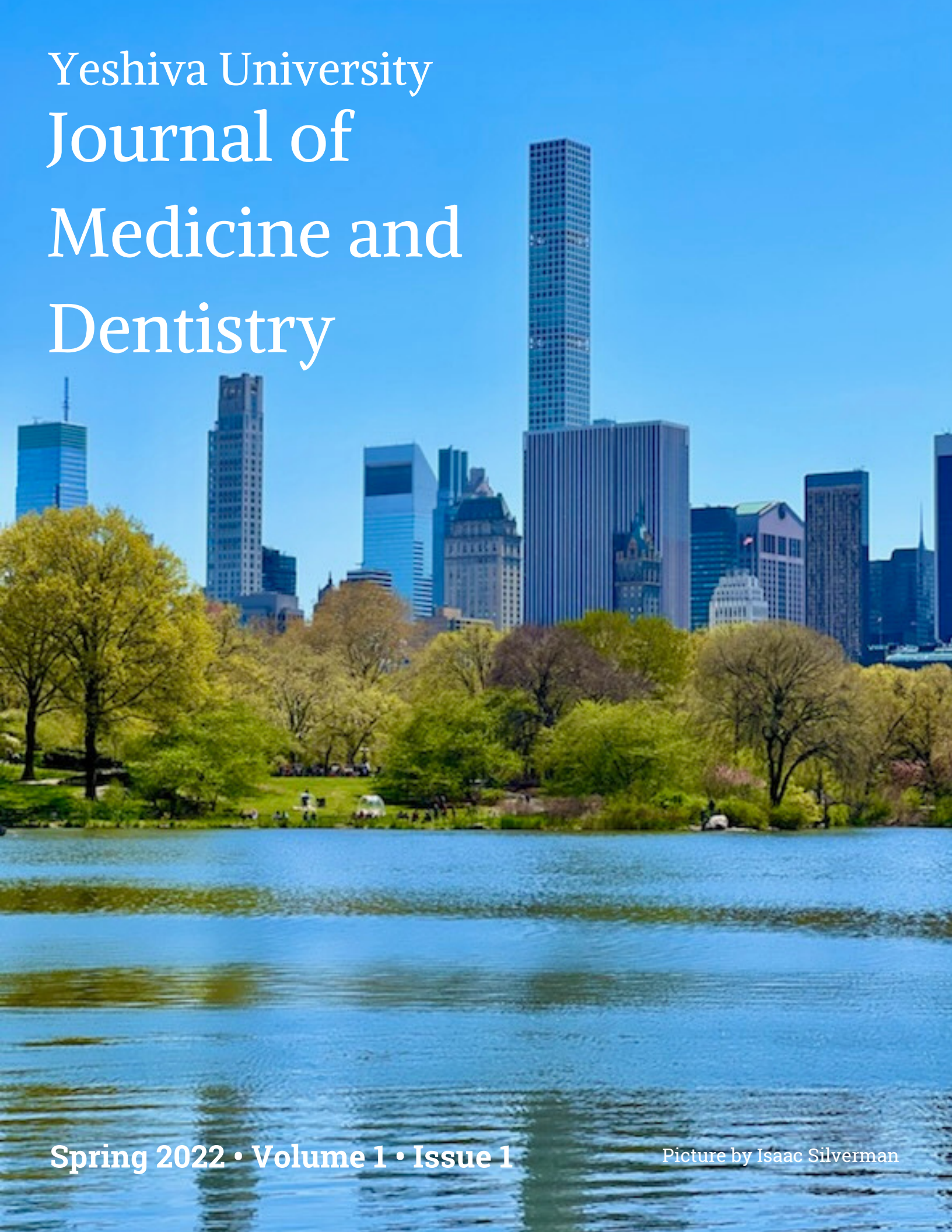
Yeshiva University

Journal of Medicine and Dentistry



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Picture by Isaac Silverman

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Mission Statement

The Yeshiva University Journal of Medicine and Dentistry was created for the student body and faculty of Yeshiva University as well as for the broader communities with which the university is associated and for the world at large. It is designed to serve as an outlet for all those interested in publishing work for a public audience. The editorial board consists of undergraduate students from Yeshiva College and Stern College for Women. Writers share analytical essays on new medical developments as well as research they have conducted. This journal aims to foster a greater interest in and appreciation for medical and dental sciences at the university and ultimately aid in Yeshiva University's mission to serve as a "wellspring of wisdom."

BS"D

Letter From the Editors

Dear Reader,

The Yeshiva University Journal of Medicine and Dentistry presents its first issue written by students from Wilf and Beren campuses. College students write essays, literature reviews, and research papers about topics assigned in class but seldom get the opportunity to submit writing created from their passions and interests. This journal was born when two students, one passionate about medicine and one about dentistry, had the same idea – to create a community of writers interested in sharing their research. Together we created one journal, combining the two fields and uniting all healthcare-related topics.

In developing the first edition of this new publication, we needed to establish an efficient system that would lead to a successful launch. We created guidelines for writers, constructed a proper layout, designed a logo, found printing services, built an editorial board, etc. We faced challenges as we pioneered the project, but we learned to overcome them as a team. With the collective effort of the Yeshiva University community, including members of other school publications, writing center staff, biology department staff, and others, we were able to achieve our goal.

The process of recruiting writers demonstrated just how much interest there is for such a scientific journal. Within two days of sending out an interest form, we reached the threshold to open a university-funded club. We are now the first group of writers, editors, managers, and editors in chief of the Yeshiva University Journal of Medicine and Dentistry. Having the right team was a crucial part of the success of publishing this journal.

Our greatest supporter has been Dr. Maitra, an Associate professor of biology at Yeshiva University and Senior Scientist at the Montefiore Medical Center. We are incredibly grateful for Dr. Maitra's assistance with this project. She was essential in guiding us through the editing process and creating a successful publication. Additionally, she kindly reviewed all submitted articles to ensure their accuracy. As a result, we have built a strong foundation with her in this issue, and we hope it is the first of many more to come.

Onward and upward.

Sincerely,

Isaac Silverman and Naomi Fried
Editors-in-Chief
YU Journal of Medicine and Dentistry 2022-2023

Isaac Silverman Naomi Fried

Yeshiva University Journal of Medicine and Dentistry

Review

3D Printed Surgical Guides

By: Jason Frankel

Abstract

There are several methods of restoring missing teeth. The most ideal restorative method is a dental implant. Some surgeons prefer to use dental surgical guides to perform more efficient surgeries when placing these implants. 3D printed guides produce similar accuracy to other guides at a reduced cost. Additionally, consumer-level 3D printers provide similar accuracy as high-end 3D printers, making accurate guides for implant placement both cheap and accessible.

Introduction

Tooth decay is prevalent at some point in most people's lives.[1] When sugars and starches aren't cleaned from the teeth, bacteria begin to feed on them and form plaque. Plaque contains acids that remove some minerals from the tooth's enamel (the outer layer of the tooth), causing cavities. A simple dental filling may restore a cavity.[2] Once holes are formed in the enamel, the acids attack the dentin (the middle layer of the tooth), which contains tubes that connect to the blood vessels and nerves in the pulp, providing sensitivity to the tooth.[3] If the damage reaches the pulp (the inner layer of the tooth), the method of restoration may be a root canal, in which the endodontist removes the affected pulp, including the nerves and blood vessels, fills and seals the space, and usually places a restorative crown on top.[4] If there is substantial damage beyond the root end of the tooth, a root canal may not be sufficient to correct the damage. In that situation, an apicoectomy may be done for the patient as well.[5] During an apicoectomy, the surgeon cuts a flap in the gums and drills into the bone until they reach the root of the tooth. At that point, they repair the damage from the apex (root side) of the tooth without touching the crown and then suture the flap closed.

If an entire tooth is missing, a fixed partial denture (bridge) may fill the gap between teeth.[6] Bridges are made by shaving down the adjacent teeth. Crowns made of porcelain are then put onto these teeth, with a connected crown taking the place of the missing tooth. However, if the tooth to which the bridge is attached decays, it may

become unstable and nonfunctional as the foundation for the bridge.[7] In this case, the patient may now end up with additional damaged teeth. An alternative, more ideal restorative method to fill the gap between teeth may be dental implants, which are sometimes placed using surgical guides. New technological innovations, such as 3D printed guides, provide accurately placed implants at a reduced cost.

Use and Placement of Dental Implants

A dental implant is a piece of titanium, surgically placed below the gum line that acts as a substitute for the root of a tooth. An abutment is then attached to the top of the implant, and the restorative dentist builds a crown placed upon the abutment.[8] Dental implants rely on osseointegration, the direct contact between the titanium implant and the surrounding bone.[9] The osseointegration gives them similar strength as natural teeth in the bone.[10]

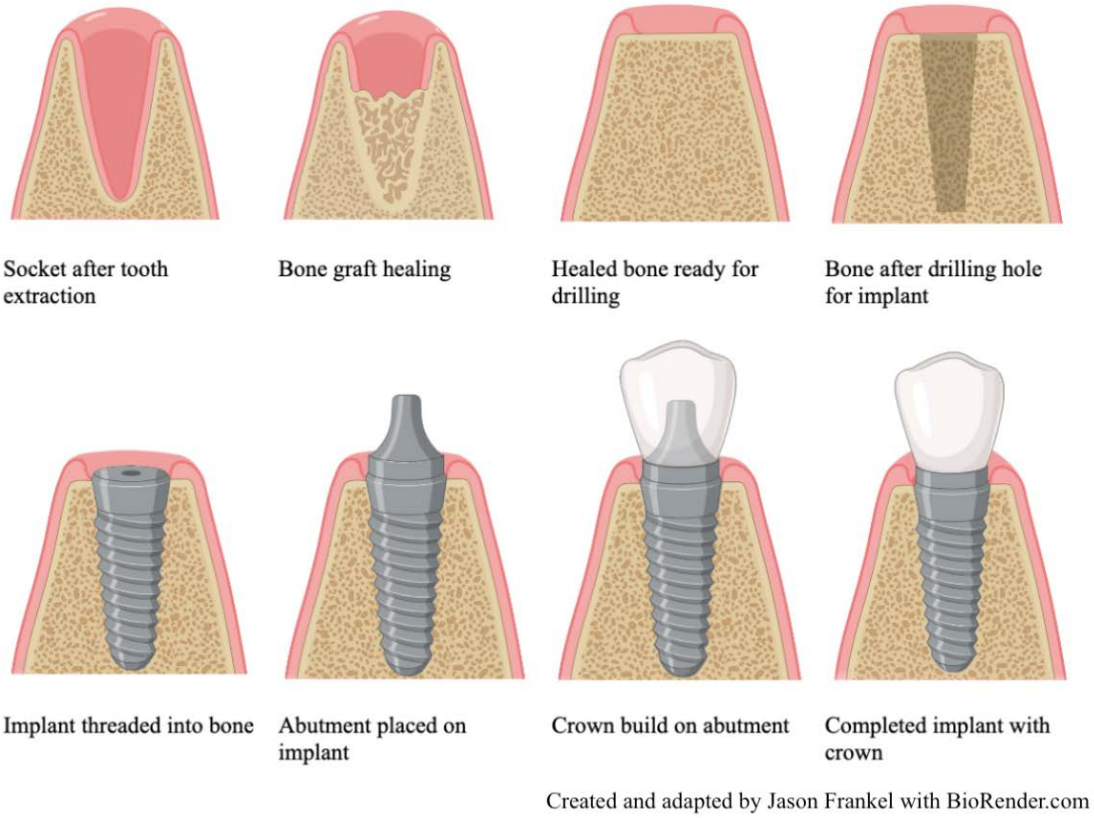


Figure 1

Dental implants are frequently done in multiple steps to ensure the most vital foundation for a new "tooth" (Figure 1). The first step is the extraction of the damaged tooth. If the implant cannot be placed right away,[11] the bone would shrink, resulting in significant bone loss. Too much bone loss in the implant's intended site may mean there is not enough bone for the implant to be placed correctly. In this case, the surgeon will rebuild the extraction socket with a bone graft and allow the bone to heal.[12] The surgeon will also take a CBCT (Cone-Beam Computed Tomography) scan to show the amount of bone available in the jaw where the implant will be placed. It also shows the adjacent anatomy to prevent damage to vital structures.[13] After the first waiting period, the patient returns for a second surgery. In this procedure, the surgeon may take an updated CBCT scan, and then will cut a flap in the gums, drill a hole into the bone, thread a titanium implant tightly into place, and finally suture the flaps to reseal them. Alternatively, surgeons can place the implant with flapless surgery, in which the drill goes straight through the soft tissue and into the bone for the implant to be threaded.[14] If there is not enough bone in the posterior upper jaw in the maxillary sinus region, a sinus augmentation may be performed, which adds bone to the base of the sinus.[15] After the second surgery, the bone is allowed to heal, and then the patient is sent back to the restorative dentist.

Dental Surgical Guides

Many surgeons prefer surgical guides (Figure 2) for these operations, while others prefer to free-hand. Dental implants have been proven highly effective, with low failure percentages. A surgical guide is a tool that is made to fit perfectly over the patient's targeted implant site to assist surgeons in the accurate placement of dental implants. The guides are designed for a metal sleeve to fit in the holes, enabling the implant to be placed at the right angle, depth, and direction into the bone.

Dental surgical guide, courtesy of BioHorizons

Figure 2

A surgical guide is made by first taking an impression or scanning the patient's desired implant site. The surgeon can then use a computer-guided implant planning system to map out the exact location of the implant in the bone. Guides can be made to either fit over the soft tissue of the implant site (for a flapless surgery) or directly over the bone (for a flapped surgery). In either case, the guides are made to fit adequately into their respective positions and allow for precision when assisting the surgeon in placing the implant.[16]

A study comparing the results from free-hand and surgical guided surgeries determined a difference between these methods. They found that free-hand surgery had a 6.42% failure rate, while guided surgeries had a 2.25% failure rate, emphasizing the effectiveness of using a surgical guide.[17]

In addition to the low failure rates, surgeries using guides have been proven to be highly accurate. Another study was done in which patients were split into three test groups. One group had a guided surgery done with a flap approach, the second had a guided surgery done with a flapless approach, and the third had their surgery also done with a flap approach, but entirely free-hand. For all three groups, their surgeries were planned based on preoperative CBCT images and allowed for pre-planning of how the surgery would take place. Then, after each patient had their surgeries done respective to their group, they had a postoperative CBCT. The surgeons then used an Osstell Mentor, a device that vibrates and calculates the frequency,[18] to determine the implant's stability[19] and found that the free-handed group was not significantly different from the guided surgeries. The preoperative and postoperative CBCTs were then compared to determine the accuracy of implant placements based on the methods used for the different groups. They also compared the distance from the planned entry point and the insertion angle into the entry point from the planned implant placement based on the preoperative CBCT and the postoperative CBCT. The results showed that the deviation

"They found that free-hand surgery had a 6.42% failure rate, while guided surgeries had a 2.25% failure rate, emphasizing the effectiveness of using a surgical guide."

of the position of the implants was more prominent in the guided flapped surgery than in the guided flapless surgery. However, both guided surgeries didn't have nearly as much deviation as the free-handed surgeries for distance from the projected entrance point and the entrance angle.[20] Although implants may fail for several reasons regardless of the placement method,[21] [22] research reveals that guided implant surgeries have proven more effective and accurate than free-hand surgeries.

Novice surgeons generally have less accurate results than experienced surgeons. However, a study found that the deviation between novice and experienced surgeons is much lower when using surgical guides than in free-handing surgeries.[23] This study further indicates the accuracy of surgical guides.

Additionally, aside from the extreme accuracy and effectiveness of using surgical guides, other benefits include reducing trauma, time of the procedure, swelling, and pain, resulting in a quicker recovery period for the patient.[16]

Potential Problem of Placing Implants with Guides

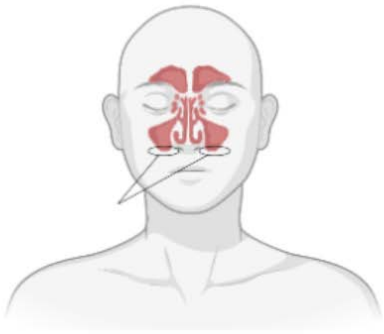
While there are many benefits of using surgical guides in assisting surgeons in placing implants, there are still some significant problems with the method. Generally, when placing dental implants, the dental assistant will irrigate the implant site as the surgeon drills into the bone. Irrigation is performed to reduce the temperature and avoid thermal osteonecrosis (the death of bone tissue due to high temperatures),[24] which would inhibit osseointegration. That being a concern, a study determined that the use of surgical guides blocks the irrigation, making it more difficult to control the temperature of the implant site while drilling.[25] However, a different study, which utilized routed irrigation (customized channels to direct the water directly to the implant site), found that the temperature was able to be held consistently below 47ºC, preventing thermal osteonecrosis.[26]

Types of Dental Surgical Guides

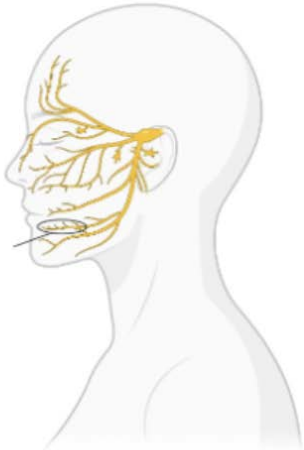
Surgical guides have thus far been proven to be the most effective and accurate route for placing dental implants. However, there are multiple types of surgical guides. Thermoforming is a traditional method used in the production of dental surgical guides.

The first step in producing these guides is by either taking an impression of the patient's teeth and casting it to form a model or taking an intraoral digital scan to print a model. A thermoplastic sheet is then heated and pressed over the model, sleeves are then placed in the guides to increase strength and ensure the implant is placed in the precise position.[27]

An alternative to thermoplastic surgical guides is 3D printed surgical guides. The first step in producing these guides is to take a CBCT scan to get a 3D image of the region.[28] In turn, the surgeon can visualize the amount of bone they are working with for the implant to be placed,[29] as well as the surrounding structures such as roots of adjacent teeth,[30] the maxillary sinus,[31] and the mandibular nerve[32] (Figure 3). Along with the CBCT, a surface scan of the teeth is also required to be converted into an STL file. The intraoral surface scan is created because some of the x-ray photons from the CBCT do not penetrate radiopaque restoration; instead, they reflect at the surface.[33] These two scans are brought together to provide a complete visual of the implant site. The surgeon then decides the length and width for the implant based on the available bone and digitally places it (in a guided implant surgery planning software) in the most optimal orientation and depth for the crown to be placed after healing.[34] After it is known where and how the implants should be placed, another STL (the guide itself) is designed to be offset from the model of the teeth (or bone, depending on the type of guide)[35] and provide holes for the sleeves[36] to place the implant in the correct position. The guide is then printed, sterilized, and ready for use (Figure 3).[37]



Base of the maxillary sinus



Part of the mandibular nerve, a division of the trigeminal nerve

Created and adapted by Jason Frankel with BioRender.com

Figure 3

3D printed surgical guides are cheaper and faster to produce than thermoplastic surgical guides. However, the most critical variable to compare the two methods is their accuracy. A study was done where some patients had implants placed with thermoplastic surgical guides while others had implants placed with 3D printed surgical guides. The results found that implants placed with 3D printed guides were more accurate in the angle of insertion and the measured distance from the planned placement of the implant than compared with those placed with thermoplastic guides.[38] The difference in cost and time of production, as well as the significantly greater accuracy of the 3D printed guides, makes them the far more efficient choice.

Surgical Guides Produced by High-End vs. Consumer-Level 3D Printers

It is inconvenient to send out the 3D-designed models and surgical guides to professional high-end printers, but it may not be necessary. A study conducted used surgical guides that were digitally designed using the same software. They then printed some guides using consumer-level 3D printers and others using a high-end professional 3D printer and compared the accuracy guides from their respective printers. The results showed that although the measured differences in accuracy between the printers were statistically significant, from a clinical point of view the deviations were negligible.[39]

Conclusion

Dental implants are the most efficient way to restore a missing tooth. Though some surgeons prefer to free-hand their surgeries, studies have proven implants placed with the help of surgical guides to be more accurate with lower failure rates. Some surgical guides can be costly, but 3D printed guides are a cheaper option with similar accuracy. 3D printers in a dental office can produce nearly as accurate guides as high-end printers, making cheap dental surgical guides easily accessible.

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Yeshiva University Journal of Medicine and Dentistry

Review

Correlation Between High Fat Diet, Cholestasis, Hepatocyte Apoptosis and the Development of Hepatic Carcinoma

By: Isaac Silverman

Abstract

Several prognoses have been determined for Hepatic Carcinoma (HCC). In this paper, I will explore the mechanism by which a high-fat diet (HFD) promotes the development of HCC. I first discuss the relationship between an HFD and the development of bile acid transport issues from the liver (cholestasis). As a result of cholestasis, an over-accumulation of hydrophobic bile acids is left surrounding the liver cells (hepatocytes). The bile acids create a cytotoxic environment for the hepatocytes. Damage done to hepatocyte mitochondria is specifically noteworthy as bile acids decrease levels of ATP as well as disrupt their physical structure. I have analyzed three pathways in which bile acids instigate apoptosis in hepatocytes, following intrinsic, extrinsic, and endoplasmic reticulum stress mechanisms. Finally, I have explored the correlation between frequent apoptosis and the development of HCC through DNA mutation in the Mcl-1 gene and other tumor suppressor genes.

Introduction

Researchers have identified Hepatic Carcinoma (HCC), the most common type of liver cancer, as the fifth most frequent cancer diagnosed worldwide with the third highest mortality rate among cancers.[1] The number of HCC cases and deaths observed have steadily increased over the past several years. In 2020, it was estimated that there were about 42,000 HCC cases and around 30,000 deaths.[2] Researchers have concluded that a correlation is present between excessive fatty tissue and gastrointestinal cancers, including HCC.[3] [4] There is a strong relationship between obesity and the development of HCC.[5] Linear regression models predict obesity

"Researchers have concluded that a correlation is present between excessive fatty tissue and gastrointestinal cancers, including HCC."

rates will increase by 33% by 2030, resulting in approximately 51% of the US population suffering from obesity.[6] Researchers have hypothesized a relationship between a high-fat diet (HFD) and gastrointestinal cancers.[7] Thus, understanding the mechanism through which HCC develops from an HFD and obesity is becoming more critical. Studies have been conducted correlating an HFD and liver damage, specifically regarding bile duct function.[8] This paper will discuss and analyze how an HFD develops bile duct dysfunction, also known as cholestasis. I have also examined how cholestasis instigates several mechanisms of excessive hepatocyte apoptosis, which promotes mutations in DNA, leading to tumorigenesis of HCC.

High Fat Diet Promotes Bile Acid Synthesis and Transport Dysfunction

First, it is important to establish that an HFD promotes bile acid synthesis and transport dysfunction, resulting in its overaccumulation in the liver. An experiment confirmed a significant correlation between an HFD and an increase in bile acid retention.[9] In the investigation, newborn C57BL/6J mice, an "inbred mouse strain" commonly used in anti-tumor research,[10] were injected with STZ (Streptozotocin), a chemical toxic to pancreatic beta cells which produce insulin.[11] The purpose of which was to develop diabetic symptoms in the mice. The mice were fed a regular diet for the first four weeks of life. At week four, a subsection of mice were introduced to an HFD. At week six, symptoms of fatty liver were observed with no signs of an inflammatory reaction. At week eight, "moderate inflammatory infiltrate" was present, including "neutrophils, lymphocytes and monocytes, and ballooning degeneration of hepatocytes," indicating an innate immune response against an HFD. At week twelve, chronic fibrosis was noted, indicating the pathology of nonalcoholic steatohepatitis (NASH), a fatty liver disease. At week twenty, all STZ-HFD mice developed HCC. Additionally, elevated levels of numerous bile acids were observed in hepatic cells of STZ-HFD mice beginning at week twelve and remained high through to week twenty (Figure 1).[9]

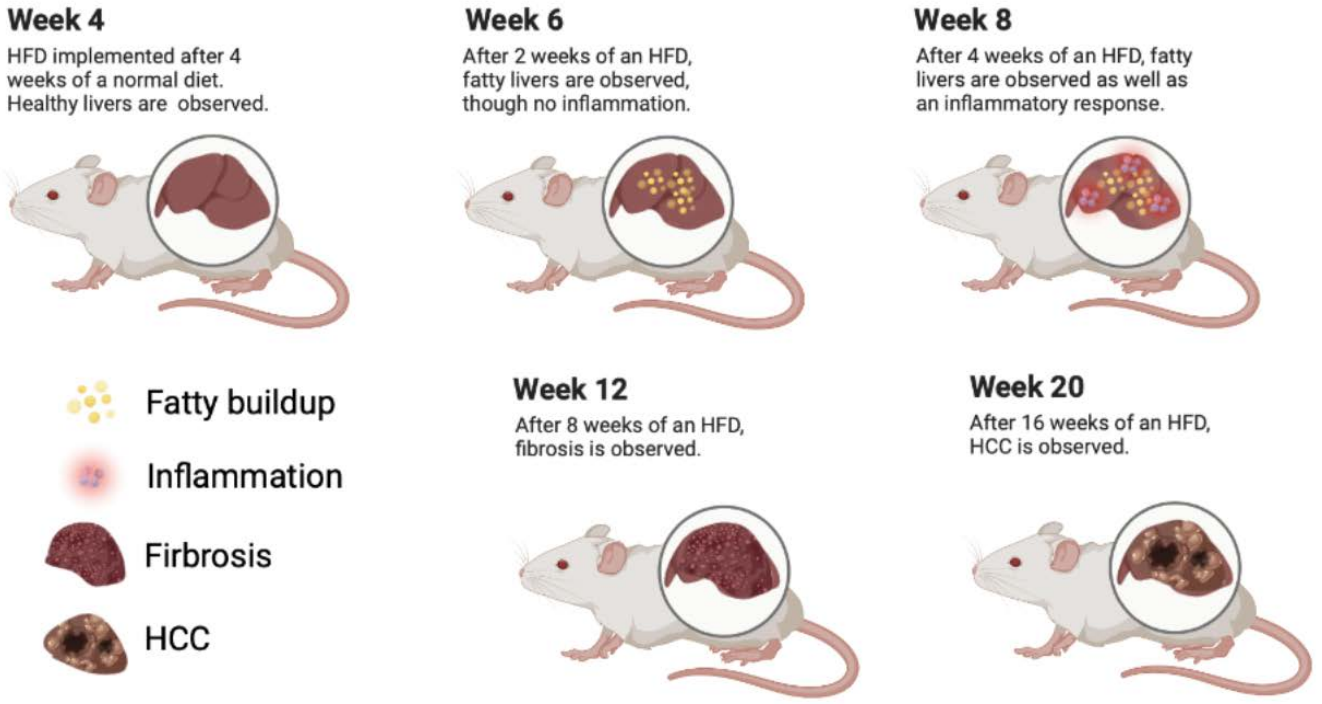


Figure 1 Adapted by Isaac Silverman from "Mouse Panels (Layout 3x2)", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

However, the International Agency for Research on Cancer had previously labeled STZ as a possible carcinogen, so these results prompted the independent studies of STZ and an HFD on the mice. This would allow firmer deduction if an HFD could be responsible for HCC. Indeed, HCC did result from an HFD alone. In fact, in all-male mice treated with STZ, HCC did not develop.[12] Thus, a relationship can be formed between an HFD and HCC. Furthermore, of the mice that developed HCC after being given an HFD, the plasma and liver bile acids such as TCA, GCA, and TCDCA showed the most "statistical significance" compared to untreated mice.[9]

In addition, raised TCDA was discovered to increase hepatocyte regeneration and decrease regulation of CEBPα, a tumor suppressor gene, indicating a carcinogenic correlation.[9]

Toxicity of Bile Acids

Hepatocytes are responsible for synthesizing bile acids directly from cholesterol by adding hydroxyl groups and oxidizing the molecules' side chain, making them less hydrophobic. The hydrophobicity of different bile acids depends on the number of hydroxyl groups added.[13] In addition, some of these hydrocycholestorols undergo further biotransformations, making them less hydrophobic.[14] The stronger the hydrophobicity of the bile acids that

accumulate in the hepatocytes, the more efficient it is in solubilizing membrane lipids[15] and having higher cytotoxicity.[16]

Numerous studies have concluded a correlation between an overabundance of bile acids and hepatocyte death. One study observed the cytotoxicity of the increased presence of the bile salts chenodeoxycholate (CDC), glycochenodeoxycholate (GCDC), and taurochenodeoxycholate (TCDC) (formed from their acid CDCA, GCDCA, and TCDCA, respectively) in rat hepatocytes. It was determined that the salt GCDC is the most toxic to hepatocytes. In an experiment, after four hours, approximately >10% of hepatocytes suspended in GCDC were viable, while approximately 40% of CDC and 30% of TCDC exposed cells were left viable. A control group of hepatocytes was also observed with approximately 70% viability. Another experiment proved that GCDC toxicity is dependent on its concentration.[17]

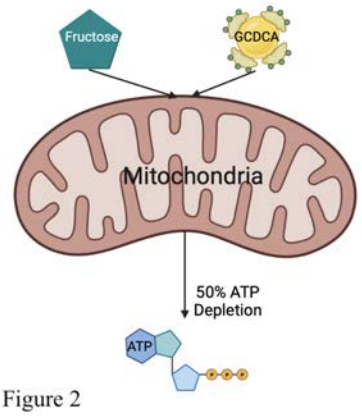
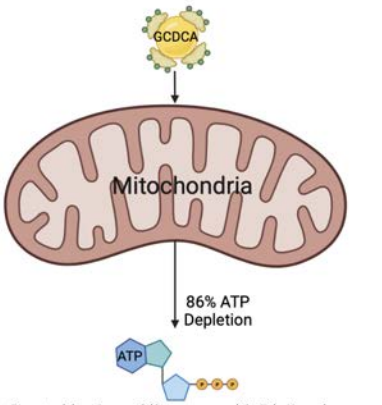


Figure 2



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Bile Acid Induced Hepatocyte Mitochondrial Damage

The previously mentioned study also demonstrated that GDCDA develops mitochondrial malfunction and is a mechanism of hepatocyte death. They observed that 86% of cellular ATP was depleted after only 30 minutes following the addition of GCDCA, compared to cells provided fructose and GCDCA, which only lost 50% of ATP. In the first experiment, the presence of ATP depletion without a "glycolytic substrate," such as fructose, indicated to researchers that an issue developed within the cell's mitochondria leading to their death via anoxia, an absence of oxygen.[17] (Figure 2)

Other studies have indicated similar abnormal mitochondrial behavior due to high bile acid concentrations, including "swelling, pleomorphism, and abnormal cristae."[18] Hydrophobic bile acids have been found to inhibit several enzymes involved in cellular

respiration, specifically in complexes I, III, and IV of the electron transport chain.[19] Additionally, hydrophobic bile acids are proven to disrupt the membrane potential of cristae[20] as well as serve as protonophores, resulting in an increased membrane solubility of H+ ions.[21] Researchers have hypothesized that non-parenchymal cell inflammation and fibrogenic responses may be attributed to such mitochondrial issues in parenchymal liver cells that emerge with cholestasis. Modified hepatocytes may emit immune signals such as cytokines, chemokines, and lipid peroxide products and signaling growth molecules, which would further an immune response. This would further lead to hepatic cell fibrogenesis, surrounding cell damage, and ultimately cell death.[22] In some cases of cholestasis, hepatocyte necrosis, as well as aforementioned mitochondrial impairments resulting in ATP depletion,[17] leading to oncolysis and cell death.

Methods of Bile Acid Induced Hepatocyte Apoptosis

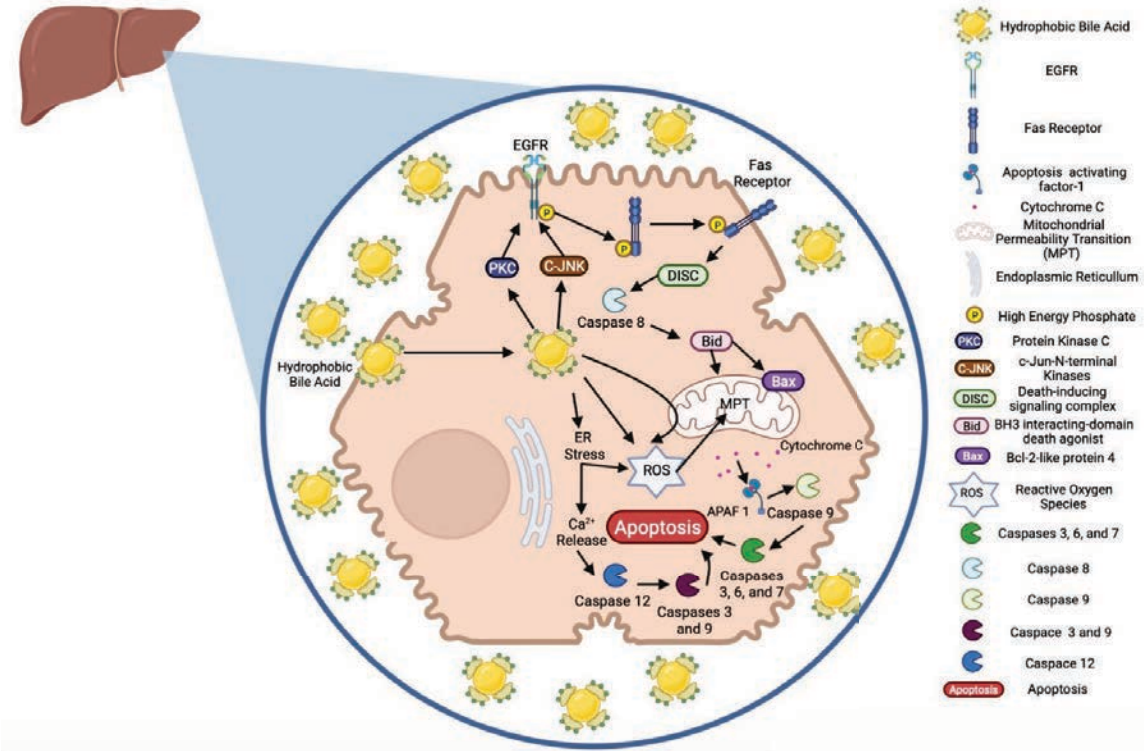

Figure 3

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Studies have indicated that hepatic apoptosis can result through several different protease cascades triggered by bile acids.[23] The first is an "intrinsic pathway," (Figure 3) which results from the mitochondrial release of apoptotic molecules due to bile-acid-induced oxidative stress.[24] Researchers discovered that "bile-acid-induced oxidative stress" may significantly impact the development of liver diseases.[25] Bile acids induce the creation of reactive oxygen species (ROS), which "oxidatively modify lipids, proteins, and nucleic acids" and ultimately end in hepatocyte apoptosis.[21] Additionally, researchers have proven that the bile acid GCDCA induces mitochondrial permeability transition (MPT).[26] Correlation has been made between MPT and ROS (Reactive Oxygen Species) generation and several other mitochondrial malfunctions, including decreased levels of oxidative phosphorylation,[27] leading to liver toxicity.[28] MPT results in the release of cytochrome c, a protein that initiates apoptosis when oxidized by ROS.[29] Cytochrome c stimulates the Bax protein (bcl-2-like protein 4) to move towards the mitochondria and release additional cytochrome c.[21] The Bax protein also interacts with IRE1α, a protein associated with creating endoplasmic reticulum stress,[30] by disrupting the homeostasis of its protein folding.[31] It also is responsible for upholding the activation of the protein-coding gene STAT3, which supports hepatocyte regeneration.[32] Furthermore, researchers have proven ROS is accountable for decreasing the number of antioxidant defenses in the cell. Among those are ubiquinone-9 and ubiquinone-10 which are antioxidants involved in the electron transport chain and prevent lipid peroxidation, the decreasing amount thus disrupts the metabolism and allows lipid peroxidation to occur.[21] Lipid peroxidation breaks down the mitochondrial membrane, which effects are previously stated, and the cell membrane.[33]

Another pathway of cholestasis-induced apoptosis is the pathway triggered by hydrophobic bile acids stressing the endoplasmic reticulum (Figure 3) by a mechanism previously mentioned. An excess amount of Ca2+ ions are released from the endoplasmic reticulum into the cytosol due to the presence of GCDCA.[17] The overabundance of Ca2+ ions stimulates an intracellular protease cascade of caspase enzymes (caspase 12 followed by caspases 3 and 9), leading to apoptosis.[34] Researchers have discovered that the presence of C/EBP homologous protein (CHOP), a

transcriptional regulator,[35] is an essential factor in hepatic cell death via cholestasis.[36] Furthermore, they observed a correlation between CHOP and the emergence of liver fibrosis due to hepatic cell damage caused by cholestasis.[36]

A third "extrinsic" pathway (Figure 3) of cholestatic derived apoptosis results from the activation of the death Fas receptor.[37] ROS instigated by hydrophobic bice acids has also proven in vitro to activate protein kinase c (PKC) and c-Jun-N-terminal kinases (JNK),[21] each of which manages cellular processes which promote tumor development.[38] [39] These molecules then stimulate the epidermal growth factor receptor (EGFR), which phosphorylates Fas and translocates it to the plasma membrane. The overabundance of Fas receptors on the membrane leaves the hepatocyte susceptible to apoptotic substrates.[37] In the event of Fas stimulation, a protease reaction is used as the apoptotic mechanism. Fas first forms a death-inducing signaling complex (DISC). As a result, caspase 8 is activated, which increases levels of cathepsin B[37], a cysteine protease, which several studies have found to be associated with tumor cell development and metastasis.[40] The activation of Caspase 8 separates and transports the pro-apoptotic Bid protein (BH3 interacting-domain death agonist) to the mitochondria, which opens the MPT pores. As previously discussed, MPT triggers the Bax protein as part of "intrinsic apoptosis." Additionally, in this pathway, as a result of MPT, the apoptotic molecule cytochrome c is released, which connects procaspase 9 with apoptosis activating factor-1(APAF-1) to activate caspase 9, which marks the point of no return in the hepatocyte death as it activates caspase 3.[41] In hepatocytes that are Fas deficient, researchers hypothesize that the death TRAIL-R2 receptor is involved with apoptosis, specifically with GDCDA.[42]

"Researchers have determined a correlation between increased levels of hepatocyte apoptosis and hepatocarcinogenesis."

Apoptosis Induced HCC

Researchers have determined a correlation between increased levels of hepatocyte apoptosis and hepatocarcinogenesis. In addition, they have discovered frequent hepatocyte regeneration with DNA damage in human common fragile sites, areas on chromosomes determined to have frequent

mutations, after large amounts of hepatocyte death.[43] Notably damaged genomic regions were FHIT, WWOX, and PARK2, all previously determined to serve as tumor suppressors.[44] These results indicated genetic dysfunction is present far before abnormal cell growth.[43]

Researchers also experimented with newborn mice mutated without the "anti-apoptotic Bcl2-family member myeloid cell leukemia 1 (Mcl-1) gene."[43] This instigated hepatocyte apoptosis and HCC tumorigenesis similar to the pathology caused by an HFD bile acid overaccumulation. Elevated levels of aspartate transaminase and alanine transaminase (AST and ALT), two liver enzymes associated with liver damage, were hypothesized to be present in these mice. Indeed, over one year of the experiment, Mcl-1 mutated mice (Mcl-1Δhep) demonstrated high ALT and AST. Over the year the ALT and AST levels decreased, being most statistically significant at 2 and 4 months when hepatocyte apoptosis and regeneration were the highest noted in mice that contracted HCC. Additionally, ALT levels in Mcl-1Δhep/HCC always remained higher than mice that had not developed HCC (Mcl-1Δhep/No HCC). In comparison, wild type mice always remained lower than all Mcl-1Δhep mice. 2-month-old mice also demonstrated a statistically significantly increased level of caspase 3 activations, indicating apoptosis, and DNA damage. Researchers determined that Mcl-1Δhep mice livers contained a significant number of genes enriched for HCC and hepatocyte apoptosis and regeneration.[43]

However, further experiments were conducted to determine if the loss of the Mcl-1 gene was responsible for the hepatocyte apoptosis and HCC or if tumor necrosis factor receptor 1 (TNFR1), a death receptor, was responsible. Crossbred Mcl-1Δhep and TNFR1 deficient mice were used to prevent "TNFR1-dependent apoptosis." At 2 months, Mcl-1Δhep/TNFR1 mice exhibited slightly lower ALT levels compared to Mcl-1Δhep mice. Additionally, both types of mice which developed HCC had significantly higher ALT levels compared to their non-HCC counterparts. Also, a larger amount of Fas receptors, activated in extrinsic apoptosis, were present in Mcl-1Δhep mice. Significantly higher levels of caspase 8 activation were also discovered in Mcl-1Δhep mice, which researchers deduced to be the dependent variable for

hepatocyte apoptosis, via an extrinsic pathway, and high ALT and AST. After 1 year, 28% of Mcl-1Δhep/TNFR1 mice displayed HCC, compared to 50% of Mcl-1Δhep, which did. Like the Mcl-1Δhep mice at 2 months, the Mcl-1Δhep/TNFR1 mice also showed statistically significant high levels of ALT and AST. Additional observations of the Mcl-1Δhep mice livers displayed an increased production of the inflammatory cytokines IL6, IL33, and IFNγ, which signal for inflammation, in contrast to Mcl-1Δhep/TNFR1 mice in which they were reduced. This experiment demonstrating the correlation between hepatocyte apoptosis and HCC tumorigenesis in mice resembles patients' similarly observed HCC development. Thus, it provides evidence for hepatocarcinogenesis due to increased hepatocyte apoptosis, which creates rapid hepatocyte regeneration with DNA mutations.[43] Other studies similarly prove that mutated gene replications have carcinogenic consequences.[45] They explain how the risk of HCC is determined by the activity of a patient's liver disease and its perpetuation,[46] including cholestasis. [17]

Conclusion

A relationship between an HFD and the development of HCC has been determined. As a result of an HFD, cholestasis promotes an accumulation of bile acids, such as GCDCA, which damage hepatocyte mitochondria and lead to apoptosis. Rapid hepatocyte apoptosis has been discovered to mutate anti-tumor genes, such as Mcl-1, which allows for HCC tumorigenesis. Numerous past and ongoing studies are focused on discovering inhibitors of bile acids over-accumulation and inhibitors of apoptotic mechanism components. Understanding the processes discussed in this article are essential to developing such inhibitors. Additionally, continuous research is in effect regarding regulating obesity and determining a healthy non-HFD which will prevent bile acid build up. HFD are especially prevalent in the United States, and these discoveries will hopefully prevent the development of HCC and possibly provide treatment to the millions already affected.

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Review

A Thorough Investigation into the Technology of Kymriah – Tisagenlecleucel A CAR T Cell Immunotherapy

By: Autumn Asen, Miriam Fried, and Rebecca Schlossberg

Abstract

Tisagenlecleucel, commonly known by its brand name, Kymriah, is a CAR T cell immunotherapy that aims to treat patients diagnosed with relapsed/refractory Acute Lymphoblastic Leukemia (r/r ALL) and Diffuse Large B Cell Lymphoma (DLBCL). Previously, the standard of care for these two hematological malignancies was chemotherapy; however, research has shown that only 50% of patients who receive chemotherapy go into remission.[1] Additionally, chemotherapy is an aggressive approach with severe side effects and inconsistent results. With the new development of the CAR T cell technology, there is hope for long term survival from these life-threatening cancers. Kymriah is one such therapy that uses CAR T technology to re-engineer a person's own T cells to attack cancer in the body. Two main efficacy studies were done for Kymriah JULIET and ELIANA that showed patients with a complete response had a 90% probability of survival at 12 months for DLBCL, and an 81% remission rate for ALL, respectively. Despite the incredible results, there are some limitations to this innovative CART cell therapy including debilitating side effects and the complete elimination of all B cells. Further research into this groundbreaking technology will surely allow for a true treatment for a debilitating disease.

Introduction

Kymriah is the brand name of the drug Tisagenlecleucel, produced by the biotechnology company Novartis. It is a CAR T cell immunotherapy that aims to treat patients diagnosed with two major cancers; relapsed/refractory Acute Lymphoblastic Leukemia (r/r ALL) and Diffuse Large B Cell Lymphoma (DLBCL). ALL is the most common childhood cancer with a prevalence of 1/1000 and an incidence of 5,960 cases annually, as of 2021.[2] ALL is an aggressive leukemia characterized by the uncontrollable proliferation and persistence of abnormal lymphoblasts or lymphocytes in the bone marrow, peripheral blood, or extramedullary

sites.[3] DLBCL is the most common type of Non-Hodgkin's Lymphoma, with 18,000 new cases reported each year.[2] It is characterized by abnormally large B-lymphocytes (B Cells) that stop responding to signals, which halt growth and division in the lymph nodes, skin, breast GI tract, brain, and bone. B cells are responsible for producing antibodies.[4]

Previously, the standard of care for these two hematological malignancies was chemotherapy. However, research has shown that only 50% of patients who receive chemotherapy to treat localized and advanced stages of DLBCL go into remission.[1] Additionally, chemotherapy is an aggressive approach with severe side effects and inconsistent results. As such, traditional methods for treating these cancers must be further explored.[5] With the new development of the CAR T cell technology, there is hope for long term survival from these life-threatening cancers. Kymriah is one such therapy that uses CAR T technology to re-engineer a person's own T cells to attack cancer in the body.

"Kymriah is one such therapy that uses CAR T technology to re-engineer a person's own T cells to attack cancer in the body."

Development of CAR T Cell Immunotherapy

The development of CAR T cell immunotherapy began with the discovery of T cells in the 1960s by Immunologist Jacques Miller.[6] In 1992, Dr. Michel Sadelain first attempted to engineer a T cell.[7] In 1993, the first generation of chimeric antigen receptors (CAR) was developed at the Weizmann Institute. Despite the CARS being successfully created, they were not ready to be used for medical purposes until proven that they could survive in the body.[8] Scientists successfully created a CAR in 2002, that could survive in the body, proliferate, and kill cancer cells by adding a co-stimulatory molecule (in this case CD28) to the re-engineered T cells.[9] In 2009 the manufacturing procedure of CAR T cells was released for use in human beings.[10] This was a major step in medicine as the FDA declared CAR T cell therapy a "breakthrough" therapy.[11] In 2017 the FDA approved Kymriah for the treatment of pediatric and adult patients with ALL, and in 2018 for DLBCL.[12] [13] Thus, the Kymriah

therapy is a result of the effort made over the span of 6 decades to find an immunotherapy that incorporates the usage of chimeric antigen receptors on T cells. In 2018, James Allison was awarded the Nobel Prize for his discoveries about the biologies of immune T cells and his invention of immune checkpoints to treat cancer.

Function of Kymriah CAR T

Kymriah CAR T cell immunotherapy functions by reprogramming a patient's T cells into CAR T cells. The Kymriah CAR allows the T cells to identify and eradicate malicious CD19 expressing cells. It is comprised of a CD19 specific, murine single-chain antibody fragment (FMC63) followed by a CD8-α hinge and transmembrane region, which is fused to both the 4-1BB costimulatory domain and the intracellular CD3-ζ signaling domain (Figure 1).[14] The 4-1BB Domain acts as the costimulatory signal required for T cell activation and is essential for the persistence, expansion, and antitumor activity of Kymriah. The technology uses a lentiviral transduction vector (LV), which can infect both nondividing and dividing cells, to deliver the transgene that encodes the CD19 specific CAR into the host cell's chromosome responsible for gene expression. The LV initiates the binding, ex vivo, of reprogrammed T cells to the CD19 receptor on target cells, which results in T cell activation as well as initiation of target cell destruction and cytokine production.[14] LV does not obtain the specificity required for in vivo applications, such as cell-specific LVs, which is currently a major drawback for the entire gene therapy field.[15] The lack of specificity can result in off-target transductions, which leads to adverse effects, such as insertion mutations causing inhibition of anti-oncogenes and activation of proto-oncogenes. Despite the potential risk factors, LVs are considered one of the safest options available, as they are associated with high levels of safety with minimal immunogenicity reported, as well

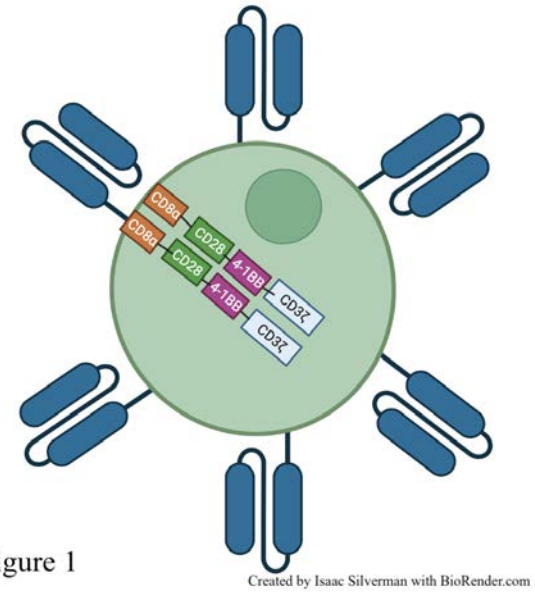

Figure 1

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as rapid induction of transgene expression. This process does not require the elimination/ disruption of any genes.[15]

DLBCL and ALL were specifically chosen as viable targets for treatment with Kymriah because they are types of B-cell malignancies that always demonstrate CD19 expression. CD19 is a cell surface protein receptor whose expression is restricted to B cells. When treating DLBCL and ALL, CD19 CAR-T cells can be utilized, as they are engineered to identify and attack the CD19 antigen on the cancerous B cells. However, Kymriah can't treat all B-cell malignancies, such as certain leukemic cells, because their CD19 expression is often masked on cancerous B cells. Since DLBCL and ALL maintain consistent CD19 expression, they were the ideal targets for treatment with Kymriah.[16]

The Kymriah CAR-T cell manufacturing process occurs in four steps and begins with the collection of non-mobilized, peripheral blood, mononuclear cells from a patient through leukapheresis. In this process, the patient's white blood cells (T cells) are extracted. All remaining cells and plasma are returned into the bloodstream. The T cells are cryopreserved within 24 hours after collection and frozen (Figure 2).[14]

The second step is cell manufacturing, in which the extracted T cells are sent to a laboratory and treated. The T cells become enriched, selected, and activated by the use of anti-CD3/CD28 antibody-coated paramagnetic beads. These beads allow for ongoing cell stimulation and are linked to higher T cell activation and cytokine production, compared to activation, another method, with anti-CD3 and interleukin (IL)-2. These T cells then get transduced

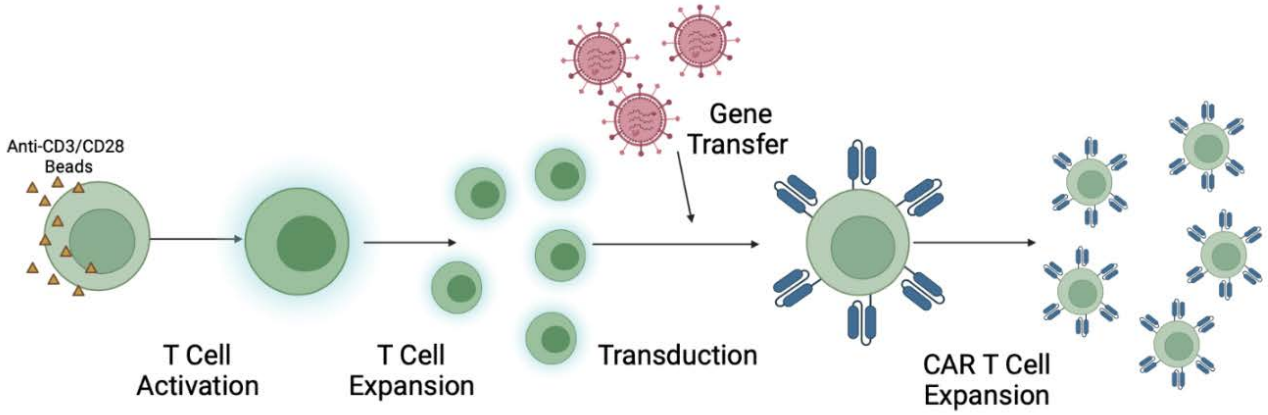

Figure 2

Created by Isaac Silverman with BioRender.com

with a lentiviral vector that is self-inactivating and contains anti-CD19 CAR transgene. Once transduced, the beads can select and activate T cells until expansion ends. This reduces the unwanted loss of antibody stimulating antibodies during the process of media exchange.[17] The unused vector is eliminated from the cells after transduction. The cells are then grown in culture. Cell expansion continues ex vivo until there are enough cells to meet the dosage requirement. The transduced T cells are harvested through isolation and separated from the paramagnetic beads, and then are processed into an infusible media.

In the third step, infusion, the infusible media, made of the re-engineered T cells, is transferred into an IV bag, cryopreserved, and shipped back to the medical facility. Thereafter, it is administered to the patient via one direct infusion. Steps one through three can take three to six weeks. Patient monitoring, the fourth step, consists of following the patient's reaction for extended periods of time.[14]

Efficacy

Two main efficacy studies for Kymriah are JULIET and ELIANA. Both were open-label, multicenter, single-arm, phase 2 global trials for Kymriah. The JULIET study targeted adult patients with r/r DLBCL to determine the best overall response rate. There were 115 participants, who had at least two previous lines of therapy- including rituximab and anthracycline. The overall survival rate is depicted from the date of infusion until the date of fatality, of the total patients that were given Kymriah.[18] Patients that exhibited any level of response had a 49% estimated probability of survival, while those with complete responses had a 90% probability of survival, at 12 months.

The ELIANA study targeted pediatric and young adults with r/r ALL to evaluate the overall rate of remission, which included either complete remission (CR) or CR with incomplete blood count recovery (CRi), with at least three months of follow up. The 75 patients deemed eligible, once receiving prior anti-CD19 therapy, were enrolled and monitored. Impact was documented for 12 months after study completion. Overall rate of remission reported was 81% for patients who received the infusion: 60% had CR, while 21% had Cri. Patient overall survival rate was 90% at month 6 and 76% at month 12.[19]

Limitations of Kymriah

Although Kymriah has given many patients a second chance, there are various limitations. Some obstacles surrounding the mechanism of the drug include debilitating side effects and the complete elimination of B cells. Side effects include cytokine release syndrome (CRS) and neurological toxicities. CRS is the result of overstimulation of the innate and adaptive immune system, which causes the release of cytokines that are toxic to other cells. The occurrence of CRS can lead to a cytokine storm, where the body is flooded with cytokines often resulting in severe toxicity, organ failure, and death.[20] Another limitation is that when the T cells are reprogrammed the inserted CAR remains in the genome in perpetuity, as such, B cells are constantly being attacked and destroyed. Individuals who receive Kymriah don't retain any B cells, resulting in a need to take daily medications to replace the lack of antibodies in their system.[21]

"CAR T cell therapies are a type of personalized medicine in which an individual is treated with their own T cells."

The treatment also has some technical limitations including long vein-to-vein time, a requirement of pre-treatments of chemotherapy, high cost, and issues with scalability. Vein-to-vein refers to the time it takes from when blood is drawn to when the blood is reinfused. Oftentimes patients with late-stage cancer cannot afford to wait. Patients also may not be able to get the treatment due to the requirement of chemotherapy, making them too weak to survive. Additionally, the high cost of treatment and limited facilities capable of processing the T cells, make it challenging for many patients to receive Kymriah.[22] [23]

Advantages of Kymriah

Kymriah has multiple advantages to the standard of care on the market today. CAR T cell therapies are a type of personalized medicine in which an individual is treated with their own T cells. As such, there is no risk of rejection and no need to wait for a donor. Additionally, the fact that the treatment only requires one infusion gives reprieve to patients who have received many treatments.[24] Most importantly, Kymriah has a higher success rate relative to classic chemotherapies, such as Clofarabine. In a study done in 2020, remission rate and success rate were studied, comparing tisagenlecleucel to Clofarabine for the

treatment of ALL. The study spanned two years and followed 183 patients who were divided by their treatment plans, Clofarabine or Kymriah. After a year, the results indicated that tisagenlecleucel performed significantly better with a 45% higher survival rate when compared to Clofarabine.[1] [25] Additionally, CAR T cell therapies also provide longer remission rates compared to chemotherapies on the market. The five-year remission rate for ALL after rounds of clofarabine chemotherapy ranges between 10%-20%, CAR T cell therapies report 46% of patients remain cancer-free and in remission at the five-year follow up.[26]

Compared to another CAR T cell therapy, Yescarta, Kymriah has shown higher efficacy and has been approved for a broader range of indications. Kymriah treats adults and pediatrics, while Yescarta only treats adults for DLBCL. Both target CD19 antigens; Kymriah T cells are programmed with a lentiviral vector while Yescarta T cells are programmed using a ϒ-retroviral vector.[20] A study done in 2019 comparing the two therapies, showed that 62% of ALL patients and 64% of DLBCL patients who receive Kymriah reach remission. Only 51% of patients with DLBCL reach remission with Yescarta.[27] Kymriah has thus far been the most successful treatment in the CAR T immunotherapy arena.

Conclusion

CAR T cell therapy has made such a significant impact that further applications are already being researched. A study called PORTIA is focused on the effects of simultaneously treating patients with Kymriah and Pembrolizumab, which is a PD-1 inhibitor. PD-L1 and PD-1 are the proteins involved in cancer development. PD-1 (programmed cell death) delivers negative signals upon interaction with its ligand PDL-1. PD-L1 has a broad range of immunoregulatory roles in the cells.[28] PD-L1 masks the cell marker for cancer by attaching to PD-1, allowing cancer to develop. Pembrolizumab is a drug that inhibits the function of PD-1, prohibiting attachment to the cancerous cell, thereby eliminating it. This study included patients who unsuccessfully underwent CAR T cell therapy as an attempt to salvage it.[29] There is also ongoing research for the usage of Kymriah with other cancers, for example, r/r Follicular Lymphoma.[30]

We have reached a point where immunotherapy treatments are a main focus within biotechnology, as there is hope to

cure diseases in a more personalized and efficient manner. Kymriah has already progressed significantly over the years, but new advancements are becoming more realistic every day.

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Review

Intraoral Imaging Techniques and the advantages of Ultrasound in the Dental Field

By: Shoshana Ellis and Jessica Schwartz

Abstract

Over the course of time, a multitude of intraoral technologies have been invented, modernized, and improved in order to create the advanced modern technology that is used in dental practices today. Since the time of the earliest dentist in the 1830's, there have been tremendous global improvements in the intricate details of dental equipment. The advancement of technology from photography to radiography to ultrasounds has developed dentistry into a prominent, impactful industry.[1] Ultrasounds have proven to be a unique area of dentistry with the potential to transform the future of the dental industry. Technological advancements regarding X-rays have had a high rate of growth in the last decade and have only continued to improve with the new technology of ultrasounds within the field of dentistry.

Introduction

The technological era began in the eighteenth century. This time period was a revolution as the development of machines were invented and the way life was lived began to shift. This time period coincided with modern dentistry as it too began in the eighteenth century. Pierre Fauchard was the first dentist to record modern practices of dentistry in his book "The Surgeon Dentist, A Treatise on Teeth." Although the technologies used back then were not the same as common practices today, it was the foundation in developing knowledge on what future dentists would eventually use to improve dental medicine.

First Intraoral Photograph

In 1839, the Paris Academy of Sciences introduced the process of photography, and thus, the first intraoral photograph was taken. Later that year, Alexander S. Wolcott paired with John Johnson and created a small business that produced optical and medical instruments. In 1840, Wolcott and Johnson designed and patented the first camera-based model known as the 'mirror camera.'As the creation of the camera was fascinating, many dentists started searching

for photographic devices to obtain intraoral images. However, there was one major issue that presented itself: there was no clear way to get light from the camera's flash within the dark crevices of the mouth. Despite using many types of close-up focused lenses, the images taken were unclear. The challenges that were encountered in early intraoral photography opened up pathways and research to discover more advanced techniques for oral photography.[2]

Discovery of X-rays

After over three decades of dental research and unsuccessful inventions, in 1895, German engineer, Wilhelm Röntgen, discovered X-rays. This was a scientific breakthrough as the world had never before seen the inside of an animal or human before. Later that year, Dr. Otto Walkoff, a German dentist, invented the first dental radiograph. The radiograph, produced by X-rays, took twenty-five minutes of exposure while having the patient lay on the floor with specified glass receptors within their mouth. The following year, Dr. C. Edminds Kells from New Orleans was the first to actively use an X-ray on dental patients. Thus, he is given the title of 'the father of dental radiography.' This new invention had an enormous influence on the world of dentistry which opened many doors for research.[3] At the age of eighteen, Dr. Frank Van-Woert invested his time in research in oral and maxillofacial radiology. He established a practical demonstration of dental radiography and presented it before the New York Odontological Society. Dr. Van-Woert discovered the first dental X-ray film, which used less radiation. This technology was groundbreaking and implemented into practices for the next one hundred years.

Advancement in X-ray Imaging

In 1987, Dr. Francis Mouyen advanced X-ray imaging by decreasing the X-ray image size. He introduced the new technology of digital imaging to the dental world by inventing a way to employ fiber optics in order to create a smaller-sized X-ray. The mechanism he used was a device called the CCD, the charge coupled device, which operates as an image sensor chip. This sensor enhanced the visualization of unseen areas that other X-rays did not have the ability to photograph. This would impact dentistry as the X-ray imaging was safer, since it emitted less radiation, and the new capabilities of fitting into a person's mouth which would make it easier to identify hard to reach crevices. Later that year, Fuji

Optical Systems of California produced the first intraoral camera. This resembled what current video cameras are like today. Due to the hard work of dentists and researchers, the advancement of dentistry globally has impacted radiography, X-ray technology, and intraoral photography.[4]

With Dr. Mouyen's technological advancement of the new X-ray, there were a multitude of processes that took place. The multi-step process required a dark room due to the chemicals that were present during the process and in order to decrease the exposure from film light. After the X-rays were placed in a dark room, they were laid on a light box in order to ensure better visualization of the X-rays. Past technologies included many harmful chemicals, time-consuming processes, wasted space, and low quality images. The process took up much space within the patient's files and was a waste of time and materials, concluding the time-consuming process with an unclear appearance of an X-ray. In order to improve these faculties, new technology such as digital X-rays have come about in recent years (Figure 1). These technologically advanced products include 3-D technology, which enhanced the imagery on the tooth anatomy, clearly revealing bone decay, bone infections, gum diseases, abscesses, and any development of abnormalities.

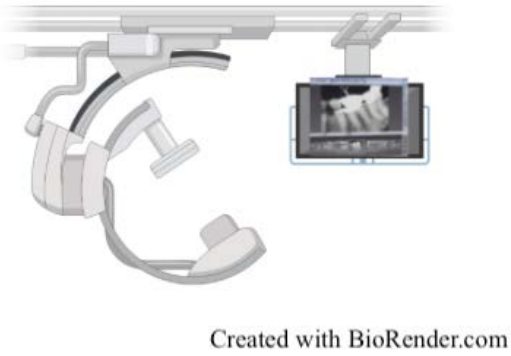

Figure 1: Advanced Intraoral X-ray

Benefits of Radiography

After this advancement, radiographs became the most common way to discover disease in a patient. Conventional radiographs work similarly to cameras. In order for a radiograph to transform a three-dimensional object into a two-dimensional image, the X-rays form a beam directed at the sample being tested. The pattern that is absorbed by the sample is projected on an analog film or a digital receptor and thus used for further studies.

Radiographic imaging provides high-quality images while also having the ability to capture images of unobstructed areas in the body that may otherwise not be visible due to the tissue and muscles that lay above it. Additionally, this type of imaging allows for

a snapshot of bone, skull, and many other hard structures.

When it comes to root canal therapy, radiographs play a key role in diagnosis, treatment, and follow-up. Given that an X-ray is the most accurate objective method, diagnosing a patient for diseases affecting the maxilla and mandible was a common practice in endodontics.

Negatives of Radiography

There are several downsides to radiographic imaging. Firstly, in terms of root canal therapy, the X-rays do not accurately depict the size of the lesion or how it fits in relationship to the surrounding teeth and crevices in the mouth. Secondly, in order to capture these radiographic images, the patient needs to be exposed to small quantities of radiation. Both of these pitfalls can harm the patient as it can cause indirect pain and long term negative effects towards them.[5]

Combining the modalities of both intraoral scanning and radiographic imaging can allow for a safer, more efficient way to successfully prepare for surgical restorative dentistry cases. In dentistry, intraoral scanning provides dentists with high-quality images of the patient's oral environment, specifically tooth structures.

Ultrasonography Technology

While radiographic imaging and intraoral scanning work efficiently enough to get the job done, scientists are always looking for ways to improve their methods in order to make a patient's visit more pleasant and less painful. "Majlesi was looking to solve this issue, to do so he compared and conducted a study on 72 patients with pain in upper trapezius muscle." The main objective of this study was to resolve spasms and reduce pain. The test resulted in an increase in blood flow within the tissue causing the initial pain. After the test results were conclusive they discovered how to relieve the pain by using a combination therapy followed by tens therapy to relieve the muscles spasms. [6]

Currently, researchers are attempting to implement the use of diagnostic ultrasound in dentistry. Diagnostic ultrasound is a non-invasive technique that is used to take images inside the body. Anatomical ultrasounds produce images of the body's internal organs and functional ultrasounds compile information of physical

characteristics such as the movement of blood or softness of tissue.[7]

Ultrasounds were designed by using technologies that conduct sound waves inside the body and receive information from the waves that echo back. Ultrasound probes, also known as transducers, emit sound waves that have frequencies above the threshold of human hearing. Usually, ultrasound probes are placed on the skin, in order to enhance the image quality. Although it is also common for these probes to be placed inside the body via the gastrointestinal tract, vagina, or blood vessels during urological procedures.[8]

Functional ultrasounds combined with anatomical ultrasounds produce "information maps." Information maps help doctors visualize the patient's organs externally without having to operate and see the problem internally.

An additional benefit in using ultrasounds with patients is that there is no exposure to radiation. For patients that need to be monitored frequently, constant exposure to radiation, even at small amounts, can be dangerous, making ultrasound a good alternative.

Ultrasonography in Different Specialities of Dentistry

Studies have shown the positive effects of using Ultrasounds in multiple areas of dentistry. Although this technology is fairly new, it is forecast to be implemented in many offices over the next few years (Figure 2).

"Ultrasound was capable of detecting auto-immune diseases, mucosal growths and had a high rate of detecting oral cancer lesions."

With studying Ultrasonography in oral medicine, it was found that Ultrasound was capable of detecting auto-immune diseases, mucosal growths and had a high rate of detecting oral cancer lesions. Additionally, there was a specific focus on Sjoren's syndrome, which is an auot-immune disease that is identified by dry eyes and dry mouth, and it showed positive results when tested.

In orthodontics, Ultrasonography was found beneficial in reducing the size of root resorption due to the multiple orthodontic forces that were done to that area. Also,

when ultrasonography was used, it reduced the patient's treatment time.

When ultrasonography was compared to MRI, and magnetic resonance imaging, it was found that Ultrasonography is more resourceful as it gets results faster and more accurately. This was true specifically for the study of diagnosing temporomandibular joints in patients.[9]

Therapeutic Ultrasounds

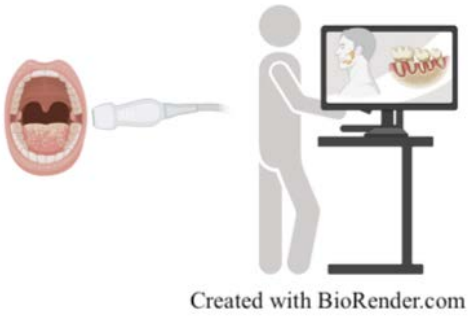

Figure 2: Intraoral Ultrasound

Additionally, another advantage to Ultrasonography is it can be used in a therapeutic manner (Figure 2). This is resourceful for dentists as many times they encounter patients with discomfort in the facial region as well as patients with facial syndromes such as Oral Submucous Fibrosis and Myofascial pain.

Oral Submucous Fibrosis is a disease in which the oral cavity is inflamed and the submucosal tissues have progressive fibrosis. When there is a severe case, this results in difficulty in movement and opening of one's mouth. In 2018, Vyoma Bharat Dani et al conducted a study with patients with this disease, his results were that movement improved with Ultrasonography and exercises compared to exercises alone.

Myofascial Pain is a chronic muscular pain disorder that is triggered by a stimulus like muscle tightness. This can result in TMJs and TMDs which dentists come across frequently. Grieder et al conducted a study in patients with TMD and Ultrasonography resulted in accelerated improvement when added to the normal muscle exercises.[10]

MRI Technology

In addition to Ultrasonography, MRI in dentistry leads a future towards radiation free imaging. Magnetic Resonance Imaging has been used to diagnose intraoral clinical problems. The application has been proven successful and aims to improve the efficacy of diagnosis and treatments. These treatments include mapping implants, jaw lesions, diseases such as TMJ, orthodontic

treatments, and endodontic treatments. The non-ionizing radio frequency electromagnetic radiation in the presence of controlled magnetic resonance imaging is a non-invasive technique that can detect abnormalities and diagnose elements in the soft tissue. The vital technology can help Doctors and Dentists alike to improve their diagnostic accuracy. [11]

Conclusion

Ultrasonography is the new and improved technology in the field that has created many opportunities for more advancements in treatments. It is efficient in intraoral photography that will better advance the accuracy as well as the efficiency of an oral X-ray. Over the years, the new technological advancements have enhanced the imagery of soft tissue visualization as well as clarified the images within the mouth, specifically gum tissue. Ultrasound technology has created a catalytic change in intraoral photography.[12]

As technology continues to progress, ultrasound machinery is only the beginning of the new developments that will collectively revolutionize and improve global healthcare industries. The forward technology is an-impressive process that uses a simplistic tool that has the capabilities that encapsulate the intraoral cavities and measure the gum tissue. This establishes an advanced, more efficient procedure. Today's advanced research comes to prove how the field of dental mechanisms has catalyzed. The way in which procedures are conducted are consistently improving and updating in order to limit pain, recovery, and treatment time. The broadened spectrum of forwarding thought will impact radiation-free imagery, as it continues to improve the future of medical technology.

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Yeshiva University Journal of Medicine and Dentistry

Review

Metallopharmaceuticals

By: Zaelig Averch

Abstract

The role of inorganic molecules in therapeutic medicine has evolved over the last century. Specifically, metal ion-containing drugs have been applied to a wide range of uses including anti-pathogenic and anti-cancer treatment. Although key to their versatility, the therapeutic use of these compounds is hampered by the toxicity of the metal ions they contain. This systematic review aims to describe the history of metallopharmaceutical chemistry, their advantages, disadvantages, and whether or not they may still serve a purpose when organic alternatives are available.

Introduction

Medicinal inorganic chemistry deals with the application of inorganic compounds, especially transition metal complexes to therapeutic uses. It's still an extremely young field in the discipline of medicinal chemistry, but it holds enormous promise. The introduction of metal ions into human biological systems has proved to be useful both diagnostically and therapeutically. For example, Magnetic Resonance Imaging (MRI), one of the most widely used diagnostic techniques, utilizes the lanthanide transition metal gadolinium (III) as a contrast agent.[1] Therapeutically, there are a myriad of applications of metallodrugs, including in anti-cancer drugs, mood stabilizers, antidiabetics, antiarthritics, antimicrobials, antivirals, and antiparasitics.

The diverse applications of metallodrugs is due, in part, to the great variety and complexity of ligands that can be used within a single metal complex. Additionally, the expanded valence of transition metals allows coordination of up to twelve ligands, making metallodrugs extremely versatile tools in clinical settings. These treatments, however, are not without their drawbacks. The successful implementation of metallodrugs is made difficult by the toxicity of the metals used and their resultantly narrow therapeutic indexes.

'Magic Bullet' Treatments

Paul Ehrlich, a German-Jewish chemist, helped pioneer the field of therapeutic metallodrugs with his development of

Salvarsan in 1912. It was proven to be an effective treatment against syphilis and has subsequently been recognized as the first modern antimicrobial agent.[2] Prior to the development of Salvarsan, the primary treatment for syphilis utilized mercury, which is extremely toxic and has been demonstrated to cause cognitive decline after acute exposure.[3] After its introduction in 1912, Salvarsan rapidly replaced mercury treatment, despite the fact that Salvarsan is an organoarsenic compound, making it also quite toxic, with a tendency to 'burn up veins'.

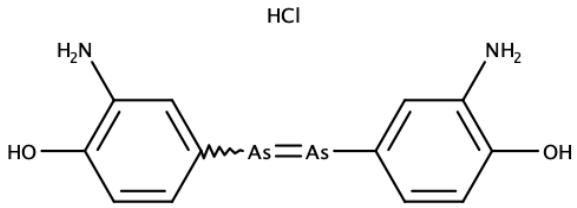

Figure 1. Molecular structure of Salvarsan (Arsphenamine)

Salvarsan offers a good historical example of the drawbacks of metallodrugs and the challenges that face their successful implementation. The medical application of metallodrugs is severely limited by the toxicity of many transition metals compared to organic compounds. This is why sixteen years later, in 1928, the organic antibiotic penicillin quickly replaced salvarsan for the treatment of syphilis and many other diseases. While metallodrugs are very attractive to pharmaceutical researchers because of their amazing versatility of structure and function, these treatments must be cautiously implemented because of their potential toxicity.

As a result of the toxicity risk, the most well-researched applications of metallodrugs are for the treatment of potentially terminal diseases, for which patients often don't have any other recourse. Additionally, the cytotoxicity of metallopharmaceutical drugs can, in fact, be an advantage, especially in their application as antimicrobials and anti-cancer drugs. One of the most famous examples of a metal-containing anti-cancer agent is cisplatin (Platinol) which was discovered in 1965 by Barnett Rosenberg and Loretta VanCamp at Michigan State University. Since its discovery, cisplatin has been used for the treatment of bladder, head and neck, lung, ovarian, and testicular cancers. It's widely effective against many types of cancers, including carcinomas, germ cell tumors, lymphomas, and sarcomas.[4]

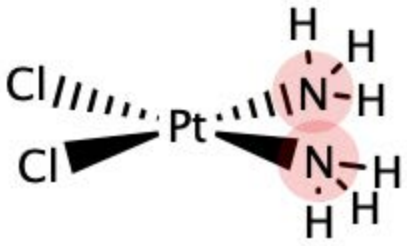

Figure 2. Molecular structure of cisplatin (Platinol)

The mechanism of action of cisplatin highlights the way toxicity can be used to a therapeutic advantage. Upon entering the cell, cisplatin hydrolyzes, losing two chloride ions (Figure 2) and becoming a potent electrophile with a 2+ oxidation state that is able to react with the nitrogen atoms in nucleic acids. Specifically, cisplatin is able to bind to the N7 reactive center of purine residue nucleotides like guanine and adenine. Since the oxidation state of hydrolyzed cisplatin is 2+, it is able to bind to two nucleotides, causing an irreversible blockage to DNA replication, triggering apoptotic cell death. This method of action may kill healthy cells in addition to cancerous ones, and has been correlated with many of the toxicological effects of cisplatin, including nephrotoxicity,[5] hepatotoxicity, cardiotoxicity,[6] and congestive heart failure.[7] Despite these potential side-effects, cisplatin is a highly effective anti-cancer agent. This is because its method of action affects DNA replication, which occurs more frequently in rapidly dividing cancerous cells than in normally functioning somatic cells.

Cisplatin and salvarsan are good examples of metallodrugs created as ‘magic bullet’ treatments. Both are highly cytotoxic drugs intended to target a specific foreign attacker, either rogue cells that cause cancer or the bacterium *treponema pallidum* that causes syphilis. This ‘magic bullet’ type of treatment was relatively common with early metallodrugs, reflecting the difficulty of balancing the versatility of organometallic pharmaceuticals with their potential toxicity. This issue revolves around the difficulty of limiting the cytotoxic effects of these drugs only to target cells, which has the potential to result in a number of side effects potentially even more serious than those cited regarding cisplatin. For example, the antiparasitic arsenic-containing drug melarsoprol has been used since 1949 to treat trypanosomiasis (African sleeping sickness), despite the fact that it causes encephalopathy; damage or disease affecting the brain and cognition.[8] This incredibly deleterious side-effect would completely eliminate the therapeutic use of melarsoprol

if it wasn't for the fact that untreated trypanosomiasis is nearly 100% fatal.[9]

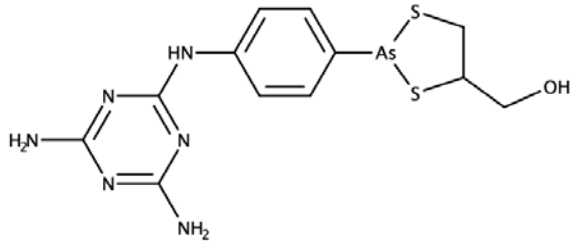

Figure 3. Molecular structure of Melarsoprol

BMOV, BEOV, and BBOV

The problem of toxicity is exacerbated in certain clinical applications that require high or repeated doses of medication many times a day. Type I diabetes is a good example of a serious autoimmune disease that requires frequent redosing of medication to maintain normal levels of insulin in the body throughout the day. In 1977, it was discovered that two vanadium salts, bis(maltolato)oxovanadium(IV) (BMOV) and bis(ethylmaltolato)oxovanadium(IV) (BEOV), were able to reverse most diabetic symptoms in diabetic lab rats. This promising initial finding was quickly tempered during pre-clinical trials. Vanadium is a potent toxin and can, in high enough blood concentrations, inhibit sperm motility, causing infertility in males.[9b, 10] Vanadium toxicity has also been linked to gastrointestinal and urinary disease, as well as fetus malformation. These side effects became distinctly problematic because the repeated doses necessary to maintain proper insulin release resulted in the accumulation of vanadium in the pancreatic tissues of lab rats. Importantly, successive research on vanadium-containing anti-diabetic drugs has yielded the synthesis of compounds like bis((5-Hydroxy-4-oxo-4H-pyran-2-yl)methy lbenzoatato)- oxovanadium(IV) (BBOV) which was synthesized in 2013, and has half the oral toxicity, with the effective dose lowered by a factor of 1000.

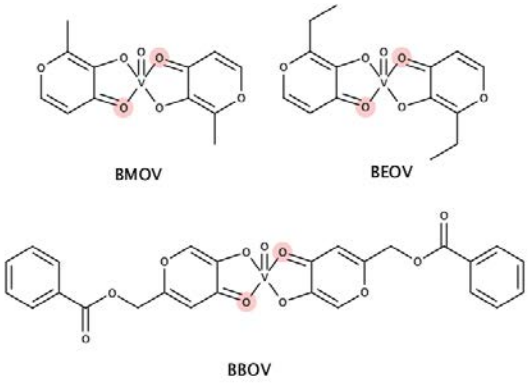

Figure 4. Molecular Structures of BMOV, BEOV, and BBOV

The evolution of BBOV from BMOV and BEOV showcases an important part of the way these drugs are developed, similar to the way salvarsan replaced mercury treatments for syphilis. Typically the first generation of a metallodrug is fraught with

toxicity issues, either eliminating their usefulness entirely, or limiting it as a last line of defense treatment. Successive generations, however, improve upon the structure to decrease the necessary dose to the point where the amount of heavy metal consumed per dose is less than the amount the human body can excrete in between doses.

Toxicity and the Therapeutic Index

The toxicity of metallodrugs is linked to the difficulty with which they are excreted.[11] The metabolism of water-insoluble heavy metal complexes also depends on the presence of cellular antioxidants that are able to quench free radicals by suspending the activity of enzymes like catalase, peroxidase, and superoxide dismutase. Excretion for water-soluble complexes, on the other hand, is correlated with their ability to be successfully filtered from the blood via the kidneys. Many small metals like sodium pass easily through the nephron, but larger heavy metals are often unable to diffuse into the urine as easily, making nephrotoxicity a common side effect of overdose on many metallodrugs (Table 1).

This delicate balance between effective doses and excretion is well described by a drug's 'therapeutic index' which is defined as the window between a drug's therapeutic and toxic effects. As soon as a therapeutic index can be determined, that is, as soon as a specific dose is found to be consistently more therapeutic than toxic, the treatment can be widely implemented. In fact, the window between therapeutic and toxic effects doesn't even need to be very wide for a drug to be approved for treatment. Take the example of lithium carbonate, an oral mood stabilizer that was FDA approved in 1970 to reduce suicide risk and mood swings in bipolar patients.[12] Lithium's exact method of action is yet unconfirmed. However, it has been proposed that it reduces cellular levels of myoinositol, high concentrations of which are found in the neurons of bipolar patients during manic and depressive episodes. Unfortunately, myoinositol is a necessary growth-promoting factor in mammalian cells, and a deficiency can result in intestinal lipodystrophy (AKA Whipple's disease) which can be fatal if untreated.[13] No matter the mechanism of toxicity, lithium has been widely demonstrated to be toxic in doses very near the therapeutic dose for bipolar patients. As a result, lithium carbonate has a very narrow therapeutic index.[14] So narrow, in fact, that many bipolar patients prescribed

lithium also undergo lithium decontamination during or after treatment.

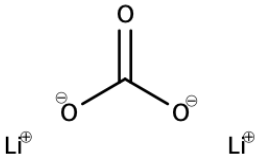

Figure 5. Molecular structure of lithium carbonate

Further illustrating the difficulty of a narrow therapeutic index, lithium carbonate has been examined as a potential treatment for neurodegenerative diseases like Alzheimer's disease. Lithium ions exert a neuroprotective effect on the amygdala, hippocampus, and prefrontal-cortical regions of the brain.[15] This treatment, while being potentially groundbreaking for patients suffering from Alzheimer's or Parkinson's disease, has been deemed unsafe due to the necessity of lifelong intake of lithium in moderate to high doses.

A major reason that so many metallodrugs have such narrow therapeutic indexes is a result of their complicated coordinated structures. The method of action of many of these drugs is poorly understood as a result of the complex steric and coulombic interactions between the charged metals and their often bulky ligands. As a result, many drugs in addition to lithium carbonate, like auranofin which contains a gold (I) center, have been heavily criticized despite their clinical efficacy.[16] Auranofin in particular, like a number of other gold (I) compounds, has been shown to slow the progression of rheumatoid arthritis by inhibiting several cathepsin proteases implicated with the disease.[17] The exact mechanism by which these cathepsins are inhibited is poorly understood however, leading practicing physicians to prescribe these gold medications as a last resort when other treatments have failed.

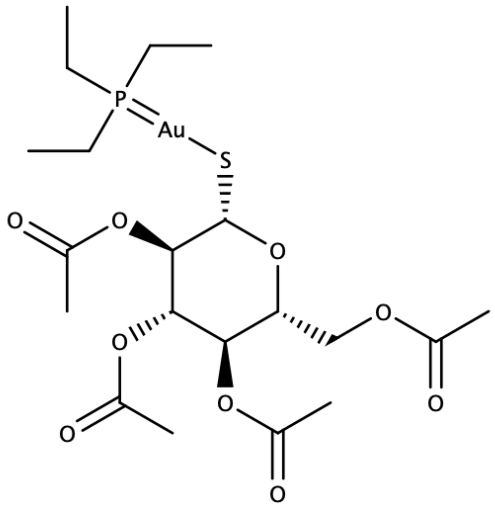

Figure 6. Molecular structure of Auranofin

Conclusion

Despite the field of metallopharmaceuticals rapidly expanding over the last hundred years, it still remains less fully developed

than traditional organic treatment routes. There are twelve metals essential to human physiology, and our bodies have developed a complex and sensitive system of pathways for their transport and utilization. This complexity poses a core challenge to the development of novel metallodrugs. The human body's sensitivity to these metals is both a blessing and a curse for organometallic pharmaceutical researchers. While these drugs can be incredibly potent and their unique geometry makes fine-tuning their functionality a tantalizing possibility, their toxicity and often extremely narrow therapeutic indexes demand caution when developing any treatment involving the use of heavy metals.

Appendix

Table 1. Toxicological comparison of of metallodrugs

Drug	Treatment of:	Metal	Toxicologic symptoms
Gadolinium	(MRI contrast agent)	Gadolinium	- Nephrotoxicity - Hematoxicity - Hepatotoxicity - Pancreatitis - Neurotoxicity
Salvarsan	Syphilis	Arsenic	- Inflammation - Vomiting - Nausea - Nephrotoxicity
Mercury	Syphilis	Mercury	- Nephrotoxicity - Hemorrhagic gastroenteritis - Abdominal pain - Neuropathy
Cisplatin	Cancer	Platinum	- Nephrotoxicity - Peripheral neuropathy - Nausea - Vomiting - Myelosuppression
Melarsoprol	African Sleeping Sickness	Arsenic	- Encephalopathy - GI/skin reactions - Pyrexia - Peripheral neuropathy
BMOV	Diabetes mellitus (DM)	Vanadium	- Infertility - Urinary/GI disease - Fetal defects
BEOV	DM	Vanadium	
BBOV	DM	Vanadium	Same toxicology as BMOV/BEOV, but 1000x lower effective dose
Lithium carbonate	Mood disorders; inc. bipolar disorder	Lithium	- Nephrotoxicity - Whipple's disease - Nausea - Vomiting - Neuropathy
Auranofin	Rheumatoid arthritis	Gold	- Anemia/leukopenia - Proteinuria - Hematuria - Inflammation - Nephrotoxicity - Hepatotoxicity

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Yeshiva University Journal of Medicine and Dentistry

Review

Correlation between ABO Histo Blood Groups and Covid-19 Susceptibility and Outcome

By: Yitzchak Stein

Abstract

Since the onset of the Covid 19 pandemic, a disease caused by the virus SARS-CoV-2, research has connected ABO histo-blood group antigens and their role in susceptibility, outcome, mortality, and viral uptake of infected particles. Sample populations studied showed decreased susceptibility and mortality in patients of the O negative blood type. Research is still underway to determine ABO polymorphism and its role in susceptibility and outcome of the disease. Research conducted with SARS-CoV-1disease manifestation has paved the way for proposed mechanisms in which ABO polymorphism was established to affect susceptibility and outcome. The host range of Covid-19 is determined by its Spike protein. Anti-A or Anti-B antibodies from the ABO blood groups bind to the S protein, blocking its connection with angiotensin-converting enzyme 2. ACE2 is the host receptor for SARS and Covid-19. This supports the idea that binding could decrease susceptibility to Covid-19. The S proteins subunit's essential role in receptor binding has become a key research focus in antiviral therapeutic development. Research studying the mechanical mechanism of viral uptake of SARS-CoV-1 and statistical analysis of groups infected with Covid-19 indicates a correlation between ABO histo blood groups and susceptibility and outcome.

Introduction

Correlation between ABO histo-blood group antigens and increased susceptibility and illness in infectious disease has been identified in adenocarcinoma,[1] thrombosis,[2] and esophageal carcinoma.[3] The outbreak of Covid-19 in December 2019 resulted in a 2% mortality rate and an overall hospitalization rate of 2.1%.[4 5] This reached a peak of 9.2% for those over 60.[6] Through membrane microdomains[7], blood group antigens facilitate intracellular uptake of viral particles[8]. Blood types A, B, AB, and O are carbohydrate epitopes present on the surface of red blood cells. Antigenic determinants of blood types A and B are the

trisaccharide moieties GalNAcα1-3-(Fucα1,2)-Galβ- and Galα1-3-(Fucα1,2)-Galβ-, while O blood antigen is GalNAα1,2-Galβ-.[9] Research on the correlation between ABO histo-blood groups and SARS-CoV has led scientists to research the molecular mechanisms of blood antigens present in Covid-19 because of its similarity to SARS-CoV in respect to ACE2.[10]

Blood Type Susceptibility and Mortality

Multiple studies have been conducted spanning different populations researching the correlation between blood group antigens and susceptibility to Covid-19, many of which have proved a correlation present. The largest study conducted to date was in Ontario, Canada. Infection was determined by viral RNA polymerase chain reaction testing, with the second outcome of severe illness or death. They studied a sample population of 225,556 people possessing a mean age of 54. The adjusted relative risk for O blood antigen group in respect to blood antigen groups A, B, AB, was 0.88 (95% Cl, 0.84 to 0.92; absolute risk difference, -3.9 per 1000 [Cl, -5.4 to -2.15]).[11] Rhesus negative antigens in regard to Covid-19 was adjusted relative risk 0.79 [Cl, 0.73 to 0.85]; absolute risk difference, -6.8 per 1000 [Cl, -8.9 to -4.7]. Those that were O negative showed an increased protective response of adjusted relative risk, 0.74 [Cl, 0.66 to 0.83]; absolute risk difference, -8.2 per 1000 [Cl, -10.8 to -5.3]. These results show a direct association between O and Rh-negative blood groups and lower susceptibility to infection of Covid-19.[12]

In China, research conducted by Jiao Zhao studied the correlation between blood type susceptibility and mortality in a sample population of 1,775 patients. The patients had a blood type distribution of A, B, AB, and O of 37.8%, 26.4%, 10%, and 25.8%. The proportion of blood group A among the patients positive for Covid-19 was higher in the control group, with 37.8% in the former versus 32.2% in the latter $p<0.001$). Those in the O blood group were lower than the control group, 25.80% versus 33.84% ($P<0.001$). Covid-19 susceptibility increased for blood group A (odd ratio 1.279, 95% Cl 1.136~1.440) and decreased for blood group O (odds ratio 0.680, 95% Cl 0.599~0.771).[13]

Researchers applied this distribution pattern in studying increased risk for deceased patients. The distribution for blood groups A, B, AB, and O in a sample of 206

deceased patients was 41.3%, 24.3%, 9.2%, and 25.2%. Lower risk of death was associated with blood group O versus non-O groups, with an odds ratio of 0.660 (95% Cl 0.479~0.911, P=0.014). Blood group A was associated with a higher risk of death versus non- A groups with an odds ratio of 1.482 (95% Cl 1.113 ~1.972, P = 0.008).[14]

Covid-19

These studies propose a trend in the correlation between ABO blood groups and the severity of Covid-19. However, research is still underway on the molecular mechanism by which ABO polymorphism plays a role in susceptibility and severity. Research conducted connecting ABO blood groups, and SARS-CoV-1 has paved the way for studies concerning Covid-19 and the molecular mechanism by which antigens play a role in susceptibility and outcome. Severe acute respiratory syndrome, also known as SARS, was first recognized in Guangdong Province, China, in November 2002.[15] In June 2003, the epidemic was finally contained,[16] possessing a case fatality rate of 11%.[17]

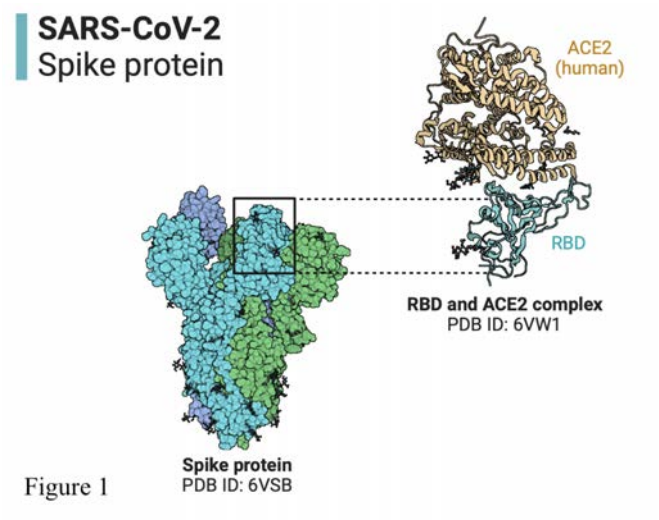

Figure 1

Spike Proteins and ACE2

ABH antigens present themselves on platelets from a person with the corresponding phenotype.[18] They are found on epithelial cells, commonly in the upper respiratory tract, nasal epithelium, and trachea.[13] These cells can synthesize ABH carbohydrate epitopes. The host range of Coronavirus is determined by its S protein (Aka spike protein) (Figure 1).[19] S proteins are type I transmembrane and class I fusion proteins possessing an N-terminal domain and receptor binding domain, making up the S1 subunit, C-terminal domain, fusion peptide, heptapeptide repeat sequence 1, HR2, TM domain, and cytoplasmic domain, making up the S2 subunit.[20] These domains function for receptor binding and virus-cell fusion (Figure 2).[21] Because of their nature, researchers proposed in a study that the S

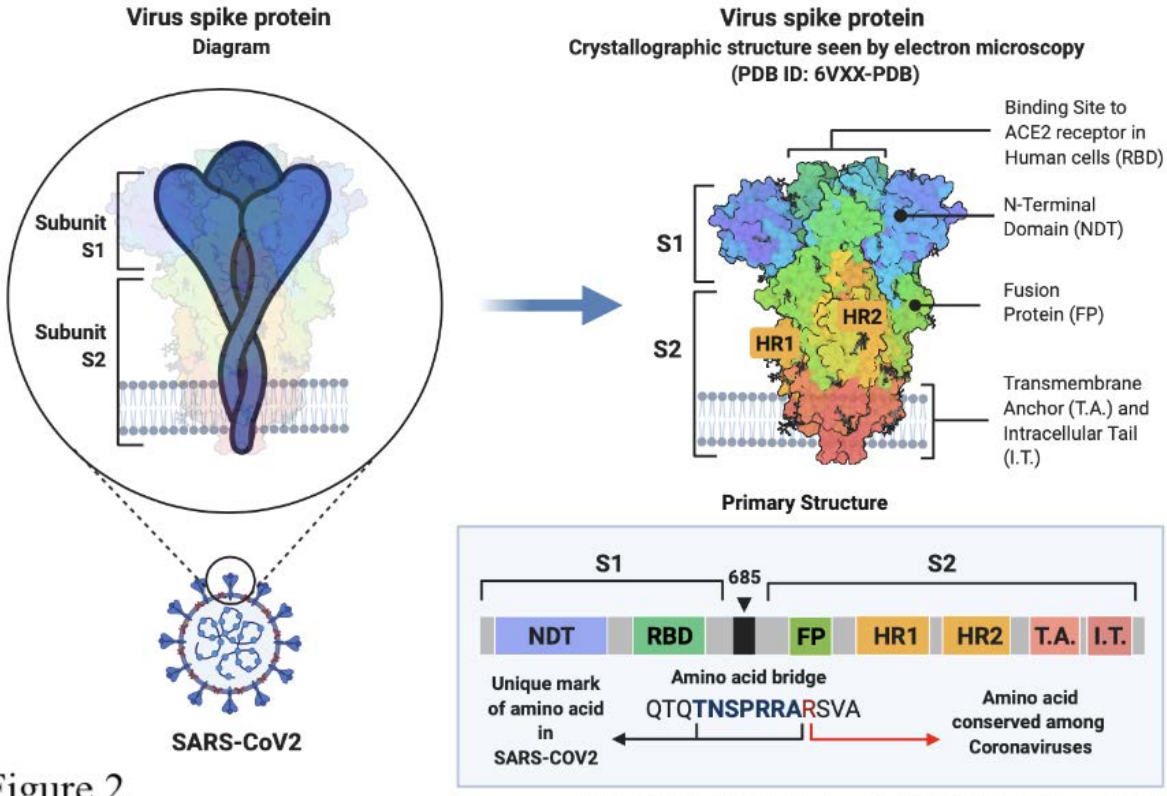

Figure 2

"An In-Depth Look into the Structure of the SARS CoV2 Spike Glycoprotein", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

protein of virions produced by either A or B blood groups would be able to be decorated with A and B carbohydrate epitopes. The anti-A or anti-B antibodies from the ABO blood groups could bind to the S protein and block its connection with angiotensin-converting enzyme 2 (ACE2). Since ACE2 is the host receptor for SARS-CoV-1,[22], inhibiting the binding could decrease susceptibility to SARS-CoV-1. This hypothesis was tested using a cell binding assay that reconstructed the interactions between S proteins and ACE2.[23] It was indicated that the interaction between the S proteins and ACE2 could intervene in adhesion between ACE2 and cells possessing S proteins and A histo-blood groups. This would be inhibited by anti-A-antibodies, reducing susceptibility.

Cell adhesion is used to screen molecules that block the transfusion of a virus without the use of infected molecules. This method displayed monoclonal anti-A antibody or natural plasma anti-A inhibiting SARS-CoV S proteins bonding to ACE2.[10] Correlating studies confirmed groups of glycosylation sites are located around the RBM of the SARS-CoV S protein.[24 25]

Antibodies can bind to these glycans and inhibit the interactions between ACE2 and S proteins by interfering with their viral replication cycle.[26] This block decreases susceptibility to SARS-CoV. These studies support the idea that ABO-histo-blood group antigens, through the actions of natural antibodies, could inhibit the transfusion of infectious particles, causing complement-mediated-neutralization.[27] [10]

SARS-CoV-1 Blood Type and Susceptibility

During the outbreak of SARS-CoV-1, a study conducted in Hong Kong, China, modeled the effect of natural anti-A and B antibodies and the susceptibility of the virus. Three transmission patterns, each possessing different transmission probabilities, according to the amount of protection provided by anti-histo-blood group natural antibodies presented themselves. The stronger the group effect, the more protection offered. Researchers assessed group effect based on a population of infected individuals possessing different blood type frequencies—the more substantial the impact of the group, the stronger the delay of the epidemic. Results displayed a strong group effect in groups with high frequencies of blood type O. To confirm the results, they ran simulations consisting of A, B, and AB blood groups, and the results were unmodified.[28] This study shows mathematical evidence supporting ABO histo-blood groups' contribution to viral transmission in SARS-CoV-1.

"ABO-histo-blood group antigens, through the actions of natural antibodies, could inhibit the transfusion of infectious particles, causing complement-mediated-neutralization."

Heptad Repeat Domain Dependency

This research conducted on S proteins and their role in severe respiratory syndrome has led to correlating studies and clinical research into antiviral drug development for Covid-19 and its illness caused by it, Covid 19. The S protein, and its subunits, S1 and S2, play an essential role in receptor binding and entry of infectious viral particles into its host cell (Figure 3), making it an important focus of research into producing antiviral medication.

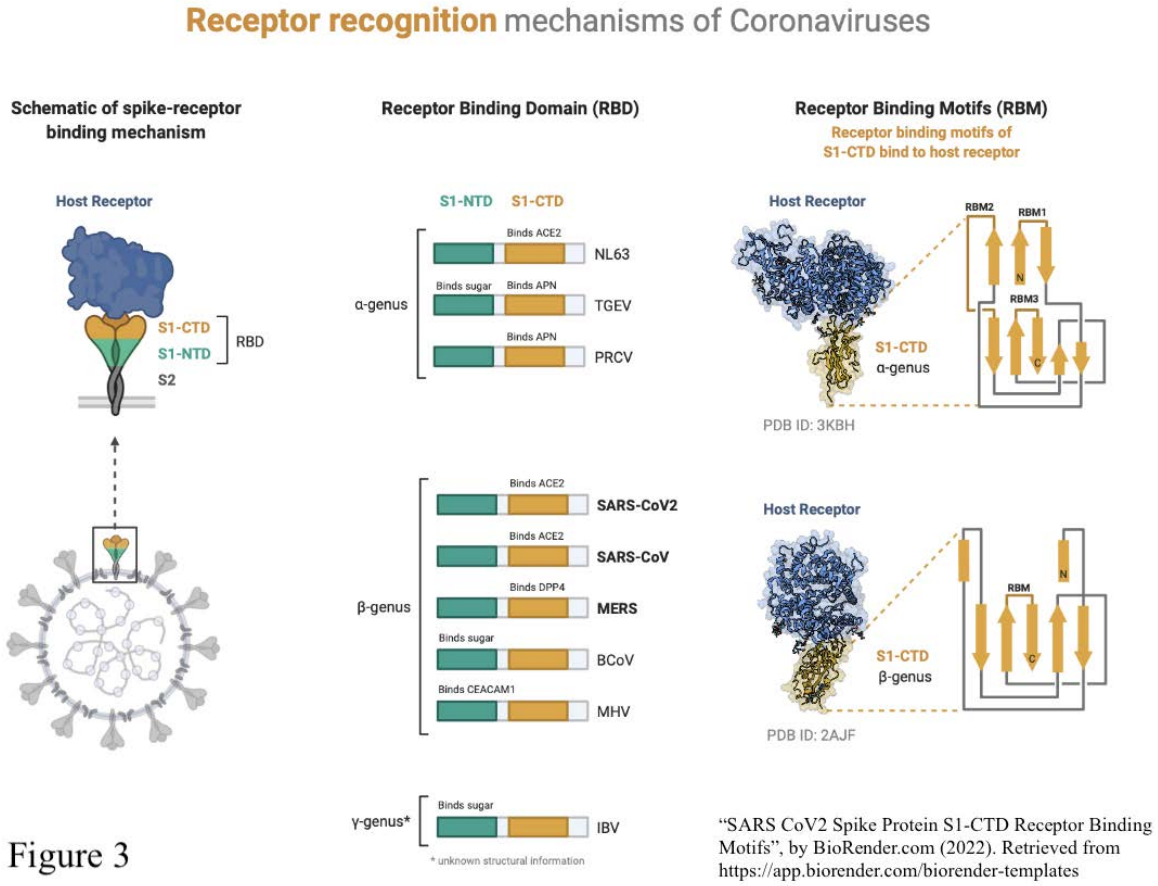

Figure 3

"SARS CoV2 Spike Protein S1-CTD Receptor Binding Motifs", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

In the S2 subunit, the heptad repeat domain has been a critical focus in research for antiviral therapeutic medication. The heptad repeat area in the S2 subunit plays a crucial role in HCV infection. As a mode of interaction for HR1 and HR2.[29] In a similar study, a synthetic peptide created from the stem region of a Zika virus envelope protein inhibits the infection and other flaviviruses in vitro, disrupting the integrity of the viral membrane.[30] This study indicates higher antiviral efficiency when peptides taken from conserved regions of viral proteins are utilized in developing the treatment. A study further testing this theory found that peptides are taken from the HR2 part of the S2 subunit formed class 1 viral fusion proteins of enveloped viruses competitively bound to viral HR1, resulting in an inhibition of infection mechanisms.[31] This opens the door for developing a therapeutic drug that works as a fusion inhibitor to treat Covid-19 (Figure 4).[32] Clinical trials are currently underway researching the dependency of Covid-19 on the S protein.[33] [34] [35]

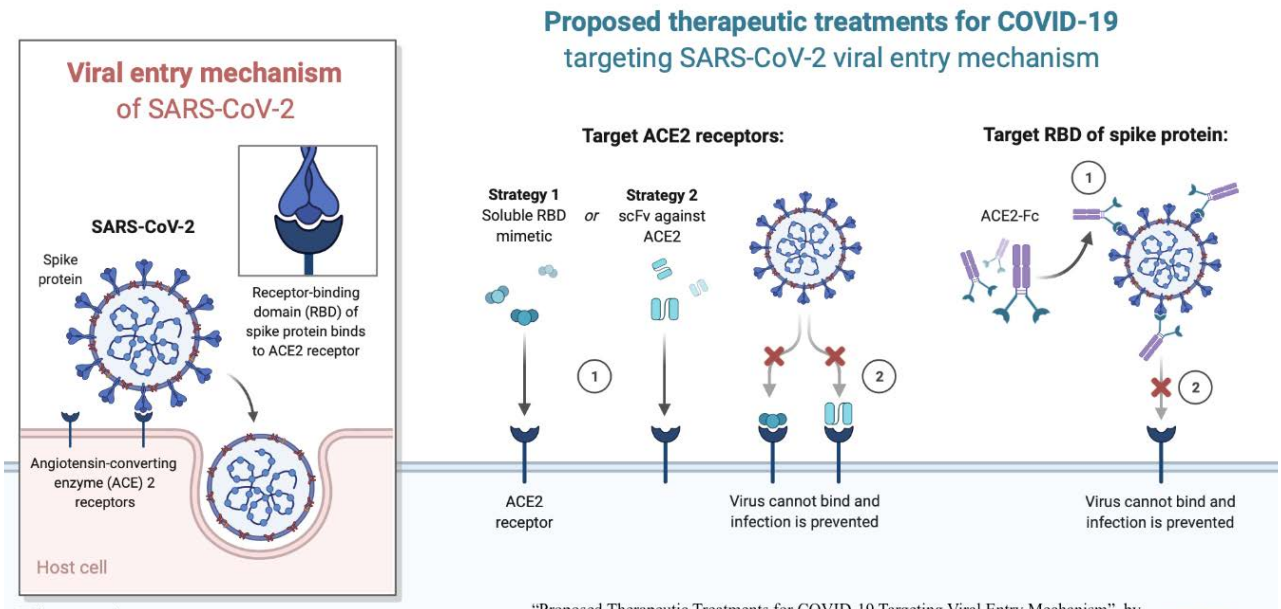

Figure 4

"Proposed Therapeutic Treatments for COVID-19 Targeting Viral Entry Mechanism", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

Conclusion

The correlation between S proteins and ABO histo-blood groups supports the theory that blood type plays a role in susceptibility and outcome regarding Covid-19. Statistical analysis of infected and noninfected populations show a correlation between blood type O- and a decrease in susceptibility and mortality rate of infected individuals. Blood type O displayed an odds ratio of 0.680, 95% Cl 0.599~0.771 regarding susceptibility and an odds ratio of 0.660 (95% Cl 0.479~0.911, P=0.014) regarding mortality of infected individuals. As research continues to further the understanding of the mechanisms of infection, better therapeutic antiviral medications for individuals exposed will become a reality, combating the mortality rate and susceptibility of Covid-19.

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Yeshiva University Journal of Medicine and Dentistry

Article

The Role of SIRT2 Expressed by Oligodendrocytes in Increasing Axonal ATP

By: Yannay Kaplan

Abstract

Axons require sufficient energy to be healthy, otherwise neurodegenerative diseases result. A recent study shows that oligodendrocytes (OL) send SIRT2 proteins in exosomes which increases axonal ATP levels. The delivery of Sirtuin 2 (SIRT2) was found to be critical to increase ATP axonal energy by using in vivo samples comparing wildtype OLs to SIRT2-deletions, by using neurons from knockout mice, and by preventing the expression of SIRT2 from the exosome. Only wildtype OLs exhibited strong acetylation of mitochondrial proteins ANT1 and ANT2. Additionally, SIRT2 was found to revive the mitochondrial ability of knockout mice. Using this data, a potential way to increase ATP production in neurodegenerative disease has been discovered.

Introduction

The brain is composed of several types of cells, including neurons, oligodendrocytes, glial cells, and astrocytes. Oligodendrocytes (OL) are responsible for covering neurons with a myelin sheath, which allows signals to be sent quickly throughout the body. A recent study demonstrated OLs are fundamental to ensure neurons can produce ATP.[1] This research provided a better understanding of a mechanism in which OLs allow axonal mitochondria to produce ATP. A lack of neuronal supply of ATP is connected to neurodegenerative diseases such as Alzheimer's, Parkinson's, and MS.[2,3] Previous studies have shown that OLs are vital to the function of an axon and that having the myelin sheath intact without OLs is insufficient and axons will die.[4,5] These studies have demonstrated a link between the health of an axon, ATP, and OLs.[6] The purpose of this paper is to explain how researchers in a study analyzed the mechanism in which OLs cause axonal mitochondria to increase the production of ATP.[1]

OL Enhancement of ATP Production in Axons

It is first imperative to establish a significant correlation between OLs and ATP production levels in axons. Chamberlain et al extracted neurons from mice and separated the axons from their cell bodies using a microfluidic chamber. Axons were divided into two groups. A control group was created containing only the axons. In a second experimental group, OLs were added. In each group of axons, ATP levels were measured using a method called Fluorescence resonance energy transfer (FRET) which uses fluorescent markers to detect ATP binding. A significant increase of ATP ($p<0.0001$) in the experimental group was observed in comparison to the control group, demonstrating that OLs have an important role in ATP production in axons. The advantage to FRET is that it is highly specific, allowing for the determination of the specific organelle in which ATP generation occurs. Specificity ensures that the conclusions reached will be more precise, and that doubts regarding the accuracy of some previous studies and their corresponding conclusions are allayed.[3] Additionally, using this method, results are received in real-time. Non-myelinating OLs were used for most of the experiments described, which increases the information regarding how non-myelinating OLs in gray matter support axonal ATP production.[1]

Next, they determined if the type of signal transferred between the OL and the axon which led to the eventual increase in ATP in an axon is contact dependent or secretory based. To understand the process undergone by OLs to increase ATP, the researchers left pure OLs on media/broth for 24 hours to condition the media. After 24 hours, they removed the OLs and incorporated the conditioned media with the axons. The media caused a similar increase in ATP in the axons. Due to ATP production also increasing while OLs were not present, the researchers concluded that a secretory mechanism was used by OLs to transmit energy to axons. As will be described later, SIRT2 is being expressed by OLs. Based on the above, the expression of SIRT2 by OLs is not based on axon need but is instead a continuous release of the protein.[1,7]

Possible Effect of Lactate on Axonal ATP Production

Lactate is an important energy source for axons. The molecule is broken down into pyruvate and assists mitochondria to create ATP through oxidative phosphorylation.

One source of lactate for axons are OLs.[8] It was hypothesized that if lactate was added to media containing only axons, axonal mitochondria would absorb the lactate and increase production of ATP. When lactate was applied to the media, no change in fluorescence was observed in the axon, indicating the addition of lactate to axons had no significant effect on axonal ATP levels.[1] Thus, another mechanism responsible for increasing ATP must be found.

Possible Effect of OL Exosomes on Axonal ATP Production

OLs release exosomes responsible for the development of neurons and their maintenance. Further research was conducted to determine whether exosomes possess an influence on the production of ATP in neurons. Media was conditioned with wildtype OLs and incorporated into a plate containing axons. A second plate of axons also received conditioned media from OLs as well as a neutral sphingomyelinase inhibitor to inhibit the expulsion of exosomes from the multivesicular membrane. FRET was used to measure ATP binding. A significant difference in ATP production was observed when OLs were added to the respective plates. This difference demonstrated that exosomes are an important variable in an axon's energy production. Additionally, exosomes were stained and detected in axons. Researchers determined that exosomes from mature but not necessarily myelinating OLs increase axons' capability to produce more ATP.[1]

Enrichment of SIRT2 in OLs and Secretion in Exosomes

Once researchers determined that a secretory mechanism is used by exosomes, they investigated the protein SIRT2 as a possible protein being secreted. SIRT2 is a deacetylase commonly found in OL exosomes (it is found 40x more in OLs than neurons) and has been recently found to travel to the mitochondria and deacetylate mitochondrial proteins.[1] Acetylation of mitochondria is known to control ATP production.[9] Neuron cultures taken from mice containing both OLs and axons were stained to assess the expression of SIRT2 in OLs. The finding was that only mature OLs expressed SIRT2.[1 7]

Scientists found a directly proportional connection between myelination from mature OLs and SIRT2 levels. They observed SIRT2 present in both the brain and spinal cord of mice. A later experiment

examines this further. Through this experiment, researchers concluded that OLs secrete exosomes containing SIRT2, which increases ATP production in neurons.[1]

Levels of ATP Increased by Presence of SIRT2

The next experiment conducted consisted of three parts, all suggesting that SIRT2 is the vital component which causes an increase in ATP production in neurons. One part examined the effect on axonal energy when OLs containing exosomes without SIRT2 were grown with axons, compared to when OLs with SIRT2 expressing exosomes were used. The former batch of OLs were taken from general SIRT2 knockout mice. No increase in axonal energy was found when OLs without SIRT2 were used, while a significant increase of axonal ATP was measured when OLs expressing exosomes containing SIRT2 were used.[1]

"Scientists found a directly proportional connection between myelination from mature OLs and SIRT2 levels."

In the second stage, a trial was conducted in which SIRT2 was placed in a viral vector and added to axons. No OLs were used in this stage. If no SIRT2 was added to the vector, the level of ATP in an axon did not change; however, when SIRT2 was added to the vector, axons increased their production of ATP. Thus, the researchers concluded that the vital component in the process of increasing production of ATP in an axon is due to SIRT2 in an OL's exosomes (Figure 1).[1]

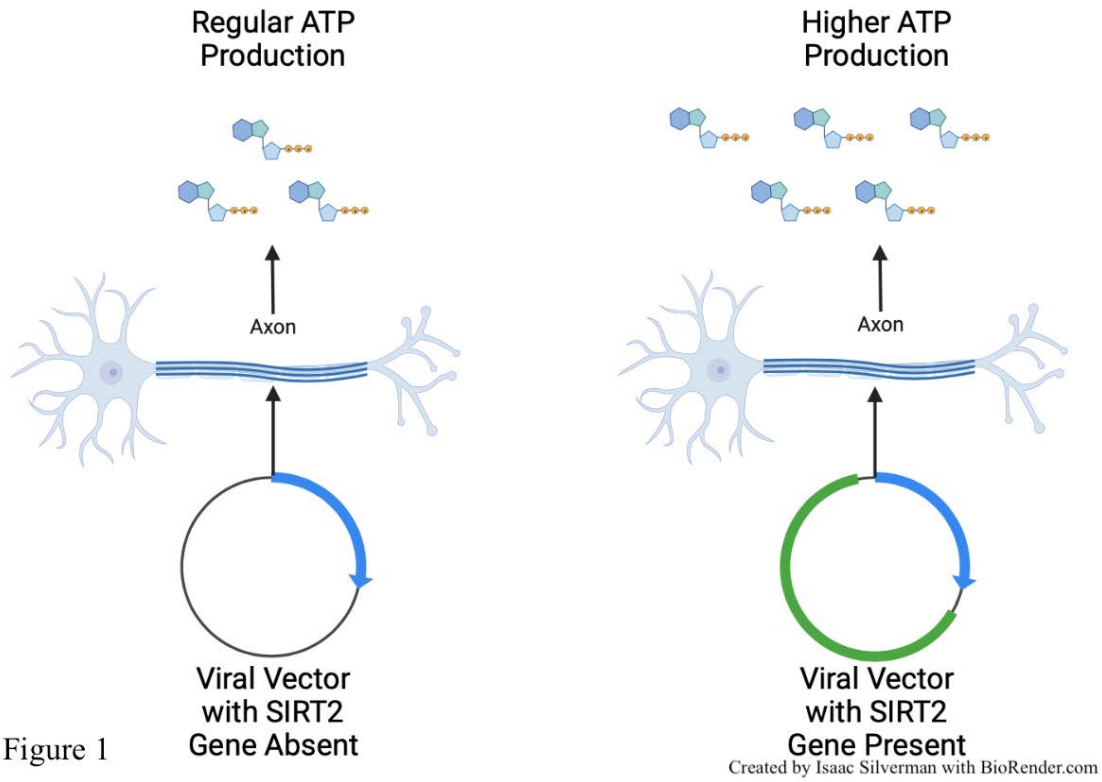

Figure 1

The third stage used a small molecule of RNA which targets SIRT2 mRNA and causes degradation. The exosome of the OL loses a majority of its SIRT2 protein. As a result of the significant decrease of SIRT2 expression, cultures which contained SIRT2-deficient OLs and axons caused similar ATP production levels as individually cultured axons. This was in contrast to axons which were cultured with OLs which expressed SIRT2, which showed higher ATP production levels. All three experiments described show a directly proportional correlation between SIRT2 expression and ATP production.[1]

Neuron Mitochondrial Deacetylation Caused by SIRT2

After establishing a correlation between OL exosome secretion of SIRT2 and an increase in axonal ATP, further experiments were done to determine the mechanism in which the presence of SIRT2 in a neuron's axon causes ATP production to increase. Two groups of neurons were produced, one with wild type OLs in media and the other with SIRT2 knockout OLs present, which previously showed no increase in ATP compared to axons alone. Subsequently, mitochondrial proteins were isolated from neurons through centrifugation and

mitochondrial fractionation. These mitochondrial proteins were placed in two batches of OLs – one which expressed SIRT2 and one which did not – and the quantity of deacetylated proteins was measured. As aforementioned, SIRT2 is known to be a deacetylase, so the wild-type batch was hypothesized to contain more deacetylated proteins. In addition, research has shown that deacetylation of mitochondrial proteins increases their activity, which increases ATP production. As predicted, the researchers found that the wildtype axons produced more ATP, signifying that SIRT2 deacetylated proteins.[1]

Subsequently, the experimenters chose specific mitochondrial proteins which they hypothesized SIRT2 would bind to that may cause an increase in axonal ATP. The proteins chosen included ANT1, ANT2, PHB2, ATP5A, and NDUFA5. They found that SIRT2 deacetylates ANT1 and ANT2 but not PHB2, ATP5A, and NDUFA5 (Figure 2). The reasoning behind the inconsistency of protein deacetylation is left open to future experiments. The study researchers suggest that perhaps other sirtuin proteins are responsible for deacetylating the proteins left untouched.[1]

Revival of Mitochondrial Function via SIRT2

In a final experiment, the researchers assessed SIRT2 expression in myelinated axons using knockout mice which do not have OLs that express SIRT2. Although these mice have a similar number of mitochondria in their axons as compared to wildtype mice, their mitochondrial function is compromised. Mitochondrial membrane potential – which is correlated with the health and ATP production of an axon – was measured using a dye which was inserted into the spinal cord. Researchers introduced exosomes from wild type mice into the spinal cords of the knockout mice. Using the dye that shows mitochondrial health, they

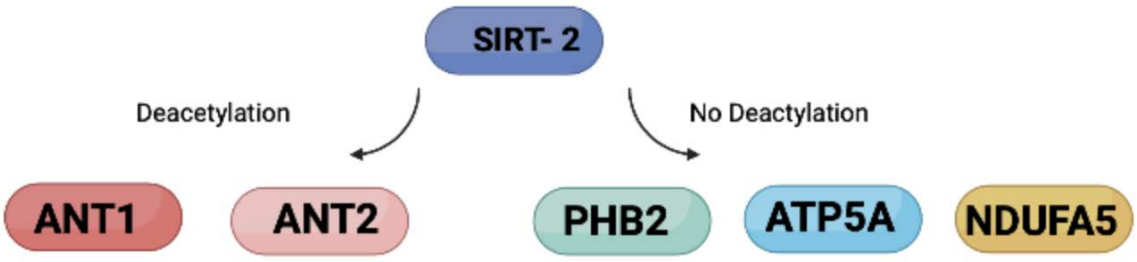

Figure 2

Created by Naomi Fried with BioRender.com

observed that the injected SIRT2 exosomes in knockout mice increased mitochondrial ATP production. This increase was not seen with a separate group of control mice.[1]

Conclusion

OLs have been found to increase axonal ATP production by secreting SIRT2 via exosomes. In axons, SIRT2 deacetylates certain mitochondrial proteins which cause ATP production to increase. Further research is still required to discover whether the SIRT2 protein interacts with the mitochondria via a signal cascade or by entering the cell. Furthermore, SIRT1 has been implicated in changing histone acetylation.[10] Additional research could be conducted to determine if SIRT2 enters the nucleus and changes histone acetylation as-well.[7] Additional experimentation with SIRT2 expression between white and gray matter oligodendrocytes will be helpful to understand the applications of this knowledge. Clarity with regards to how axonal energy is generated and increased will be helpful in developing methods to combat neurodegenerative diseases.

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Yeshiva University Journal of Medicine and Dentistry

Opinion

Does it matter where you take Psychedelics?

By: Chloe Schreiber

Introduction

Psychedelics, a category of substances defined by their hallucinogenic, perception altering properties, have recently been gaining recognition in mainstream medicine for their benefits in treating psychiatric conditions such as addiction,[1] Post-Traumatic Stress Disorder (PTSD),[2] Obsessive-Compulsive Disorder (OCD),[3] depression,[4] and anxiety.[5] Despite these substances having only recently been recognized for their ability to treat these conditions, psychedelics have been around for much longer and are used in a myriad of different practices and applications around the world.

While some naturally derived psychedelics like ayahuasca and psilocybin, which are commonly known as magic mushrooms, have been around for centuries and used for spiritual ceremonies in Eastern and indigenous cultures. Others like Lysergic acid diethylamide (LSD) and 3,4-Methylenedioxymethamphetamine (MDMA), commonly known as ecstasy, were developed by Western scientists Dr. Alexander Shulgin and Dr. Abert Hofmann in the early 20th century. During the 1960's, psychedelics became widely used in western culture as recreational drugs known for their "trip" inducing qualities and were associated with the hippie counterculture movement. This increased use of psychedelics led many countries, as well as the United Nations, to label psychedelics as Schedule 1 controlled substances which prevented these drugs from potentially being utilized for medical purposes.

For decades, the interest in or usage of psychedelics was considered taboo due to their illegal status, leading those in search of a remedy for their psychiatric conditions to seek out these substances in other countries in illegal ways.

The Psychedelic Renaissance

However, in recent years there has been a psychedelic renaissance, and although still illegal and labeled as Schedule 1 drugs, these drugs are now being provided in a variety of ways and viewed as more than just a way to "get high." From a clinical

perspective, psychedelics are being evaluated for their therapeutic and medicinal qualities by doctors and therapists, and currently encouraged by the FDA,[6] possibly foreshadowing the legalization of these substances for medical usage.

One way in which psychedelic medicines are provided is through "wellness" centers and retreats, advertised as immersive psychedelic experiences leading to growth and well-being. There are also providers known as "sitters" or "guides" who serve as impartial "supervisors," ensuring a user's safety or offering necessary guidance during an experience. The Eastern and indigenous ayahuasca and psilocybin experiences still exist today, led by spiritual leaders or shamans who provide an ancient spiritual psychedelic experience.[7]

Safety, Efficacy, and Ethical Considerations

With the increase in popularity of psychedelic medicines, their safety and efficacy must be established, as well as the outcome of the experience. Based on a review of psychedelic literature, it became evident that while there have been studies conducted which analyze the safety and effectiveness of psychedelics in treating certain psychiatric conditions, there is also a lack of research comparing the different contexts in which psychedelics are taken. In addition to the safety and efficacy considerations, there are ethical issues in some of these settings that can potentially compromise the very vulnerable and impressionable person who is under the influence of psychedelics.

Timothy Leary, a Harvard professor, conducted research on psychedelics at Harvard in the 1960's and developed the Set and Setting hypothesis which states that the primary determinants of a psychedelic experience are the internal set- intention, expectation, motivation- and the external setting or context, including the presence of a guide or therapist.[8] Set and Setting is a primary and crucial aspect of psychedelic treatment. It is therefore imperative that we be conscientious and sensitive to our choice of psychedelic context, specifically where one ingests psychedelics, and evaluate these different environments.

"Set and Setting is a primary and crucial aspect of psychedelic treatment."

Shamanistic Ceremonies

When organizing psychedelic experiences, the shamanistic and religious rituals appear to be the most ethically challenged when compared to the more traditional research contexts. Shamanistic ceremonies typically contain a group of around 10 people, take place for a few consecutive nights, and are led by a shaman or religious leader who has had extensive training in their tradition. During the ceremony, there may be chanting, rhythmic dancing and music, a prayer service or silence. The ceremonies also take place in dim light in order to induce visions. Depending on the medicine used- typically ayahuasca or psilocybin- there is also a purging component, as the medicine causes some users to become sick and vomit. Before and/or after the ceremony, there is usually a preparation and/or review to clarify and frame the users' experience. The specifics of how the experience is presented is dependent on the tradition of the group leader or shaman, but generally it is framed as accessing metaphysical realms or worlds via spiritual beings and interacting with 'good" and "bad" spirits. Also, the purging in these ceremonies is framed as the removal of spiritual toxins from the body. This purging, in conjunction with interactions with spirits and the feeling of being separated from one's physical body in another realm, is part of the process of leaving the user with a healthier and stronger body.[9] Some of these religious retreats also contain daytime activities like tai-chi and yoga or fasting, and as their popularity has grown in recent years, more westernized, tourist-attracting versions have been created.

The religious nature of these ceremonies may be viewed as unethical by some, since it seems that the shaman is pushing religious beliefs on the ceremony participants while they are in a vulnerable and impressionable state. However, this spiritual way of framing the experience may have a positive effect, arguably replacing psychotherapy and psychopharmacology, and helping participants to work through and interpret what may be confusing experiences. The ancient traditions of these ceremonies also place emphasis on the significance of set and setting. Their rhythmic beats, which help participants minimize the chance of getting stuck in an unwanted experience, and the presence of a trained leader are both intended to ensure a meaningful and productive experience. While a study exists which analyzes many individual aspects of ayahuasca ceremonies and the experiences

and outcomes of participants,[10] further research should be conducted to determine if there are any short- or long-term consequences of these religious ceremonies attributed specifically to religious indoctrination.

Risks of Psychedelic Usage

Additional concern may also be due to the lack of a health screen before and after these ceremonies, ensuring that all participants were not at risk for complications during the ceremony or had chances of adverse reactions. While some psychedelics have been shown to be among the safest recreational drugs,[11] they do, like all things, have some contraindications and risks. Psychological risks include a bad experience with the potential for developing paranoia, fear, panic, or dysphoria. Distressing effects in modalities such as sensory, somatic, metaphysical, or personal/psychological, can result in the user having disturbing illusions, troubling thoughts, or hyper awareness of physiological processes. These effects, although extremely rare, may lead to dangerous behavior as a result of intensified emotional experience. Although the accuracy of the relationship is still unclear, those who may be suffering with premorbid mental illness may be at increased risk for prolonged psychosis and psychological difficulties post psychedelic session. Additionally, lasting perceptual abnormalities such as Hallucinogen Persisting Perception Disorder (HPPD) or flashbacks to "trip-like" sensations may occur, although incidence is extremely rare. It has also been shown that incidence of these conditions appears to be much lower in contexts with careful screening and preparation.[12] In terms of physiological risks, many psychedelics can raise blood pressure and heart rate, therefore those with hypertension should consult with a doctor before using these medicines.[13]

"Psychological risks include a bad experience with the potential for developing paranoia, fear, panic, or dysphoria."

Sitters

Seemingly on the other end of the spectrum of ethical psychedelic providers are "sitters" or "guides." A part of the underground psychedelic movement, a sitter is anyone who is present for another's psychedelic experience and serves as an impartial resource for them, ensuring the users safety

and helping them through their experience when necessary. In other words, they serve to maintain a set and setting which is conducive to the user having, ideally, an uninterrupted and uninfluenced experience. A guide can be an experienced psychedelics user, a licensed therapist, or someone who has never taken these medicines at all. There are many individuals and groups which offer their own certifications or proposed instructions to follow, however there is no official certification or training that is technically necessary to be a sitter, and no official manual to reference. Using a guide for one's psychedelic experience may be viewed as the most unadulterated and ethically sound route, as the one-on-one experience and absence of third-party interpretations allow the user to have a completely uninfluenced experience. However, the lack of training of the guide, lack of health screen or follow up, and lack of supplemental therapies or activities must be considered.

Additionally, due to the informal nature of sitters and their use in the underground scene, there is an absence of any data and research for the method.

FDA Trials

In terms of available data and detailed protocols, the FDA's clinical trials on psychedelics are arguably the safest providers of psychedelics. Due to the psychedelic renaissance in recent years, the FDA has been performing clinical trials on the safety and efficacy of various psychedelics to treat different conditions. The exact nature of one's psychedelic experience in a trial, i.e., the drug being used, the number of sessions, dosage, supplemental activities, accompanying psychotherapy, etc. will differ trial to trial, but common factors include the multiple, spread-out sessions taking place over a few weeks, taking place in quiet, neutral settings, with trained staff and medical personnel available if necessary. Short term follow ups with participants were performed at various points after the session(s) to assess the efficacy of the drug. Health screens are also performed before each trial, however not all trials tested for the same criteria. While most have the exclusion criteria of certain psychological conditions like schizophrenia or bipolar disorder, not all trials perform extensive medical testing to screen for conditions such as hypertension that are possible contraindications for taking certain psychedelics. Additionally, not all

trials perform long-term follow ups or post-session health screens to detect any adverse events. That there are trained personnel present measuring outcome data in real time is something that does not take place in the informal settings described above.

Wellness Centers

Additional psychedelic providers which have become popular are wellness retreats. Located around the United States and across the world, these retreats are marketed towards both first-time as well as experienced psychedelic users. Somewhat of a culmination of the previously mentioned psychedelic providers, they have the greatest diversity in offerings- group or solo sessions, pre- and post- session psychotherapy and integration, and supplementary activities like yoga and massages, allowing for the user to tailor the experience to his or her needs. However, depending on the nature of a specific retreat, the ethical and safety concerns of the previously mentioned distributors may apply as to it well. The staff of these retreats usually have some third-party certifications in and/or experience with psychedelics, but as stated previously, there is no agreed-upon standard for what it means to be qualified in this field. Interestingly, these retreats commonly have medical staff such as psychiatrists and nurses that are very involved with participants, performing detailed health screening and therapies throughout the process to ensure a safe and productive experience.

Conclusion

As more of these psychedelic providers are emerging, and in research settings, the safety and efficacy of these medicines have been proven time and time again, it is imperative that the contexts of these psychedelic providers are studied to ensure ethical, safe, and efficacious delivery of psychedelic medicines to those in search of a treatment for conditions like PTSD, depression, anxiety, for an introspective experience, or even for those just curious about psychedelics. Factors like set and setting, pre- and post- session health screening, training of the provider (the shaman, the sitter or the guide), supplemental therapies, religious agendas, and session follow ups are only some of many factors which must be reviewed.

Acknowledgements

I am grateful to be participating as a member of a private research group[14] that is currently

looking to explore the differences between the methodologies of psychedelic providers and laying the groundwork for more research to be done. This research represents but one step towards the possible legalization of psychedelics and provision of help to those suffering with psychiatric conditions. Current medications have unfortunately fallen short of treating psychiatric conditions and psychedelics represent a potential new paradigm for psychopharmacology in the very near future.

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Letter

Red Hair In Genetics

By: Eta Goldstein

Introduction

Appearance is the most crucial part of making first impressions. With vision being our most robust sense, what we see immediately drives us to draw conclusions. Hair color is one of these features, the most unique of them being red. Terms like "Fiery redhead" and comments like "Oh, he/she has a red-headed personality." are common. People with natural red hair have been subjected to these stereotypical remarks for years. Another example is that people with blonde hair are often associated with the term "dumb blonde." Different aspects of one's personality can be associated with his or her hair color, but do these terms have any aspects of truth to them? Is there any scientific backing to suggest that there is a real correlation between hair color and the stereotype? Genetically, how can having red hair actually affect one's health and overall personality?

Redheads in History

A few times throughout the history of the Bible, it implies that a person had red hair. Esau, for example, is described as "red, covered with hair like a fur coat."[1] Through his actions, he is seen as a rather impulsive character, compromising his status as the oldest son in a moment of hunger. The second person in the Bible, and arguably one of the greatest Jewish monarchs, David, was thought to be a redhead as well.[2] He used his passion and his fire to kill in the name of God and to be a leader to his people. Other famous leaders throughout history include Alexander The Great, Queen Elizabeth I, Winston Churchill, and George Washington.[3] All of these figureheads were able to use their fierce characteristics, stemming from their hair color, to guide their people. Why does red hair correlate with leadership? What biologically could cause this correlation?

To further investigate these ideas, one may wonder why redheads are even prone to impulsivity? What gives them the qualities to lead based on their hair color? The basis for this stereotype comes from a chemical exchange in the human body. Red-headed people produce more adrenaline than non-redheads while also having the ability to

more readily and quickly access this adrenaline, leading to an intensified fight-or-flight reaction.

Genetics of Red Hair

How does someone obtain the unique characteristic of red hair? Red hair is an autosomal recessive trait. Thus, this gene must be in both parents' chromosomes for a child to express red hair. There are various causes for the different effects associated with having red hair, which all stem from a genetic mutation in the MC1R gene, which encodes for the melanocortin-1 receptor. This gene is pleiotropic, and can be expressed in multiple phenotypic traits. Biochemically, the development of red hair is caused by the synthesis of more pheomelanin pigment, the typical primary pigmentation responsible for the black-brown hair color gradient, than eumelanin pigment. The larger amount of pheomelanin, instead of eumelanin, is responsible for the red hair color.[4] This, coupled with excessive prenatal exposure to estrogen, facilitates the expression of red hair during fetal development. Additionally, red hair is hypothesized to be an evolutionary adaptation for sufficient photosynthesis of provitamin D in conditions of low intensity of UV-B radiation, which is common throughout Europe. Statistics prove that one to two percent of the European population has red hair. Researchers discovered red hair to be the most prevalent color to have emerged through evolution. Therefore, corrective evolution did not have sufficient time to have taken place, which would have otherwise neutralized the multitude of adverse side-effects associated with the existing red hair alleles.[5]

"Researchers discovered red hair to be the most prevalent color to have emerged through evolution."

Causes for Red Hair

Red hair can be a result of climate adaptation. Mutations in the gene MC1R also regulate melanogenesis which is responsible for low melanin levels. Eumelanin is a yellow-red pigmentation responsible for red hair, which replaces the black color from pheomelanin in skin, hair, and the iris. Pale skin also results from congenitally low eumelanin in the skin, which explains the often combination of fair skin and red hair. Pheomelanin cannot protect the body from UV radiation and thus promotes mutagenic and cancerogenic influences. Many genes involved in skin

pigmentation also affect the vitamin D 25(OH)D3 concentration in the human body. In a study with controlled sun exposure, vitamin D 25(OH)D3 had a much greater effect on the 73 redheads than on the 130 subjects who had various other hair colors. Sun exposure was found to have a minimal effect on 25(OH)D3 concentrations in redheads yet had a positive effect on the levels of 25(OH)D3 of non-red-heads. The decrease in the eumelanin-pheomelanin ratio in red-headed people apparently was an adaptation synthesis of sufficient amounts of vitamin D in regions with low UVB radiation because redheads were found to have a greater 25(OH)D3 concentration. Many redheads, often born with fair skin, avoid sun exposure. As a result, they maintain their fair skin by preventing sunlight from inducing the formation of vitamin D. The intensity of redness is an adaptation to low-intensity radiation, causing inadequate sunlight-induced photosynthesis of vitamin D in the skin. Redheads obtained the ability to synthesize sufficient 25(OH)D3 even with their minimal levels of sun exposure.[6]

Physiological Impacts of Red Hair

Now that the reason for red hair is understood, the different effects of the character trait on different genders can be investigated. Research proves that men with red hair are prone to develop colorectal cancer scientifically. Regarding performance in health studies, men with red hair did better in three categories and worse in three categories compared with those men who have black, brown, or blonde hair colors.[5] Parkinson's, decreased platelet function, and defects in the immune system were all found to be associated with both genders. Many interesting elements correspond specifically to red-headed female women. These women are found to have higher levels of pain sensitivity and are prone to endometriosis. In comparing red-headed females and females with other colored hair, red-headed women did better in three specific categories and worse in ten other categories when equated to one another. Other negative associations for female redheads include colorectal cancer, cervical cancer, uterine cancer, and ovarian cancers. This can be explained by the excess transference of estrogen from the mother to the baby during fetal development.[5] Estrogen is known to influence the development of these fetal reproductive organs. Subsequently, fertility problems were found to be at greater incidence in those with red hair, coinciding with the higher incidence of endometriosis

that was reported. Red hair, as previously discussed, is associated with having fair skin, causing a higher vulnerability to UV radiation, leading to sunburns, and ultimately potentially skin cancer. Despite this, more reproductive lesions and cancers were reported than sun cancer cases despite this. Interestingly enough, along with being more prominent in red-headed women, people with richer red hair color were found to report greater incidence. Furthermore, those with richer red hair reported a higher rate of severe disorders, including musculoskeletal disorders, heart and vascular problems, cancer, fertility issues, metabolic illnesses, sexual dysfunction, genitourinary disorders, osteoporosis, obstetric complications, and neurological problems.[5] These conclusions resulted from studies performed using many red-headed women with different shades of hair color. Red hair was found to be more frequent in women than men. Although many problems are clearly associated with red hair, which results in selection against redheads, there is a counterbalance of positive sexual selections in favor of redhead women, maintaining red hair frequency at a low but stable balance. This may provide reasoning for the conclusion that red-headed people, regardless of gender, are found to have more children.

"MC1R, the gene for red hair, is also the gene that regulates pain pathways."

Not subjected to redheads, pain is not something that most people like to endure. However, redheads specifically have greater anxiety levels in regards to pain, especially dental pain.[7] MC1R, the gene for red hair, is also the gene that regulates pain pathways. The melanocartinergic pathway is also involved in anxiety-like behaviors, and the MC4R gene, which is involved in producing both red hair and fair skin, is implicated in anxiety.[8] Anesthesia is reduced in people with the MC1R variant. This resistance to subcutaneous local anesthesia leads to an increase in anxiety, leading to avoidance of dental care. This anxiety also leads to a greater perception of pain, causing the necessity for larger amounts of anesthesia. This can be explained by the emotional amplification of the somatic experience.[8] Interestingly, women were also found to be more sensitive to cold pain perception, cold pain tolerance, and heat pain. Those with darker shades or more variants correlated with greater levels of dental anxiety.[8] Additionally, patients

with red hair reported significantly greater pain in regards to needle insertion.[7] Medically, there is no block on the inferior alveolar nerve, which is the injection for anesthesia, thought to be the cause for the greater necessity, but rather it is the emotional amplification due to the increase in anxiety coupled with extreme pain perception which causes the greater need for anesthesia in red-headed peoples.

Conclusion

After discussion of the multitude of associations of traits linked to the red hair gene, the positive and negative outcomes can be evaluated. Red-headed people do not choose their hair color, but it is overall a desirable trait because it is visually appealing. The choice of how to use the inborn qualities given to red-headed people can be observed throughout the red-headed characters in the Bible. One can use the impulsive nature and passion to be a positive leader of people, guiding them towards growth. The bloodthirst can be productive by obtaining a job such as a surgeon, where cutting is ultimately to heal and provide life instead of death. Red hair is an interesting phenomenon that has much-unexpected research accompanying it. Although hair color is not a choice, and some aspects which are correlated are not in one's control, there are elements of being a redhead that one can use to benefit oneself and help others ultimately. Characteristics associated with being red-headed are not set in stone. Through developing one's personality and traits, these characteristics can be worked on to only be used in effective ways. With the right amount of self-discovery and work, anger can turn into passion.

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