Undergraduate Research Journal
University of California, Merced

Novel Medical Treatment using known Vaccines and further Investigation to Research a Cure for: Herpes Simplex Virus – Type 2

By: Jaskanwaljeet Kaur

University of California, Merced

Author's Note

Jaskanwaljeet Kaur, School of Natural Sciences, University of California, Merced
I would like to thank Miriam Barlow, who provided insight and expertise that greatly assisted my research topic and for comments that greatly improved the manuscript over all.
Correspondence concerning this research should be addressed to Jaskanwaljeet Kaur.
Contact: jaskanwaljeetkaur@gmail.com

Abstract

Viruses were first discovered in the 18th century, but many aspects of viruses are still questionable, such as the way some viruses replicate, infect or reproduce. Overtime, the scientific community has been able to demystify the virus by first describing its structure, the various types of viruses present in the environment and eventually discovering vaccines. Albeit this research, there are still many incurable viral infections, many of which are sexually transmitted. One such sexually transmitted infection is Herpes Simplex Virus, Type 2. The Herpes Simplex Virus is a highly contagious virus that is passed on from one person to another via direct contact, i.e. sexual contact with multiple partners, having another sexually transmitted infection (STI) or a weakened immune system. Once contracted, the HSV-2 will always live in an infected individual's nerve cells, and can cause regular outbreaks of symptoms in the genital areas. There are a few medications that can possibly be used in order to control the spread of the virus, but none that can diminish or eliminate the virus completely overtime. Thus, in order to design a vaccine that can potentially cure the virus is the primary goal. This paper defines the various techniques previously used in order to diagnose and treat the virus, and goes on to further explain by giving experimental techniques of how those methods can be applied together to research a possible cure for HSV-2.

Novel Medical Treatment using known Vaccines and further Investigation to Research a Cure for: Herpes Simplex Virus – Type 2

Genital herpes, a double-stranded DNA (dsDNA) virus, is one of the most commonly sexually transmitted diseases throughout the world, and is also the primary cause of genital ulcer disease (GUD) in both underdeveloped and developed countries. Genital herpes is caused by herpes simplex virus type 2 (HSV-2), and approximately fifty to sixty million adults in the United States are infected. Although millions of people suffer from HSV-2, there is no set cure for the disease, but there are vaccines, such as acyclovir or valacyclovir, to control the spread of the virus in the body (Roxby, 2011). HSV-2 is a problematic virus, mainly because getting infected with it, invariably makes the body susceptible to human immunodeficiency virus type 1 (HIV-1). This especially has implications during pregnancy for women and their children; and if caused later in the pregnancy, potentially result in neonatal encephalitis[1] (Roxby, 2011). In a study conducted in Niarobi, Kenya, a total of 296 pregnant and postpartum women were evaluated for being either: HSV-2 seropositive[2], HSV-2 seronegative or suffering from GUD. These three criteria were evaluated in order to understand the implications these aspects have on HIV-1 transmission, as well as transmission of opportunistic infections (OI) that often occurs with many of the sexually transmitted infections. It was thus seen, that women who had GUD or were HSV-2 seropositive, were more likely to experience an OI (Roxby, 2011). But HSV-2 seropositive women with GUD

[1] **Neonatal encephalitis** is inflammation of the brain due to an infection in an infant's brain.
[2] A **Seropositive** result that indicates a positive response to blood serum levels, in response to the presence of viruses.

may differ from co-infected women with asymptotic[3] HSV-2; it is also possible that symptotic genital ulcers may identify with co-infected persons with varied immunologic characteristics from those who are HSV-2 seropositive but do not develop/recognize GUD. Therefore, GUD could be a potential marker for HIV-1 disease progression (Roxby, 2011). In another study cited by the paper, patients who had GUD, noticeably had higher plasma viral loads than those who were HSV-2 seropositive without GUD. Thus, if a patient does have GUD, treatment for it has been shown to increase the long-term survival of HIV-1 infected patients (Roxby, 2011). This study suggests that women who have GUD are a subgroup that may benefit from the anti-herpes treatment, and the treatment may be helpful with keeping co-infections from occurring or spreading. There has been a significant amount of research performed recently on HSV-2, in order to come up with a cure or better vaccine for it. Some ways for encountering the virus are by: using siRNA[4]-based microbicide, balancing $CD4^+$ and $CD8^+$ glycoprotein responses in the body to fight against the virus, using non-replicating dominant-negative HSV-2 virus vaccine, and using subtilosin from other bacteria's like *Bacillus amyloliquefaciens.*

In the research article, *An SiRNA-based Microbicide Protects Mice from Lethal Herpes Simplex Virus 2 Infection*, the main purpose was to show vaginal instillation of small interfering RNA's (siRNA) targeting HSV-2 (UL27 and UL29 genes), which in turn protected the mice from lethal HSV-2 infection (Palliser, 2005). The usage of siRNA's was tolerated well by the mice

[3] **Asymptotic** infection is the ability of some viruses, such as HSV -2, to be present and transmissible without causing any outward symptoms, causing the body to not recognize the virus as a foreign invader.
[4] **siRNA**, also known as Small interfering RNA's are a commonly used RNA interference tool, employed in order to induce short-term silencing of protein coding genes.

because it did not induce interferon-responsive genes or cause inflammation and protected mice when administered before/after lethal HSV-2 challenge. The results of this paper, thus suggested that siRNA's might just be useful and active component of a microbicide designed to prevent viral infection or transmission (Palliser, 2005). Another way of going about the cure for herpes was researched through the enhancement of the cytotoxic T-cell response, which was accomplished through polypeptides that were modified with the usage of codon optimization algorithm. The reason behind using this algorithm was to show how these codon-optimized ubiquitinated[5] and non-ubiquitinated constructs encoding the same viral envelope protein. Glycoprotein D (gD2) was then induced to B and T cell responses to help protect against the lethal viral challenge and reduce ganglionic latency (Dutton, 2013). The results demonstrated that the mixing of codon-optimized ubiquitinated and non-ubiquitinated gD2-encoding constructs produce a balanced humoral and cellular response, which were seen in the article by the high survival rates in the mouse model of HSV-2 infection and to reduce latent viral DNA load in the dorsal root ganglion (DRG), which gives hope that a potential vaccine could help reduce viral latency and shedding (Dutton, 2013).

The next suggestion for a cure stems from the article, "*A Herpes Simplex Virus 2 (HSV-2) Glycoprotein D-expressing Nonreplicating Dominant Negative HSV-2 Virus Vaccine Is Superior to a GD2 Subunit Vaccine against HSV-2 Genital Infection in Guinea Pigs*". This article highlights that instead of using the gD2 subunit vaccine, the HSV-2 expressing non-replicating dominant-

[5] An **ubiquitinated** molecule is modified or degraded, based on the attachment of a small protein, ubiquitin, which serves as a tag for ubiquitination

negative vaccine can possibly be used. The potential viral vaccine investigated in this paper is called CJ2-gD2. It expresses gD2, a major HSV-2 antigen, as efficiently as the wild-type HSV-2 infection, and can lead to a 500-fold reduction in wild-type HSV-2 viral replication in cells co-infected with CJ2-gD2 and wild-type HSV-2 (Pengwei, 2014). This article depicted that CJ2-gD2 evokes a much stronger response to various HSV-2 antigens, and is very effective in preventing primary/recurring HSV-2 genital infections/disease in immunized guinea pigs. The article was able to prove via experimental methods with guinea pigs that using CJ2-gD2 could be useful in the future, but further research to enhance its response might help come up with a better solution (Pengwei, 2014).

The most recently researched cure or enhanced vaccine for herpes is known as subtilosin, which is a ribosomally synthesized peptide by bacteria *Bacillus subtilis* and *Bacillus amyloliquefaciens.* Subtilosin is a bacteriocin[6] that is of importance because of its application in contraception and reproductive health due to its unique properties such as: natural origin, biodegradability, antimicrobial properties, overall safety and ease of production (Quintina, 2014). To see how subtilosin works, a virus yield inhibition assay was used and its results indicated that noncytotoxic concentration of subtilosin inhibits HSV-2 replication in Vero cell[7] cultures. Subtilosin was able to strongly inhibit extracellular and total virus production. Even though the viral glycoprotein D (gD) expression level remained unaffected by subtilosin, an altered pattern

[6] **Bacteriocins** are proteinaceous toxins produced by bacteria in order to inhibit the growth of similar or closely related bacterial strains.

[7] **Vero cells** are lineages of cells used in cell cultures and are primarily isolated from kidney epithelial cells extracted from an African green monkey.

of gD intracellular localization was detected in subtilosin-treated culture. Therefore, it was concluded that subtilosin displayed antiviral and virucidal actions against HSV-2, and it affected the late replicative cycle such as viral glycoprotein intracellular transport (Quintana, 2014). Overall, there have been many several attempts made in order to diagnose to determine a cure for herpes, but so far the techniques that are known are not thorough enough and require further research in order to perform better results. A question that might help further HSV-2 research is: Is it possible for the above described research methods, to merge together in order to obtain a cure that is able to diminish HSV-2 overtime in infected individuals?

Experimental Procedures

Experiment #1: Using Cohorts to Study HSV-2

1. Obtain vaginal HSV-2 samples and plasma samples from the following groups of people: sexually active women (between the ages of 18-25) and pregnant women. Check for any other STI's beforehand along with HIV-1 infections, since this could indicate how much the virus has spread throughout the body. Samples should contain ~200 individuals in each category.

2. Detect the HSV-2 antibody using enzyme-linked immunosorbency assay (ELISA[8]) and repeat results with western blot[9] to make sure the results are correct.

3. Check for other STI's as well since they could potentially contribute to a bigger viral load.

[8] **ELISA** also known as enzyme-linked immunosorbency assay is laboratory method that measures the concentration of an analyte, mainly antibodies or antigens, in a given solution.

[9] The **Western blot** technique is used to identify specific amino-acid sequences in proteins.

4. Once all their history is performed give them HSV-2 vaccines, such as acyclovir and/or valacyclovir, and monitor how these vaccinations help the patients and especially monitor if they do not work on certain patients, since those patients most likely posses a different strain of herpes.

5. Lastly, perform this study for about one year in order to detect any changes in the bacterial load with the viruses given to both healthy individuals and pregnant women. Pregnant women should be monitored past the first year, merely to see if the baby contracted herpes through transmission inside the mother's body.

All in all, this experiment will detect any new strains of the virus that humans might have to fight against. This experiment also helps with seeing transmission of the virus between the mother and the baby. The transmission does not always occur, but when it does, it is proven to be deadly for the baby. Checking for other STI's is a good idea before performing the experiment, because knowing that there other are other infections in the body besides herpes gives a new understanding of how the virus replicates in the body. HSV-2 makes the body susceptible for HIV-1 transmission. Thus, if for example, an individual has both HSV-2 and another STI, it makes the body even more vulnerable to HIV-1. Therefore, the experiment is potentially showing us: any new strains of HSV-2, whether all the babies of pregnant women studied had HSV-2 transmission from their mother's, and how the bacterial load is affected with the known medications.

Experiment #2: Designing a Long-Term Vaccine

Vaccines for viruses are difficult to produce, primarily because viruses have the potential to replicate quickly and can change their replication methods such that vaccines and cures eventually become ineffective. Albeit this, researchers continue to test various new vaccines against the HSV-2. The best animals to test HSV-2 on are guinea pigs, mainly due to their similar lesions to the ones in the human body when infected with HSV-2. Recently discovered viral vaccine (CJ2-gD2) and subtilosin from the *Bacillus* species, show that there might just be a way to use both of these simultaneously in order to produce a better cure for HSV-2.

There are 3 ways in which to infect guinea pigs with these potential viral vaccines:

1. Inject a small amount (~10µl) of just CJ2-gD2 into an infected guinea pig.
2. Inject a small amount (~20µl) both CJ2-gD2 and subtilosin into an infected guinea pig
3. Inject subtilosin (~20µl) of just subtilosin into an infected guinea pig.

Once all of the guinea pigs are injected with the potential vaccines, all of them should be monitored for the results that they yield. Subtilosin is a vaccine that could be better used (if used individually) in the later stages of HSV-2 infection, as stated in the paper. If subtilosin was injected in early stages of the HSV-2 infection it did not work as efficiently. But when it was affected in the later stages of the HSV-2 infection, it was able to lower the viral load significantly. This is the main reason why CJ2-gD2 and subtilosin could work together: CJ2-gD2 is a vaccine that helps reduce the viral load as soon as it is injected in the test animal, i.e. guinea pig, and subtilosin could be used to keep the viral load low if the virus were to resurface again. This way, if both the vaccines work together individuals who have HSV-2 could very well just take a pill and

be able to keep the viral load at bay. This does not mean that HSV-2 could be fully cured. There is still more research needed in order to be able to fully rid the human body of the virus.

Experiment #3: Designing a new Type of Microbicide

Microbicides are compounds that can be applied inside the vagina or the rectum to protect against sexually transmitted infections. A good way to start a new microbicide is to look at various components that are needed in order to make it successful. The female vagina already has a low pH, so that pathogenic organisms are unable to survive and thrive there, yet there are still persistent STI's and viruses that are able to penetrate that low pH barrier. Thus, to start with, products that are able to enhance the natural vaginal defenses such as acid-buffering agents or antimicrobial peptides could be used. The main reason that the female body is susceptible to STI's and viruses is due to the neutralization of this acidic environment by alkaline semen for up to six hours after intercourse (Keller, 2005). The best to prevent the HSV-2 infection is to stop it from establishing in the first place.

1. Using a model organism, potentially guinea pigs because their similarity to humans is helpful, inject it with nonoxynol-9 (N-9). N-9 is primarily a spermicide, a good barrier to pregnancy because it attacks the acrosomal surface of the sperm, effectively breaking it down and rendering the sperm unable to fertilize the egg.

2. Once the organism has been injected with N-9, infect it with the HSV-2 strain.

3. Then, monitor the organism for a few hours before sampling the organism to see whether HSV-2 was able to infect the organism despite the N-9 barrier against viruses and STI's.

This experiment should be helpful in understanding whether a simple microbicide is good enough against transmission of various sexually transmitted viruses and bacteria. If not, other known microbicides could be used together, or newer ones could be diagnosed to help prevent the transmission process, which after all, occurs due to the neutralization of the vagina after intercourse.

Conclusion

Overall, there has been a lot of research previously performed in order to find a cure or a long-term vaccine for viruses for a long time. For example, HIV-1 evolves so often that there is a new vaccine for it every few years. The constant evolution of HIV-1 thereby makes it a difficult virus to research a cure for since it changes so often. The research being undertaken to cure sexually transmitted viral infections is premature, and there is a long way to go before a definite answer can be given in terms of constant vaccine, let alone a cure, but till that time anti-viral vaccines are being manufactured constantly, despite the fact that they are a long way from permanently curing a given viral infection, such as HSV-2.

References

Dutton, Julie L., Bo Li, Wai-Ping Woo, Joshua O. Marshak, Yan Xu, Meei-li Huang, Lichun Dong, Ian H. Frazer, and David M. Koelle. "A Novel DNA Vaccine Technology Conveying Protection against a Lethal Herpes Simplex Viral Challenge in Mice." *Plos One:*. N.p., 3 Oct. 2013. Web. 28 Nov. 2014.

Palliser, Deborah, Dipanjan Chowdhury, Qing-Yin Wang, Sandra J. Lee, Roderick T. Bronson, David M. Knipe, and Judy Lieberman. "An SiRNA-based Microbicide Protects Mice from Lethal Herpes Simplex Virus 2 Infection." *Nature.com*. Nature Publishing Group, 23 Nov. 2005. Web. 28 Nov. 2014.

Quintana, V. M., N. I. Torres, M. B. Wachsman, P. J. Sinko, V. Castilla, and M. Chikindas. "Antiherpes Simplex Virus Type 2 Activity of the Antimicrobial Peptide Subtilosin." (n.d.): n. pag. *Wiley Online Library*. Journal of Applied Microbiology, 23 Aug. 2014. Web. 1 Dec. 2014.

Roxby, Alison C., Alison L. Drake, Grace John-Stewart, Elizabeth R. Brown, Daniel Matemo, Phelgona A. Otieno, and Carey Farquhar. "Herpes Simplex Virus Type 2, Genital Ulcers and HIV-1 Disease Progression in Postpartum Women." *Plos One*. N.p., 26 May 2011. Web. 28 Nov. 2014.

Zhang, Pengwei, Lining Xie, John W. Balliet, Danilo R. Casimiro, and Feng Yao. "A Herpes Simplex Virus 2 (HSV-2) Glycoprotein D-expressing Nonreplicating Dominant-Negative HSV-2 Virus Vaccine Is Superior to a GD2 Subunit Vaccine against HSV-2 Genital Infection in Guinea Pigs." *Plos One*. N.p., 30 June 2014. Web. 28 Nov. 2014.

Keller, Marla J., Ana Tuyama, Maria Josefina Carlucci, and Betsy C. Herold. "Topical Microbicides for the Prevention of Genital Herpes Infection." *Journal of Antimicrobial Chemotherapy* 55.4 (2005): 420-23. *Oxford Journals*. Medicine and Health, 2 Mar. 2005. Web. 1 Dec. 2014.