THE DEVELOPMENT OF AN EDIBLE VACCINE TO PREVENT HEPATITIS B

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***Abstract*— *Hepatitis B is a virus that affects the liver, is spread through bodily fluids, and can be fatal if left untreated. This disease impacts millions of people around the world, especially in impoverished countries, despite it being preventable by vaccine. To address this issue, an edible vaccine to prevent hepatitis B is being developed with the aim to be a more stable and easily distributed alternative to the injectable vaccine. To create the vaccine, specific genes are introduced into plants, which causes them to produce the desired protein, in this case, the antigen for hepatitis B. Plant cell walls protect the antigens from breaking down in the stomach acid upon ingestion of the vaccine, which is why vegetables were chosen as the form of delivery. The edible vaccines are being tested in clinical trials, but none have been approved yet for the market. Clinical trials conducted using potatoes containing the hepatitis B surface antigen showed increased antibody concentration in subjects who ate the antigen-containing potatoes, which proves that edible vaccines are a viable alternative to the injectable immunization. Questions arise in regards to how to create a uniform product, if it can be sustainable, as well as how practical it is to eat raw potatoes, among other vegetables. Additionally, resistance to genetically modified organisms (GMOs) and vaccines is affecting the development of edible vaccines. In spite of these doubts, scientists see the potential of edible vaccines to reach groups of people who do not have access to standard, injectable vaccines. As a result, the prevalence of preventable diseases including hepatitis B could be lowered, and millions of lives could be saved.*

Key Words—edible vaccines, engineered plants, genetically modified organisms, hepatitis B, surface antigen

AN INVESTIGATION INTO HEPATITIS B AND EDIBLE VACCINES

The most prevalent infectious disease in the world today is hepatitis B. Hepatitis B is a liver disease that spreads virally through contact with blood, or other bodily fluids, from an infected person. It is estimated that over 292 million people are currently living with a chronic hepatitis B infection [1]. The World Health Organization reported that in 2015, 887,000 deaths occurred as a result of this disease [2]. The majority of the population affected by hepatitis B is located in developing countries where the lack of vaccination is a widespread issue. Many factors contribute to this, including insufficient funds, supplies, and medical access. The infrastructure of these areas is often poor, and the storage of the vaccines, which requires the use of refrigeration, can be an issue. Vaccination is a huge medical advancement that has saved a countless number of lives and could potentially save many more.

Vaccines have aided humanity in making significant advances in the fight against infectious diseases over the last few decades, eliminating diseases such as smallpox and polio almost altogether. Regardless of how vaccines are administered, the goal remains the same: to prepare the immune system to fight off a disease if contact occurs. A new innovation being developed, edible vaccines are created by genetically introducing antigens into a specific food. They are in the clinical trial phases with the goal of providing a better alternative to the traditional, injectable vaccines which possess many drawbacks. Scientists believe edible vaccines have the potential to eliminate many of the drawbacks due to their unique qualities. The hope is that an edible form of vaccines will allow for higher immunization rates in developing countries. Additionally, edible vaccines are more sustainable than injectable vaccines in terms of productivity and environmental impact. Overall, edible vaccines create less waste, require less energy, and lead to a more productive world because fewer resources are expended on people who are ill from vaccine-preventable diseases [3]. Before the vaccine can be released to the market for use, it must go through years of clinical trials and testing, all monitored by the FDA. Edible vaccines could be the solution to the lack of vaccination against hepatitis B [4]. To investigate whether edible vaccines have the potential to become a widespread hepatitis B prevention method, the emerging technology will

be analyzed in terms of its development process, clinical trials, and advantages.

HEPATITIS B

The Disease

Hepatitis B (HBV) is a viral infection of the liver that impacts an estimated 400 million people around the globe. The virus can be spread through contact with infected bodily fluids, having unprotected sex, or by using contaminated needles [5]. Adults who contract the disease have a higher chance of the disease simply going away, compared to children who will most likely develop it for life. For infants who are infected, there is a 90% chance of a chronic infection developing. This risk drops to 50% for children ages 1 to 5 and 5-10% for healthy adults over the age of 19 [1]. To confirm diagnoses, two proteins in the blood are tested for: the hepatitis B surface antigen (HBsAg) and surface antibodies (anti-HBs). The HBsAg proteins show up in the body after exposure, and if they do not disappear after six months, then the condition is classified as chronic. The antibodies (anti-HBs) show up in the blood after the HBsAg proteins disappear, and they make the individual immune to hepatitis B for the remainder of his or her life. The appearance of the antibodies indicates that the individual has already fought off the disease. Complications from hepatitis B include scarring of the liver (cirrhosis), kidney disease, and liver cancer. There is currently no cure for hepatitis B, which makes its prevention crucial [6].

Current Methods of Treatment

The first step of most treatment plans is to give the infected patient the hepatitis B vaccine plan and a hepatitis B immune globulin shot that boosts the immune system to help fight off the disease. The vaccine is injected through the use of a syringe and needle, both medical instruments that can only be used once. Patients are advised to avoid substances that are harmful to the liver such as alcohol and acetaminophen. According to a swiss study on smoking and hepatitis B, “alcohol increases HBV replication, promotes damage to the liver and increases the likelihood of developing cirrhosis” [7] Moreover, “smokers with hepatitis B have a higher rate of liver cancer than nonsmokers” [7]. Since the liver is already damaged from the hepatitis B virus, it is important to reduce the burden that is placed upon it. The best-case scenario for someone infected with hepatitis B is that the disease goes away rather than becoming chronic. This is indicated by the presence of anti-HBs in the blood and means the individual is now immune to hepatitis B. If the disease does become chronic, many medications such as entecavir, tenofovir, and lamivudine are available for patients. These medications can slow the damage done to the liver by fighting off the virus [8]. As with the use of any medication, there is the potential for negative side effects. Also, not every patient with chronic hepatitis B needs to go on medication. If medication is given, the medication will need to be taken for the rest of the patient’s life [9]. Those with chronic hepatitis B can live long productive lives, provided they care for their health and see a doctor regularly [10]. Hepatitis B can become fatal if left untreated and not monitored by a doctor. From 2010 through 2014, there was a steady hepatitis B related mortality rate of 0.5 deaths per 100,000 population [11]. Prevention by vaccine is critical to lower the risk of contracting hepatitis B.

PREVENTION

The Approach to Prevention

The hepatitis B vaccine is the primary form of medical prevention for the disease. The original vaccine became available in 1981, and has since been used over one billion times, lowering rates of chronic infection among children around the world [1]. Until recently, there was only a vaccine that was effective for infants at birth and for children up to 18 years old. The immunization plan for these two groups consists of three shots received over short period of time. In 2017, a new injection plan was approved by the FDA for use on adults age 18 and older, which consists of two shots received over a one-month period [12]. Contrary to public belief, an individual cannot contract hepatitis B from receiving the vaccine. An individual can receive the vaccine after exposure to HBV, preferably in the first 24 hours after exposure, and this can essentially prevent the infection. After a certain period, an infected patient can receive the vaccine with no additional harm, but it will be of no benefit to the patient [9]. With over one billion doses administered, the hepatitis B vaccine is deemed to be one of the most safe and effective vaccines ever developed [12].

The Struggle for Prevention

Although Hepatitis B is preventable by vaccine, immunization rates in developing countries remain low. In 2017, countries such as Chad and Nigeria reported that the vaccination rate of children in their country was below 60%, while the United States had a rate that was between 95-100% [13]. Since the 1990s, universal vaccination programs have led to a decrease in the frequency of vaccine-preventable diseases by focusing on six major diseases: measles, tuberculosis, diphtheria, pertussis (whooping cough), tetanus, and polio. In Africa, for example, vaccination of children has increased from 5% in 1980 to 77% in 2014, but the vaccination plan is still ongoing. Millions of children die every year from vaccine-preventable illnesses. Africa has fallen behind in their vaccination goals and reasons for this struggle include lack of trust in healthcare, insufficient

resources, no access to care, lack of public awareness to vaccination benefits, and poor infrastructure [14].

In general, in countries where hepatitis B is still widespread, government healthcare systems often struggle to keep up with managing the virus. Challenges facing vaccination in developing countries include inability to afford vaccines, high cost of production, lack of sterile conditions, and insufficient availability of medical personnel. One of the main issues preventing further globalization of immunization is the need for refrigeration for the vaccines. The vaccines cannot sustain long exposures to warmer temperatures [15]. To address these issues, an alternative to injectable vaccines is being developed which can be taken orally. Edible vaccines are a promising solution to the challenge of global immunization.

EDIBLE VACCINES

How They are Produced

Edible vaccines are the transportation method through which antigens are delivered into the body. An antigen is "any substance that causes the immune system to produce antibodies against the disease" [16]. These antibodies remain in the immune system, ready to attack the virus if it enters the body. Proteins and peptides are the two categories of antigens which are administered into the body. Either the full-length protein or a peptide fragment of the protein acts as the antigen. The choice to use either the protein or the peptide antigen is case specific and depends on many factors [16].

There are two main methods used to express the immunogenic protein or peptide in the host plant; both were developed based on plant viruses. The first is epitope presentation systems, and the second is polypeptide expression systems. Epitope presentation systems use "short antigenic peptides fused to the coat protein (CP) that are displayed on the surface of assembled viral particles" [17]. Polypeptide expression systems "express the whole unfused recombinant protein that accumulates within the plant" [17]. In figure 1, the diagram illustrates the process of creating vaccine potatoes in simple terms. Shown in the image, the chosen plant leaf is exposed to bacteria which delivers an antigen gene and an antibiotic resistant gene into the leaf cells. The leaf containing these new genes is then exposed to an antibiotic to kill cells that do not possess the new genes. The cells that have the new antigen genes are protected by the antibiotic resistant gene. The gene-altered cells will multiply and form a clump of cells over time. This clump of cells, called a callus, will grow shoots and roots and then must be planted in the soil. After a few months, the callus will grow into an antigen-containing vaccine plant, specifically a potato in this example.

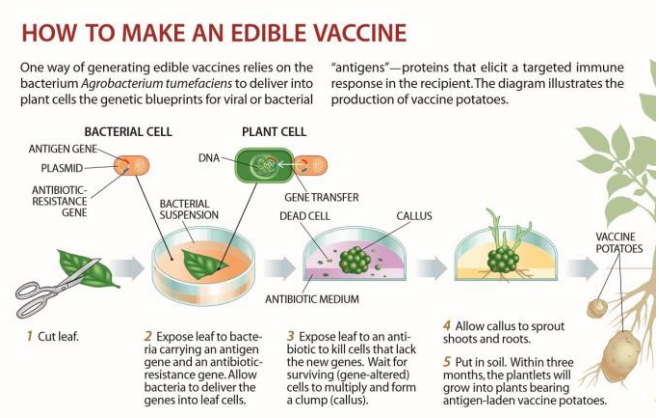

FIGURE 1 [18]
Diagram Showing Simplified Process of Making Edible Vaccines

Not all plants can be used to express proteins, due to their individual limitations, so choosing the optimal host plant to express the protein for the intended application is an important matter [19]. According to a recent article from BioTechnology Notes, "foods under study of edible vaccines include bananas, potatoes and tomatoes as well as lettuce, rice, wheat, soybean and corn." It is important that the plant chosen to contain the vaccine is pleasant-tasting, hardy, and possesses a high nutritional value. Additionally, choosing a plant that is native to the country in which it will be used is ideal [20].

The theory behind using a plant to contain the antigens is based on the rigidity of the plant cell walls. When the edible vaccines are consumed and come in contact with the stomach acid, the strong cell walls protect the antigens from breaking down. The antigens are then released in the intestines, and the mucosal immunity is stimulated in the mucosal membranes [21].

Immunity is the ability to resist an infection through specific antigens, and there are two relevant types: mucosal and systemic. Mucosal immunity is important in the gastrointestinal and respiratory tracts of the body, and receives antigens through tissue. Systemic immunity receives antigens through lymph or blood [19]. Upon introduction of the antigen into the respiratory system, large immune responses occur. Systemic responses are also stimulated, but the mucosal effect is much larger in comparison. Due to edible vaccines having the potential to stimulate both mucosal and systemic immunity, they potentially offer a higher level of protection than traditional vaccines [21].

The Federal Food and Drug Administration (FDA) Center for Drug Evaluation and Research establishes strict guidelines which must be followed when a company is attempting to have a drug approved for market release in the United States. From initial development to the end of the approval process, drugs generally take about ten years to enter

the market. There are five main phases of the drug development process: discovery and development, preclinical research, clinical research, FDA review, and FDA post-market safety monitoring. All of these steps are taken to ensure that the drugs made available to consumers are as safe as possible [22].

Clinical Research

After phase I (discovery and development) and phase II (preclinical research), phase III (clinical research) follows. Preclinical research describes the evaluation of the potential drugs in terms of toxicity and drug dosage. After detailed evidence has been collected, the drug is evaluated to determine if it is safe to begin testing on humans. The process then moves on to actual clinical research. Clinical research is typically the longest and most important part of the drug approval process. This is where the drug is tested on humans to observe how the drug interacts with the body. Trials follow a strict protocol and results must answer specific questions set forth by the FDA [22].

Recently, a large number of plant-based vaccines have entered into preclinical research along with clinical research and development. The first hepatitis B (HBV) vaccine in a plant was derived from yeast in the late 1900s. The yeast derived rHBsAg has been compared to the plant-produced HBsAg antigen, and has been tested as an injectable vaccine on mice. A study was conducted aiming to test the effect of using a potato vaccine containing the HBV antigen (HBsAg). In the trial, over the course of three weeks, mice were fed peeled potatoes containing 42 µg HBsAg once a week. Anti-HBsAg antibodies were observed in the mice after the second dose of the potato-based antigens, but not in the mice fed the yeast derived HBsAg. The antibody levels reached their maximum seven weeks into the trial and eleven weeks later, they decreased to the baseline level. The control group of mice which were fed regular potato did not experience an increase in anti-HBsAg antibodies [20]. Following the first trial, a similar trial was conducted using mice primed with potato derived HBsAg which were then boosted with yeast-derived rHBsAg. The goal of the second study was to determine if memory B cells had been established. Memory B cells are formed after initial infection and remain in the body to generate a stronger antibody response in the case of re-infection. The mice demonstrated a robust secondary response lasting over a duration of five months [20].

A double-blind and placebo-controlled clinical trial conducted on humans was carried out using a plant-derived HBV vaccine. Previously vaccinated volunteers were fed uncooked potatoes containing the antigen, with a dose of approximately 8.5 µg/g HBsAg. Over half of the volunteers, who consumed one hundred grams of the antigen-containing potato, demonstrated a significant increase in anti-HBsAg antibodies. None of the control group experienced an increase in HBV antibodies [20]. The results of the mice and human studies exhibit the potential of plant-derived vaccines to continue advancing in the research phase and eventually further. Clearly, there is still much to be learned about edible vaccines before they are released to the market for use, but with each trial the process moves further and further in that direction.

After preclinical and clinical research is collected, the process enters phase IV (FDA review) and an official application is submitted to the FDA requesting to be approved for the market. The "application contains clinical results, labeling information, safety information, drug abuse potential, patient information, and directions for use" [22]. Over the course of six months to a year, the FDA reviews the application and clinical trial data. Often times the FDA requests more information and companies have to work with them to resolve the issues before approval. Once the drug is approved, the company works with the FDA to create prescription information and then moves on to phase V: FDA post-market safety monitoring. In phase V, the FDA continues to monitor the drug and its safety to deal with any issues that may arise. They also regularly review the manufacturing and advertising related to the drug to ensure everything follows regulations [22].

Advantages and Benefits of Edible Vaccines

Edible vaccines possess significant advantages that cannot be overlooked. When compared to traditional, injectable vaccines, the benefits of edible vaccines become clear. Many of the drawbacks of injectable vaccines can be resolved by edible vaccines.

The most apparent characteristic of edible vaccines is that they can be consumed. On the surface level, this eliminates the pain and the fear of pain that some patients experience when getting injected vaccines. Furthermore, this feature makes administration easier which promotes compliance, especially among children. The edible means of administration also means there is a decreased need for medical professionals and a reduced need for sterile conditions. Edible vaccines are heat stable, so refrigeration is not required, and they can be stored near the site of use. Because of the reduced need for specific conditions, edible vaccines are economically favorable. Moreover, edible vaccines are much less expensive than traditional vaccines to mass produce and transport [20]. Another benefit of edible vaccines is their increased safety due to utilizing subunit preparation. Subunit preparation only uses a small part of the virus, rather than the entire virus, typically the part that does not pose as much of a threat [23]. With an injectable vaccine, there is a chance that the very disease that is trying to be prevented may develop within the body. Thus, the use of subunit preparation can prevent this from happening and lead to improved safety [4].

Edible vaccines exhibit another very important benefit which is their sustainability: less waste is created, less energy

is used, and societal productivity increases as more people are vaccinated. Each injectable vaccine requires energy to create, while an edible vaccine plant can be produced once and sprout new plants continuously. There is little to no waste remaining after the edible vaccine is administered, compared to injectable vaccines which require careful disposal of needles and syringes. US hospitals alone produce more than 5.9 million tons of medical waste per year, much of which comes from administering vaccines [24]. Energy used to transport this waste to a proper disposal site [25]. Eliminating the need to use energy to remove the waste can save close to 6 billion dollars each year [24]. Additionally, energy is saved because edible vaccines do not need to be refrigerated or cooked. Energy is still used to transport the edible vaccine to the desired location, however, the vaccine has the potential to be grown close to the needed location which could eliminate further use of energy. As edible vaccine researcher Jihong Liu Clarke said, "you don't need much more than a greenhouse and some seeds" [26]. This eliminates the need for transportation and the safety precautions that accompany transporting an edible vaccine, making edible vaccines a more sustainable option than traditional vaccines, which must be shipped to where they are administered.

By far the greatest benefit of edible vaccines is their ability to generate both systemic and mucosal immunity. The drawbacks of traditional, injectable vaccines contribute a significant number of people in developing countries not being vaccinated. Edible vaccines offer a solution to many of the disadvantages of traditional vaccines. Their benefits are driving the research farther towards making total vaccination against hepatitis B a reality [20].

Connection to Sustainability

Sustainability is a broad term which covers areas from construction and development to population growth and technology. When discussing vaccines, sustainability relates to creating a productive society by providing a necessity for life. The UN Report of the World Commission on Environment and Development states that "sustainable development requires meeting the basic needs of all and extending to all the opportunity to satisfy their aspirations for a better life" [27]. This means that vaccines are sustainable for society because they free people from the burden of sickness, allowing them to pursue their goals.

The effects of vaccination lead to a more productive world. The International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) is striving to eventually achieve universal vaccination, because they recognize the value of vaccination. In an article on vaccines' sustainability from the IFPMA website, the interrelated effect of vaccination on a community is simply put: "Parents can be more productive if they spend less time taking care of their sick children. Children achieve more in school if they are healthy" [3]. This idea also applies to medical professionals and supplies, which are put under strain during outbreaks. Vaccines prevent disease outbreaks through herd immunity. Herd, or community, immunity refers to when enough members of a society are immunized that the community is unlikely to face an outbreak, even if certain members are unable or unwilling to get the vaccine. Moreover, if someone in the community does become infected, the disease is unlikely to spread due to the large proportion of the community being vaccinated [28]. Vaccines reduce the frequency and intensity of disease outbreaks, allowing those affected to better receive care. FPMA effectively summarizes the sustainability of vaccines: "A vaccinated community is not only healthier; it is stronger and more productive" [3].

APPLICATIONS BEYOND HEPATITIS B

Additional Diseases

In addition to preventing hepatitis B, edible vaccines have applications for other diseases such as malaria and measles, and for stopping auto-immunity. Similar to hepatitis B, malaria is still prevalent worldwide and is a major cause of death in developing countries, with an estimated 1.5-2.7 million deaths per year. A plant-based malaria vaccine is currently being developed and three different antigens are being explored: merozoite surface protein (MSP) 4, MSP 5 from Plasmodium falciparum, and MSP 4/5 from P. yoelii.

Measles is a respiratory infection which is caused by the paramyxovirus. The disease is extremely contagious and is spread through saliva or mucus which can be transported through the air through a sneeze or cough. Measles is rare in the United States due to the effective vaccination of most of the population. Only 372 cases of measles were reported in 2018 [29]. However, approximately one million children die each year from measles around the world, and the survivors often have long lasting health effects [29]. To combat this disease, studies attempting to express the paramyxo virus protein in plants have been performed. Reports from a study conducted by Claude Muller reveal success in expressing the antigen in transgenic carrots [30].

Autoimmunity is the reaction of the immune system against healthy cells and tissue. Type I diabetes is an autoimmune disease, and over the past decade, a plant-based diabetes vaccine has been in the development process. The vaccine aims to help people predisposed to Type I diabetes. In a study, the antigen contained in potatoes and tobacco plants was fed to mice which were not obese. The results showed increased levels of the antibody IgG which is related to proteins that repress harmful immune responses, therefore suppressing the body from attacking its own health cells. The plant-based vaccines fed to mice which were diabetic "helped to suppress the autoimmune attack and to prevent the delay of high blood sugar" [20].

Use in The Veterinary Field

Edible vaccines have been applied in the veterinary field to protect domestic animals against infections caused by parasites. Similar to humans in developing countries, animals are under-protected and immunizing them is a challenge. Plant-based vaccines for livestock and domestic animals can be administered with less processing in a cruder form than those for humans. Certain parasitic diseases such as Fasciolosis, Schistosomiasis, and Cysticercosis affect cattle, sheep, swine, and sometimes humans. The livestock may become ill, unable to reproduce or grow, and even die. Edible vaccines containing antigens to immunize against these diseases have been studied in clinical trials. Researchers found that the edible vaccine for Fasciolosis generated an immune response, however further trials will need to be conducted to determine if the response was sufficient to protect against the disease. Similarly, the clinical trial for Schistosomiasis introduced a specific protein to mice via alfalfa and showed possibility of further developing the vaccine. The edible vaccine trial for Cysticercosis was similar in that it suggested an effective vaccine could be developed. The trials that have been conducted on plant-based edible vaccines for animals reveal a promising future [18].

The sustainability of edible vaccines is also showcased by their use in the veterinary field. By preventing disease in animals, edible vaccines help to ensure there is enough food to feed the population. Many resources are necessary to raise livestock such as land, money, and feed. There is enough grain in the world to feed all of the people on Earth, however, in many countries where people are starving, the available food is being fed to animals. Animals are highly valued and are in high demand in developing countries. Although raising livestock for food rather than crops seems to be contributing to the problem of hunger, it would be a disaster if something were to affect the livestock. Since so many resources are expended on raising the animals, it would be a huge waste for the animals to die from diseases. Edible vaccines are a sustainable, cost-effective solution to preventing such a catastrophe from occurring [31].

QUESTIONS AND OPPSOTION

Questions arise as to how a uniform product with the correct dosage will be manufactured, and how to ensure the same dosage will be present from plant to plant and generation to generation. There are also concerns regarding the practicality of eating raw vegetables, such as potatoes, as raw vegetables can carry diseases. Cooking the plant could potentially weaken or even destroy the antigen within, however there is little data thus far from which to make any conclusions. There is a new idea, however, that the edible vaccines within plants, such as bananas or potatoes, could be processed into a powder form. Scientists believe that the powder could maintain consistency, therefore making the vaccine more usable [32]. Selection of the optimal plant is a difficult task, as detailed previously. The plant is selected based on the specific antigen, the intended application, and location. Clearly, choosing a plant that meets all of the specifications is challenging. Another limitation of edible vaccines that should be recognized is that the stability of the antigen in the plant is not known. No data has been collected on the long-term survival of the vaccine, likely because they are such a new technology.

Increased resistance to genetically modified organisms (GMOs) is affecting the process of developing edible vaccines [21]. Furthermore, the increased opposition to vaccines in recent years could affect the future of edible vaccines. Those who oppose vaccines, dubbed ‘Anti-vaxxers’, believe the risks that vaccines pose is greater than the benefits, and that vaccines could harm their children. Individuals who forgo receiving vaccines to preventable diseases put themselves and others without vaccines at risk [33]. Diseases that were common in the past, and that many people died from, are still being seen among small groups. Although these diseases have been almost eliminated by vaccines, outbreaks still occur, and spread quickly, among those without the vaccine. For instance, in 2014, the United States observed 23 measles outbreaks. The most notable situation was an outbreak in Ohio among 383 mostly Amish individuals [34].

Because there are significant differences between edible vaccines and traditional vaccines, there is a possibility that those opposed to traditional vaccines would accept edible vaccines. However, without concrete data, only speculations can be made [24].

In spite of these doubts, scientists still see the potential of edible vaccines to reach groups of people who do not have access to standard, injectable vaccines. As a result, the prevalence of preventable diseases including hepatitis B could be lowered and millions of lives could be saved.

A BRIGHT FUTURE FOR EDIBLE VACCINES

In simple terms, edible vaccines are created by inserting a specific antigen into a plant. Currently in the clinical trial stages, the edible vaccine for hepatitis B seems well on its way to entering phase V, the consumer market.

Edible vaccines, compared to injectable vaccines, reveal advantages which many people could benefit from. These advantages including heat stability, lower cost of production, ease of transport, increased compliance, simplicity of administration, and others, solve many of the drawbacks of injectable vaccines. The drawbacks of traditional vaccines contribute to the situation of under vaccination in developing countries and the high number of deaths from hepatitis B.

The recent and ongoing research on edible vaccines has produced confident results. Studies carried out on mice that

have been fed antigen-containing plants have revealed that HBsAg antigen levels increased in their bodies following consumption of the plant. Successful immune responses to the vaccine have been documented, which continues to propel the research forward.

Edible vaccines also have applications for many other diseases and areas of medicine. For example, edible vaccines are being developed for diseases such as malaria and measles, as well as for stopping auto-immunity. Additionally, they are used in the veterinary field for protecting livestock against parasitic diseases. Studies have shown that after consuming the vaccines, the animals have demonstrated positive immune responses and antigen levels have increased. Edible vaccines have many applications beyond hepatitis B, for humans and for animals.

Questions towards specific aspects of the vaccine, as well as oppositions have surfaced. There are still unanswered questions as to how to ensure a uniform product with correct dosage, how the vaccine is affected if the plant is cooked, how the antigen survives over a long period of time, and others. Choosing the optimal plant to contain the vaccine is also very difficult. Opposition is expected from those who oppose genetically modified organisms (GMOs), which is the foundation behind the development of the vaccine. The stance on those who oppose vaccination is unknown at this time. Despite the unanswered questions and opposition, edible vaccines present an innovative, new solution to help countless individuals around the world that cannot receive traditional vaccines for a variety of reasons. Based on the advantages that edible vaccines possess, and the current clinical trial research, edible vaccines demonstrate significant potential to become a widespread disease prevention method.

SOURCES

[1] "What Is Hepatitis B?" Hepatitis B Foundation. Accessed 03.04.19 http://www.hepb.org/what-is-hepatitis-b/what-is-hepb/

[2] "Hepatitis B." World Health Organization. 07.18.2018. Accessed 03.01.2019. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

[3] "Tackling Global Health Challenges." International Federation of Pharmaceutical Manufacturers and Associations." Accessed 3.25.2019. https://www.ifpma.org/subtopics/vaccines-partnering-with-global-vaccines-community-towards-universal-immunization/

[4] W. Langride. "Edible Vaccines." Scientific American. 12.1.2006. Accessed 3.4.2019. https://www.scientificamerican.com/article/edible-vaccines-2006-12/

[5] "Prevention & Diagnosis" Hepatitis B Foundation. Accessed 03.04.19 http://www.hepb.org/prevention-and-diagnosis/transmission/

[6] "Hepatitis B: Symptoms, Causes, Transmission, Treatments, Medications & Prevention." *WebMD*, WebMD, www.webmd.com/hepatitis/digestive-diseases-hepatitis-b#1.

[7] B. Ryan. "Smokers with Hep B or C Have Higher Risk of Death." 2.21.2018. Accessed 3.7.2019. https://www.hepmag.com/article/smokers-hep-b-c-liver-cancer-higher-risk-death

[8] "Diagnosis" MayoClinic. Accessed 03.03.19 https://www.mayoclinic.org/diseases-conditions/hepatitis-b/diagnosis-treatment/drc-20366821

[9] **"**Hepatitis B Questions and Answers for Health Professionals" Centers for Disease Control and Prevention. Accessed 03.03.19 https://www.cdc.gov/hepatitis/hbv/bfaq.htm#bFAQg02

[10] "Adults Living with Hepatitis B" Hepatitis B Foundation. Accessed 03.05.19 http://www.hepb.org/treatment-and-management/adults-with-hepatitis-b/

[11] "Viral Hepatitis" Centers for Disease Control and Prevention. Accessed 03.05.19 https://www.cdc.gov/hepatitis/statistics/2014surveillance/commentary.htm

[12] "Vaccination" Hepatitis B Foundation. Accessed 03.04.19 http://www.hepb.org/prevention-and-diagnosis/vaccination/

[13] "Immunization." UNICEF. 08.18. Accessed 03.05.19 https://data.unicef.org/topic/child-health/immunization/

[14] C. Wiysonge. "Why Africa is lagging behind in child vaccination." The Conversation. 10.13.2015. Accessed 3.6.2019. http://theconversation.com/why-africa-is-lagging-behind-in-child-vaccination-48699

[15] R. Zampino. "Hepatitis B virus burden in developing countries" *World journal of gastroenterology* vol. 21,42 (2015): 11941-53. Accessed 2.5.2019 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4641116/

[16] "Antigen." MedlinePlus. 1.28.2019. Accessed 3.4.2019. https://medlineplus.gov/ency/article/002224.htm

[17] "Protein vs Peptide Antigens." Pacific Immunology. Accessed 3.1.2019. https://www.pacificimmunology.com/resources/antigens/protein-vs-peptide-antigens/

[18] W. Langridge. "Edible Vaccines." Scientific American 09.00 Accessed 03.04.19 https://www.mcdb.ucla.edu/Research/Goldberg/HC70A_W12/pdf/EdibleVaccines.pdf

[19] K. Hefferon. "Clinical Trials Fuel the Promise of Plant-Derived Vaccines." American Journal of Clinical Medicine., vol.7. Winter 2010. Accessed 3.3.2019. http://www.aapsus.org/wp-content/uploads/Clinical-Trials-Fuel-the-Promise-of-Plant-Derived-Vaccines.pdf

[20] P. Parmar. "Edible Vaccines: Applications, Advantages and Limitations." Bio Technology. Accessed 3.5.2019. http://www.biotechnologynotes.com/transgenic-plants/edible-vaccines-applications-advantages-and-limitations/627

[21] "Making 'Edible Vaccines' in Plants." Federation of American Scientists. Accessed 03.04.2019. https://fas.org/biosecurity/education/dualuse-agriculture/2.-agricultural-biotechnology/making-edible-vaccines-in-plants.html
[22] "How the FDA Drug Approval Process Works." DiabetesPac. 2.20.2018. Accessed 3.6.2019. http://diabetespac.org/fda-drug-approval-process/
[23] "Subunit Vaccines." TheVaccineMom. Accessed 03.05.19 http://www.thevaccinemom.com/vaccine-types/subunit-vaccines/
[24] R.Watson. "Waste Management Solutions for Vaccinations." Sharps Compliance, Inc. 9.21.2015. Accessed 3.25.2019. https://blog.sharpsinc.com/waste-management-solutions-for-vaccinations
[25] S. Overstreet. "INFOGRAPHIC: 10 Things to Know About Medical Waste Compliance." Sharps Compliance Inc. 01.03.18. Accessed 03.26.19 https://blog.sharpsinc.com/10-things-to-know-about-medical-waste-compliance
[26] I. Spilde. "Edible Vaccines Can Be Grown Everywhere". ScienceNordic. 8.22.2012. Accessed 3.25.2019 http://sciencenordic.com/edible-vaccines-can-be-grown-everywhere
[27] G. H. Bruntland. Chapter 2. Our Common Future: Report of the World Commission on Environment and Development. 3.20.1987. Accessed 3.25.2018. http://www.un-documents.net/ocf-02.htm
[28] vaccines.gov. "Vaccines Protect Your Communtiy". 12.2017. Accessed 3.25.2019. https://www.vaccines.gov/basics/work/protection
[29] National Center for Immunization and Respiratory Diseases, Division of Viral Diseases. "Measles (Rubeola)". 3.11.2019. Accessed 3.25.2019. https://www.cdc.gov/measles/index.html
[30] C. Muller. ScienceDirect. "Immunogenic Measles Antigens Expressed in Plants: Role as an Edible Vaccine for Adults". 6.2003. Accessed 3.25.2109. https://www.sciencedirect.com/science/article/pii/S0264410X02006060?via%3Dihub
[31] R. Oppenlander. "Animal Agriculture, Hunger, and How To Feed A Growing Population." Forks Over Knives. 8.20.2013. Accessed 3.27.2019. https://www.forksoverknives.com/animal-agriculture-hunger-and-how-to-feed-a-growing-global-population-part-one-of-two/#gs.38s9z
[32] G. Levi. "Vaccine Cornucopia". NCBI. 11.15.2000, Accessed 3.25.2019 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1083775/
[33] S. Jacob, S. Cherian, T. Sumithra, O. Raina, M. Sankar. "Edible vaccines against veterinary parasitic diseases—Current status and future prospects". Vaccine., vol.31. 4.8.2013. Accessed 2.17.2019. https://doi.org/10.1016/j.vaccine.2013.02.022
[34] S. Novella. "The Anti-Vaccination Movement." Skeptical Inquirer. 11/12.2007. Accessed 3.4.2019. https://www.csicop.org/si/show/anti-vaccination_movement

ADDITIONAL SOURCES

R. Khamsi. "Potatoes pack a punch against hepatitis B". Nature. 2.14.2005. Accessed 1.16.2019. https://www.nature.com/news/2005/050214/full/news050214-2.html
H. Marshall. "Edible vaccine for hepatitis B". ScienceDirect. 2.1.2001. Accessed 1.16.2019 https://www.sciencedirect.com/science/article/pii/S147149060001855X

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