Dana Foundation Design a Brain Experiment Competition
2015 Second Place

Exploring the HMGB1 Inhibitor Glycyrrhizin as a Potential Cure for Multiple Sclerosis

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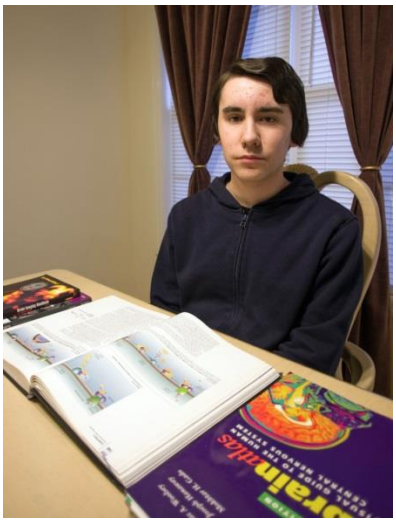

Lay Summary (Provided by the Dana Foundation):

Multiple Sclerosis (MS) is a devastating immune-mediated progressive disease of the brain and spinal cord (the Central Nervous System) with limited treatment options and no known specific cause. MS is an 'autoimmune' disease, where the body's immune system mistakes healthy tissue for foreign substances and attacks them, producing devastating consequences. In MS, the immune system targets the protective covering called myelin that surrounds nerve cell axons (communication cables) in the brain and spinal cord. The immune inflammation damages myelin and disrupts communication between cells, resulting in a wide range of physical and cognitive symptoms. Current treatments can reduce these symptoms, but many have difficult side-effects, and none directly address the cause at a neural level.

A protein called HMGB1 has been associated with inflammation in MS, and therefore serves as a "biomarker," or a target for drugs. The proposed study focuses on glycyrrhizin, an agent that binds directly to the HMGB1 protein biomarker and shuts down the immune response that produces symptoms in MS. This proposal uses an animal model of MS called experimental autoimmune encephalomyelitis (EAE). EAE in rats produces symptoms similar to those of MS. If glycyrrhizin administered to EAE in rats successfully binds to HMGB1, it would block the protein's inflammatory actions and improve symptoms. If glycyrrhizin is found to improve symptoms in the animal model, it could be tested in people with MS and possibly other related autoimmune disorders.

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Purpose:

To test the effects of the high-mobility group box 1 (HMGB1) inhibitor Glycyrrhizin on rats induced with experimental autoimmune encephalomyelitis (EAE) to mimic the effects that Glycyrrhizin might have on patients with Multiple Sclerosis. The goal of this experiment is to investigate if EAE can be effectively treated with Glycyrrhizin, and if so, if Multiple Sclerosis can be combatted using Glycyrrhizin.

Hypothesis/Question:

Employing the direct HMGB1 inhibitor Glycyrrhizin will combat the inflammatory cytokine activity of HMGB1 that is a primary etiology of demyelinating autoimmune diseases. This inhibitor will stop the demyelination and inflammation of Multiple Sclerosis.

Background:

Affecting approximately 2.3 million people worldwide, Multiple Sclerosis (MS) is one of the most debilitating autoimmune diseases ("MS the Disease"). Patients suffering from this disease begin to have obstructed communication between their brains and the rest of the bodies, making it in some cases nearly impossible to accomplish even some of the simplest of tasks— with side-effects ranging from slight gait abnormalities to more severe symptoms, such as thinking problems and blurred or doubled vision ("MS Symptoms").

It is estimated that one out of every two MS patients experiences some form of cognitive difficulty, and eight out of every ten patients experience bladder issues ("MS Symptoms"). But even more traumatizing and dangerous problems arise from MS as well. In some cases, MS can cause respiratory issues, seizures, and even complete hearing loss ("MS Symptoms"). Currently, a specific cause of MS is unknown. However, numerous proteins and substances have been speculated to be MS progenitors. In MS, body tissues are attacked by the immune system, including the protective layer that insulates our neuron's axons, which is made out of myelin ("Multiple Sclerosis: Causes", 2014). One important factor in this demyelination process has recently been discovered to be the biomarker HMGB1.

While HMGB1 is involved in normal bodily functions, such as enhancing DNA transcription, it has been found that HMGB1 can evoke numerous harms to the body such as cancer, and practically every demyelinating disease (Rosenberg, H., 2008; Sims, G. et al., 2010). HMGB1 begins wreaking havoc on the nervous system by being released from activated macrophages, and acts as a damage-associated pattern recognition molecule (DAMP). DAMP molecules can invoke immune response in noninfectious inflammatory responses. Essentially, HMGB1 causes inflammation in the body (Srikrishna, Geetha et al., 2009).

This inflammation precipitates demyelination of nerve fibers throughout the brain and spinal cord, resulting in axonal damage (Brück, W., 2005). When this demyelination occurs, the disrupted communication between one's brain and the rest of their body, a hallmark trait of MS, is triggered. Several methods may be used for the inhibiting of HMGB1, but none is better at the task than the direct HMGB1 inhibitor Glycyrrhizin, which has been implicated in the treatment of altering the pathogenesis of numerous other diseases, including Cirrhosis and Hepatitis.

Glycyrrhizin is a natural inhibitor that originates from the root of the herbaceous plant Glycyrrhiza glabra. Research conducted by Mollica et al. shows that Glycyrrhizin binds directly to the surface of HMGB1, thereby stopping the demyelination from continuing, while still having minute interference with the effects HMGB1 has on DNA transcription. As a result, Glycyrrhizin is an area of interest for combatting inflammatory demyelinating diseases. Unlike many other inhibitors of HMGB1, Glycyrrhizin also directly blocks all of the extracellular cytokine functions of HMGB1, which are responsible for the inflammation that causes nerve damage in MS. Thus, Glycyrrhizin is an ideal candidate for fighting MS.

Methodology:

In this experiment, the chosen method for investigating if Glycyrrhizin may be used as a treatment method for the demyelinating disease MS is inducing rats with experimental autoimmune encephalomyelitis (EAE). The reason for using rats is because the effects that Glycyrrhizin will have on rats with EAE is relatively homologous with that of human MS patients. EAE will be used as it is the current prevailing and most commonly used experimental model of MS. Current methods of inducing rodents with EAE use antigens such as spinal cord homogenate (SCH) and myelin oligodendrocyte glycoprotein (MOG), but these present methods only give limited imitation of MS, whereas HMGB1 has been observed to be directly involved in the demyelinating process.

This experiment will begin by giving the specimen an excess of HMGB1, which will cause inflammation and demyelination. This study will take place over the course of 12 months, because the average MS patient can expect to live 25-35 years after receiving a diagnosis of MS (Rose, J. et al.). This length of time in human lifespan is equivalent to 12 months in a rat's lifespan. Two control groups, each composed of 5 rats, will be analyzed and studied over the course of the 12 month experiment. They will be labeled Group A and Group B. Rats in Group A will be given Glycyrrhizin starting from the beginning of the second month of the experiment. Group B rats will be treated with corticosteroids at the same time. The chosen procedure of treatment is corticosteroids because they are a common method in lessening inflammation caused by MS that is used currently. By doing this, the difference in effects the two substances have on EAE can be studied simultaneously. However, corticosteroids have no effect on the overall course of MS. Group A will be studied to understand if Glycyrrhizin does effect the prognosis of MS.

Every 2 weeks, after being treated with Glycyrrhizin and corticosteroids, the two groups of rats will be scanned using magnetic resonance imaging (MRI) and will be studied closely to see if symptoms caused by EAE, such as walking difficulties, have been lessened or even cured entirely. MRI is the chosen apparatus due to its noninvasive nature. MRIs will allow for slight or drastic changes in their brain to be measured nearly effortlessly. Then the progress of their treatment can be studied in great detail. At the end of 6 months, both groups of rats will be examined rigorously to allow scientists to see if demyelinating has been stopped by Glycyrrhizin inhibiting HMGB1. By the end of 12 months, their symptoms and progress will be examined even further to understand if and how the course of EAE has been altered in them.

Results:

If this experiment goes according to plan, the desired results are to acquire a more thorough understanding of the effects HMGB1 has on MS and demyelinating autoimmune diseases in general. The best possible result would be to have enough knowledge gained through rats treated with Glycyrrhizin to have the ability to one day treat patients suffering from MS.

At the end of the 12 month experiment period, MRI scans will identify any changes in the brains of each rat. The MRIs of Group A rats will be compared to those of Group B. If a juxtaposition is noticed, then the differences obtained may be used either for possibly developing a novel drug that can treat MS with the same potency and effectiveness that Glycyrrhizin has been postulated to do in this experiment, or marketing Glycyrrhizin as an effective treatment method for MS patients.

The key factors of change which will be looked for in the MRI scans are alterations in the abundance of brain lesions, which is an apparent sign of MS. If the same amount of lesions in a specimen's brain is still present after the experiment as before it, the hypothesis of the experiment will obviously be wrong, and inhibiting HMGB1 will have been found to be unhelpful for MS patients.

If such a result occurs, then Glycyrrhizin would prove to not be of any help in the alleviation of MS symptoms. However, if a strong difference is noted, then it will be discovered that Glycyrrhizin could be a possible cure for MS.

Conclusions:

Using a readily available substance to combat one of the worst diseases in the world seems like a lofty feat to accomplish. With the results of this experiment, however, this objective may be attainable in the span of just a few years, given the proper funding and research attention. By inhibiting a root cause of demyelination and inflammation, Glycyrrhizin could substantially counter the crippling diagnosis of MS which about 200 Americans each week receive (Lee, J. 2015). Curing MS by Glycyrrhizin binding to HMGB1 and stopping its cytokine activities of inflammation and demyelination is hypothetically possible, and can be proven to be a reality with this experiment.

The millions worldwide suffering today could finally receive the long-awaited proper treatment and cure for MS. But the positive implications of Glycyrrhizin could extend far beyond just MS. Glycyrrhizin could be applied to practically any demyelinating autoimmune disease, and would allow the world to further understand the role of proteins in demyelination. If my hypothesis proves to be correct, further research about Glycyrrhizin would need to occur in order to develop a novel, low-cost, obtainable drug, which could treat a variety of different autoimmune diseases. This research could use similar methods of studying Glycyrrhizin, such as those used in this experiment. However, this experiment does lay a foundation for discovering a cure for MS, which could possibly be Glycyrrhizin. While MS is an extremely difficult disease to understand, and the number of MS patients continues to climb every single day, this experiment is one step in the fight to abate MS.

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