1 564	24.4	38 139	83	6.6	44.6	45.5	0.68	0.66	1.34	1.6
Western Pacific	1 761	13.6	23 914	38	3.2	10.3	13.2	0.00	0.76	0.76	2.0
World	6 217	20.7	128 814	81	5.2	20.7	24.0	2.28	0.46	2.73	3.4

Table 1: WHO table showing the prevalence of sickle cell anemia among various regions of the world in 2003. Significant variant refers to sickle cell trait in which the individual carries the gene but does not physically show symptoms, and α thalassaemia refers to the other mild mutations that can take place. African regions have a higher percent of individuals who are carriers of the gene over any other region. There is a much higher affected popluation in African regions who have sickle cell anemia, 10.86 per 1,000. The number of births affected are also much higher in African regions.[25]

According to the Centers for Disease Control and Prevention (CDC) August 2016 records, there are approximately 100,000 Americans who have SCD. Out of 365 African-Americans, one will have the disease. Out of every 16,300 Hispanic-Americans births, one will be diagnosed with SCD. Sickle cell trait is carried in one out of 13 African-Americans.[26] This

increases the chances of carrier parents to have offspring with sickle cell disease. Women with sickle cell disease lived o be 42 years old in 2005. Men with sickle cell disease only lived to be about 38 years old.[27]

Complications

Cardiovascular. In regards to the complications that sickle cell disease bears, its affect on one's cardiovascular system is obviously one of the most prominent. Due to the fact that sickle cell anemia is literally a disease of red blood cells makes this very evident. Furthermore, VOC can result in acute or chronic pain, blood clotting, organ failure, organ death, and early mortality.[10] The termination of blood flow due to clotting (figure 6), the lack of oxygen transport, and cell-vessel adhesion interactions, all play a role in these VOCs.[10] There is evidence that supports xanthine oxidase is a reactive oxygen species that plays a significant role in individuals with sickle cell anemia.[28] An increase of xanthine oxidase is usually evident in the endothelium of the aorta, or is possibly released from the liver and travels through blood vessels. Further investigations need to be performed to come to a better understanding of the effects xanthine oxidase has on tissues of the body, especially because not all tissues are affected in the same way.[28]

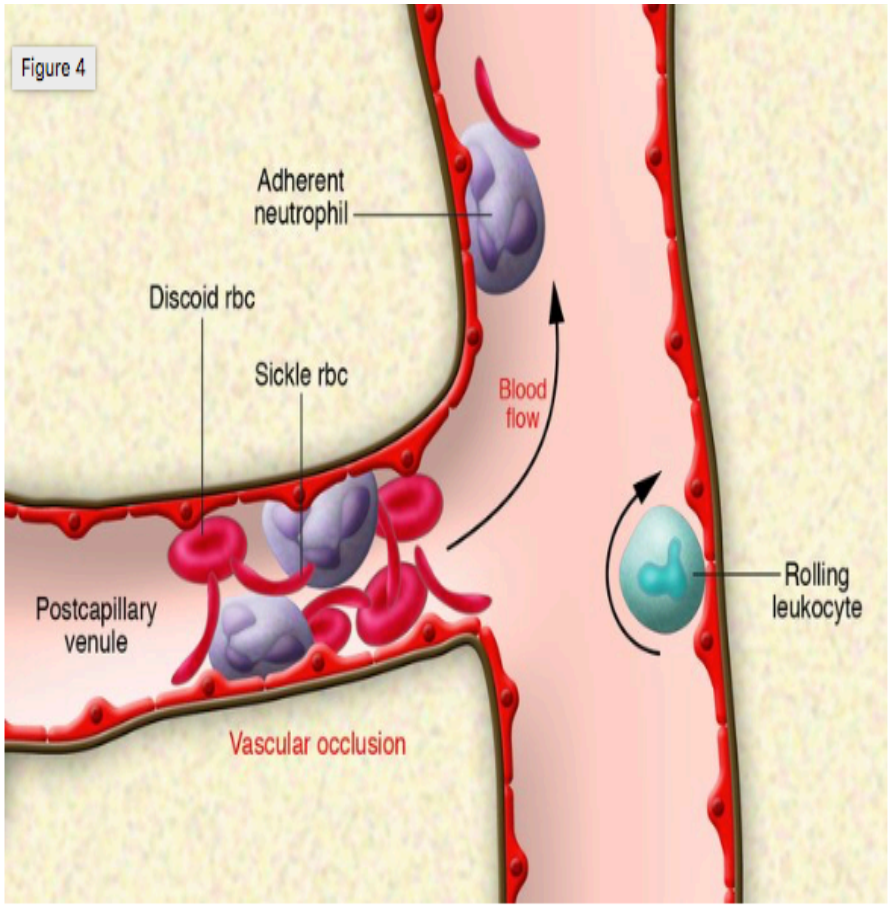

Figure 6: Blood clotting, vaso-occlusive crisis[10]

Neurological. Reactive oxygen species are reduced oxygen molecules with added electrons.[29] Typically, reactive oxygen species are regulated by anti-oxidant enzymes within individuals to ensure that one does not get harmed by inconsistencies. When these are not regulated properly, cells begin to dysfunction and die. Some of the molecules that have a role in producing reactive oxygen species include xanthine oxidase, NADPH oxidase, cyclo-oxygenase, hydroxyl radical, nitric oxide synthase (NOS), and lipoxygenase (examples shown in figure 7).[28, 29] Nitric oxide synthase is the enzyme that catalyzes the 2-step production of nitric oxide from a L-arginine to L-citrulline reaction.[29] Nitric oxide synthase is a reactive oxygen species that has an affect on individuals with sickle cell anemia. Normally, NOS produces small amounts of nitric oxide, which is used in neurons for the cascade that leads to neural transmission and relaxation of vessels, which inhibits aggregation of platelets.[30] Nitric oxide has several pathological effects that are toxic to neurons, but are not fully understood.[31, 32] Among some of the toxic effects are energy depletion and neuron death, both of which are not fully detailed or explained.[33] More research is desired specifically in neural complications of sickle cell anemia because not too much information is known about the details of these kinds of complications.

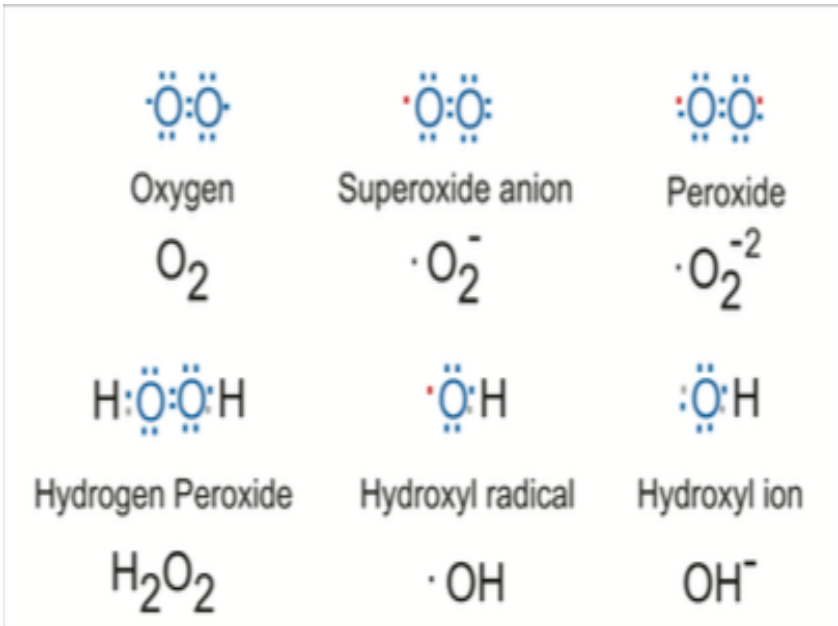

Figure 7: Examples of some reactive oxygen species[29]

CHAPTER TWO: CURRENT TREATMENTS

Introduction

There is currently no cure for sickle cell anemia because there is still more to learn about the disease. Currently, only treatments are offered to ease the complications of sickle cell anemia. Animal studies have been conducted to determine the role of reactive oxygen species, endothelium adhesion, and the role of fetal hemoglobin. To date, hydroxyurea therapy is the treatment most used and accepted for individuals with sickle cell anemia.[28] And still, research is being done on treatments that can improve the quality of life for those with the disease, as well as, treatments that ease the complications without leading to other unwanted affects.

Animal Studies

There is evidence that reactive oxygen species play a role in individuals with sickle cell anemia. When comparing anti-oxidant levels in normal red blood cell individuals and sickle blood cell individuals, there is a notable difference that can be investigated.[28] A study was done by Kaul et al in 2004 on transgenic mice with sickle cell anemia looking at and measuring the reactive oxygen species in mice with induced hypoxic crises.[34] Both xanthine oxidase and nitrotyrosine deposition are reactive oxygen species known to increase in mice with sickle cell anemia.[34] Inflammation responses in the mice were determined to be induced by nuclear factor-κB (NF-κB).[34, 35] NF-κB is a transcription factor that increases the transcription of endothelial cell adhesion molecules, which are molecules that are pro-inflammatory.[35] The activation of this particular transcription factor plays a role in increased neutrophil recruitment, which was seen in figure 5 to lead to vaso-occlusive crisis.[36, 10] Kaul et al. specifically studied sulfasalazine and its ability to treat the vaso-occlusive crisis in the transgenic sickle cell disease mice.[34] Sulfasalazine is an inhibitory agent that inactivates NF-κB in rheumatoid arthritis and inflammatory bowl disease.[37, 38] With this in mind for their study, Kaul et al. determined that sulfasalazine did decrease neutrophil recruitment and adhesion, thus allowing for better vascular blood flow; ultimately, being an effective anti-inflammatory drug.[34] By increasing reactive oxygen species inhibitors or anti-oxidant enzymes, artery dilations tend to be reduced and the leucocyte count decreases as well.[28] This suggests that reversing ROS is possible and can reduce vaso-occlusive crises.

Anemic baboons were used to test out the effects of 5-Azacytidine on fetal hemoglobin production.[39] 5-Azacytidine is a cytosine analog that cannot be methylated by a methyltransferase.[39] This molecule was used to inhibit the methylation of DNA that encodes for β-globin. Since 5-Azacytidine inhibited this methylation, a higher number of fetal hemoglobin was expressed in the baboons than adult hemoglobin expressed.[39] This was used as a push towards getting 5-Azacytidine tested on humans,[39] however, it was not studied further due to the fact it was thought to be carcinogenic.[10] Studies were then done on hydroxyurea therapy because it does not interfere with the methyltransferase of DNA.[28, 39] This encouraged small hydroxyurea trials on humans. Unlike 5-Azacytidine, hydroxyurea became tested on larger sample sizes to get more understanding of its effect on sickle cell anemic organisms.[10] Because of these studies, hydroxyurea therapy has become the most utilized therapy for SCD patients.

Hydroxyurea Therapy

Hydoxyurea is a drug that has been approved by the US Food and Drug Administration that can be used as a therapy for sickle cell anemia.[28] This drug therapy can effectively reduce sickle cell anemia symptoms such as vaso-occlusion, pulmonary events, and hospital visits, to name a few.[10, 28, 39, 40] The function of hydroxyurea therapy can be visualized in figure 8.[41] One of the mechanisms of HU induces the fetal

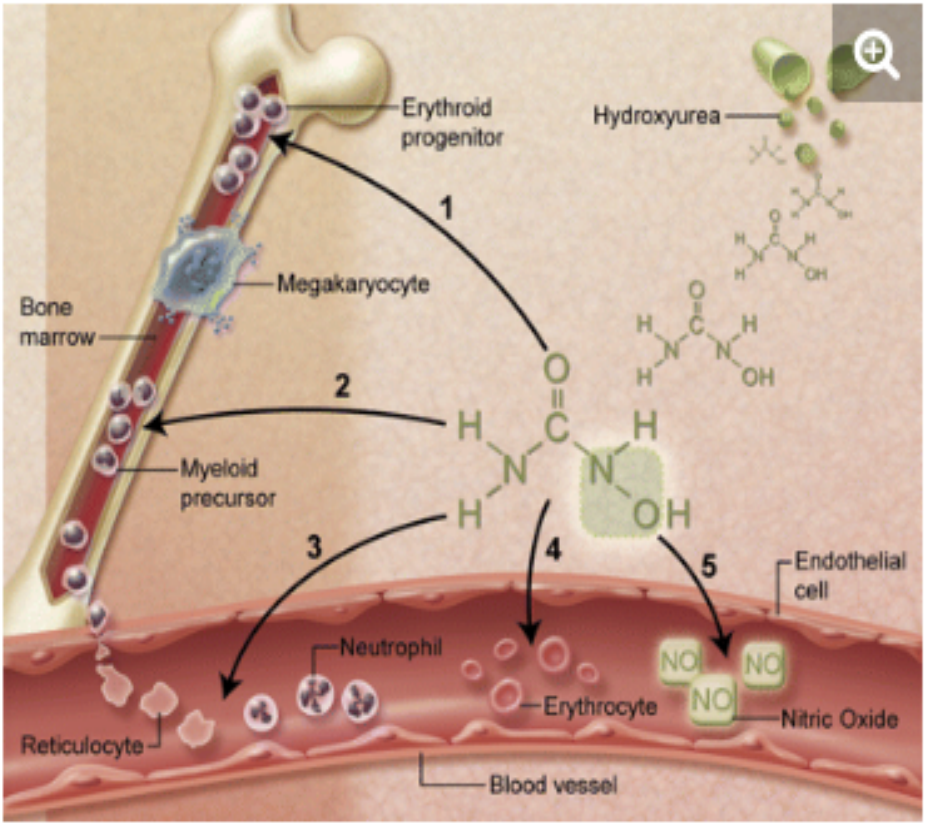

Figure 8: What hydroxyurea affects[41] although the mechanism is not known

hemoglobin (HbF) synthesis in SCD patients. This synthesis impairs the production of sickle cell formation, thus extensively reducing the VOC complications.[41] It was in 1984 when Letvin et al. studied anemic monkeys and HU therapy, determining the increased production of HgF.[42] Several VOCs are treated when patients undergo HU therapy. This therapy does not actually get rid of sickle cell anemia entirely, it just helps reduce the complications that come with the disease.[10] In regards to humans, symptoms like acute chest pain, hospitalizations, painful crises, and blood transfusions greatly declined when patients were on hydroxyurea therapy. [43] However, it is important to note that the exact mechanism HU therapy uses is unknown. Patients who have gone through HU therapy have experienced several unwanted effects. Some of those unwanted effects include, but are not limited to, body aches, flu-like symptoms, easy bruising, unusual bleeding, painful or difficult urination, confusion, hallucinations, loss of appetite, nausea, and jaundice.[44]

Other Therapies

It has been proposed to induce fetal hemoglobin in individuals with sickle cell anemia to alleviate the severity of the disease. There have been studies and observations that lead to this therapeutic proposal. Individuals with sickle cell anemia in India and Saudi Arabia have high amounts of fetal hemoglobin and much milder sickling. Also, fetal hemoglobin seems to prevent sickle hemoglobin from polymerizing and causing red blood cells to sickle. Therefore, induction of fetal hemoglobin may be a useful therapy to look further into.[10] Prior to simply inducing fetal hemoglobin, it was suggested to use 5-Azacytidine because in a small number of sickle cell anemic patients it promoted fetal hemoglobin. Yet, bigger sample sizes were not sought after to continue testing out this method of producing fetal hemoglobin because it was a potential carcinogen.[10]

CHAPTER THREE: ALDOLASE, POTENTIAL TREATMENT

Introduction

Currently hydroxyurea therapy is the most used and accepted treatment for individuals with sickle cell anemia.[28] This therapy is used because it helps to reduce the complications that arise with sickle cell anemia, such as blood clotting, organ failure, and early mortality.[10, 28, 39, 40] Individuals with sickle cell anemia who undergo hydroxyurea therapy have less chest pain, hospital visits, blood transfusions, and painful vascular cirses.[41] Sickle cell formation is inhibited due to increased production of fetal hemoglobin.[41] Although hydroxyurea is used for treatment, it is not a means of curing sickle cell disease.[10] The mechanism by which hydroxyurea functions is not known and it has several unwanted affects that it is accompanied with, such as jaundice, confusion, loss of appetite, nausea, hallucinations, and unusual bleeding just to name a few.[43] After doing some research, aldolase has been found in higher amounts in individuals with sickle cell anemia who undergo hydroxyurea therapy.[45] Due to this finding, the proposal to increase aldolase by another means is presented. Increasing aldolase without the use of hydroxyurea therapy can both improve the quality of life for individuals with sickle cell anemia, as well as, rid the unwanted affects that accompany the treatment.

Background

Fructose-bisphosphate (FBP) aldolase is a glycosomal enzyme that plays a major role in glycolysis. This enzyme can be found in three isozymic forms, defined as various forms with the same function, depending on the location of where it is found. Those three forms are aldolase A, aldolase B, and aldolase

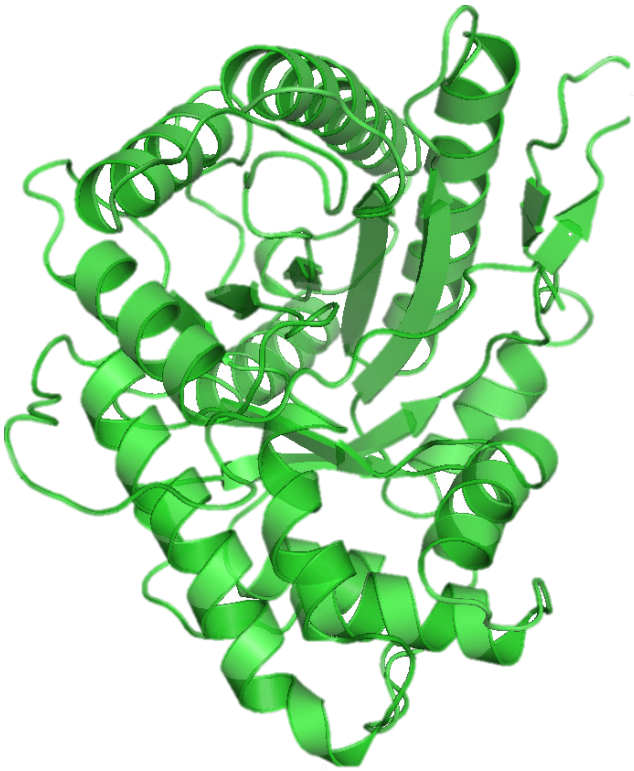

Figure 9: 2-dimensional structure of Fructose-bisphosphate aldolase[49]

C. Aldolase A is most commonly found in muscle cells. Aldolase B is most commonly found in liver cells. Lastly, aldolase C is most commonly found in the brain.[46, 47] Studies have shown that the structure of aldolase is an α/β-barrel fold.[48] The structure of aldolase is shown in figure 9.[49] The glycolytic function synthesizes ATP, which is the energy that can be used for more biological processes.[50] Aldolase A is defined based on its abundance in muscle tissues and its high catalytic activity. Aldolase B is defined based on the properties it has in the liver tissue and its low catalytic activity.[51] Because aldolase is found in the cytoplasm of cells and it is soluble, then it can be extracted by a water solution and isolated for further study.[52]

Studies have shown that there are increases in aldolase levels in patients who have undergone HU therapy.[45] These studies form the basis of my senior capstone proposal. From these studies I have formed my capstone on studying a way to increase aldolase by another means, and replace HU therapy so the patients no longer experience the unwanted effects of the treatment.

Research Strategy

Specific Aim 1: To investigate the role aldolase plays in treatment of SCD in patients who undergo HU.

Rationale: Studies have shown that aldolase levels increase in patients who have undergone HU therapy.[45]

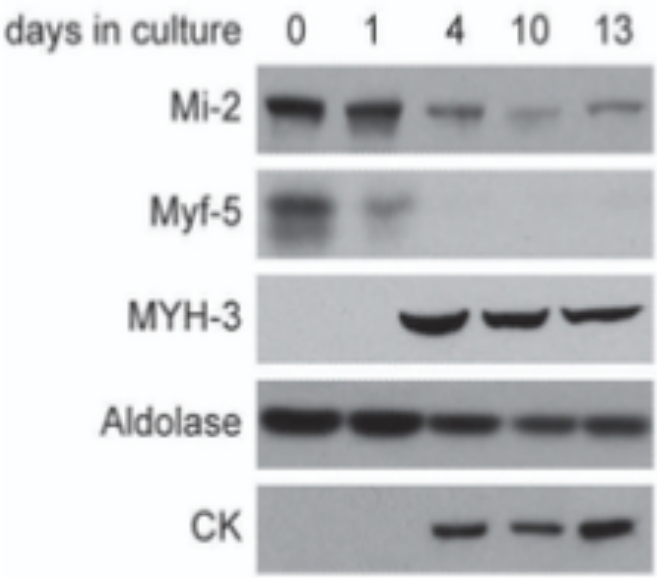

Figure 10: Example of electrophoresis[50]

Approach 1: The initial step is to measure the levels of aldolase in test subjects to confirm the increase of aldolase in my test subjects. My control group will consist of 20 transgenic mice expressing human α and β^s globins that do not undergo HU therapy. The test group will be 20 adult transgenic mice expressing human α and β^s globins that undergo HU

therapy. Blood samples will be taken and electrophoresis will be done to check the aldolase levels in each mouse. Figure 10 is an example of electrophoresis done to measure the enzyme aldolase.[50]

Proposed Results: I expect to see higher levels of aldolase in the test group of transgenic mice expressing human α and β^s globins that undergo HU therapy.

Conclusion: Aldolase is present in higher amounts in SCD mice, which may indicate its importance in SCD treatment.

Approach II: The next part of the approach to investigating specific aim 1 is to knock down aldolase in mice that undergo HU therapy by transfection of cells with siRNA. Transfection means to infect with a free nucleic acid. siRNA is considered the silencing ribose nucleic acid. Through this method aldolase is knocked down in the transgenic mouse.[53] My control group will consist of 20 transgenic mice expressing human α and β^s globins that do not do not have aldolase blocked. The test group will be 20 adult transgenic mice expressing human α and β^s globins that do have aldolase blocked. Blood samples will be taken and electrophoresis will be done to check the aldolase levels in each mouse. Sickle cell disease complications will be measured and compared. The way to measure these complications is to note the complications that each mouse encounters. There is a successful measurement when complications have been reduced.

Proposed Results: I expect to see a greater reduction in sickle cell disease complications in the control group of transgenic mice expressing human α and β^s globins that do not have aldolase blocked.

Conclusion: SCD complications will significantly decrease in the transgenic mice expressing human α and β^s globins that do not have the aldolase blocked. This will reinforce that aldolase does indeed play a key role in the treatment of sickle cell disease.

Specific Aim 2: To investigate an alternative method for increasing aldolase without using hydroxyurea to eliminate unwanted effects.

Rationale: HU therapy has unwanted effects for patients.[44]

Approach I: Measure the improvement of SCD complications by increasing aldolase through the means of myoblasts. A study was done revealing that aldolase levels increased due to myoblasts. Myoblasts are muscle cells. This can be done by getting the mice to exercise thereby encouraging the formation of myoblast and increasing aldolase levels. My control group will consist of 20 transgenic mice expressing human α and β^s globins that do not do not undergo the myoblast treatment. The treatment group will be 20 adult transgenic mice expressing human α and β^s globins that do undergo the myoblast. Blood samples will be taken and electrophoresis will be done to confirm the increased aldolase levels in each mouse. Sickle cell disease complications will be measured and compared. The way to measure these complications is to note the complications that each mouse encounters. There is a successful measurement when complications have been reduced.

Proposed Results: The treatment group with 20 transgenic mice expressing human α and β^s globins that do undergo the myoblast treatment will have increased levels of aldolase and show significant reduction of sickle cell disease complications.

Conclusion: Increasing aldolase by myoblast treatment will reduce sickle cell disease complications and eliminate the need for HU therapy and unwanted effects.

Approach II: The second part of the approach to investigating specific aim 2 is to measure the improvement of sickle cell disease complications by increasing aldolase through the means of higher fructose consumption. A study was done showing that rats have increased levels of aldolase when consuming higher amounts of fructose.[54] Figure 11 shows the results of higher aldolase levels in rats that consumed higher amounts of fructose.[54] My control group will consist of 20 transgenic mice expressing human α and β^s globins that consume the normal chow. The test group will be 20 adult transgenic mice expressing human α and β^s globins that consume

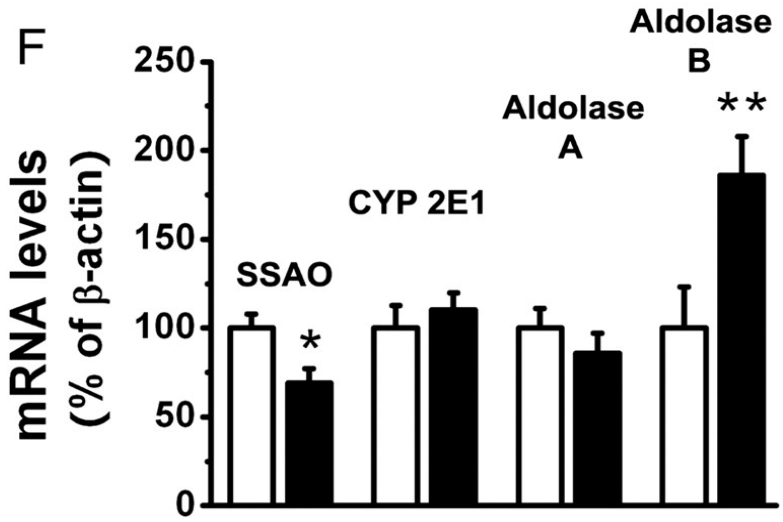

Figure 11: Results indicating higher aldolase levels in rats that consumed higher amounts of fructose[54]

chow with 60% fructose. Blood samples will be taken and electrophoresis will be done to check the aldolase levels in each mouse. Sickle cell disease complications will be measured and compared. The way to measure these complications is to note the complications that each mouse encounters. There is a successful measurement when complications have been reduced.

Proposed Results: The treatment group with 20 transgenic mice expressing human α and β^s globins that do consume more fructose will have increased levels of aldolase and show significant reduction of sickle cell disease complications.

Conclusion: Increasing aldolase by higher fructose consumption will reduce sickle cell disease complications and eliminate the need for HU therapy and unwanted effects.

Study Limitations

Some limitations of the study is that, even though we know aldolase increases in patients who undergo HU therapy, we are not guaranteed that aldolase is specifically the mechanism that HU therapy works by. Also, we do not know the other unwanted effects that may come with the proposed alternative methods that will increase aldolase. It is uncertain what the myoblast treatment or the higher fructose consumption may do to the patient. Furthermore, because we do not know the acute effects that may come along with these methods, we also do not know their long-term effects either.

Significance of Study

The significance of this particular study I am proposing is finding the mechanism that HU therapy uses to treat sickle cell disease. If this proposed study and expected results do indeed indicate that increased levels of aldolase is the key in treatment of the disease in mice models, then we are able to move to the next step of testing. Human patients can be recruited to attempt these treatments and see the effectiveness they have on sickle cell disease complications. This study will have eliminated the unwanted effects of HU therapy and helped improve patients lives.

Future Studies

A future study can be done to investigate the limitations mentioned above. In the future, the effects of the myoblasts treatment and high fructose consumption treatments can be studied and monitored. In addition to investigating the effects, a study can be done looking into a variety of dosages of the treatments. For example, instead of 60% fructose studying 80% or 50%.

DISCUSSION

As time and technology progress more becomes known about sickle cell anemia and the various factors that are involved in the disease. After the initial discovery of the disease in the United States in the 1900 by Dr. Herrick, much has been discovered about the effects of sickle cell anemia.[3] Not too long after its discovery, Dr. Vernon found the amino acid that caused the abnormality in the β-globin gene, which leads to the sickling of a red blood cell.[3] Along the years research has been done, and continues to be done, searching for a therapy or treatment for this cardiovascular disease. Symptoms such as, organ dysfunction, hospitalization, vaso-occlusion, and some others are the crisis that patients with sickle cell disease must deal with.[10] Fortunately, with the information that has been gained over the years about how sickling happens and how anemia affects the body, treatments and therapies are being tested. Hydroxyurea is among the most accepted therapies currently available with significant lessening of the complications and symptoms sickle cell anemia patients' have.[10, 28] Further studies and investigations are being done on other procedures and drugs that can possibly help alleviate the difficulties of this hereditary disease.[3]

There is still so much to learn and investigate in regards to sickle cell anemia. Most of all, beginning to view the organic chemistry behind the disease will help know how to directly interact with molecules in a way to provide treatment. Reading through the information that is already present and understanding the way that molecular interactions take place, or even being able to see how organic chemistry plays a role in sickle cell anemia can provide a unique perspective on further studies and therapies. Mutations, molecular interactions, oxygen-based reductions, electrons, and so much more, allow for treatments and therapies to be specifically aimed at providing health and comfort even with sickle cell anemia. Treatments and therapies are already present, but still can be further investigated. Hydroxyurea is currently a favored therapy with unwanted and unhealthy affects, but that is not to negate that there may be other options or forms of treatment that might just be better. It simply is a matter of knowing what to look for, knowing the molecular connections and interactions involved, addressing specific areas by taking note of the chemistry of the molecules involved, etc. For this reason, I researched and propose that Fructose-bisphosphate (FBP) aldolase is a better, less harming approach to treating sickle cell anemia. Studies have already shown the increase of aldolase levels in patients who undergo hydroxyurea therapy, but studies also show the unwanted affects that go alongside the

treatment.[44, 52] For example, jaundice, loss of appetite, body aches, and unusual bleeding, just to name a few, are some the complications that patients who undergo HU therapy must suffer through in order to alleviate VOC complications that come with SCD.[44] If an increase in aldolase is the mechanism by which the VOC symptoms are being relieved, then increasing aldolase by another means rather than HU therapy will help remove these unwanted affects of the therapy. Although aldolase cannot be a cure for SCD, based on my research, increasing aldolase can improve the overall health and condition of those who live with sickle cell anemia.

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