Metal Intake & Exposure Manipulation on Prion Aggregation and Disease Pathogenesis: Analysis & Experimental Paradigm

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Abstract

There has long been ambiguity regarding the biomolecular solutions for mitigating prion aggregation and development in vivo, much of which has stagnated in early experimental phases. To understand the main function of metallic cofactors in the development of neurodegenerative diseases, researchers have been conducting preclinical trials on mice, and have found that metal intake coupled with high oxidative stress positively correlates with increased rates of prion related neurodegeneration. The purpose of this study is to outline the general pathogenesis of prion diseases, and propose possible epidemiological and biomolecular guidelines to limit prion aggregation and prevalence rates. This study was primarily conducted through a series of literature reviews in order to consolidate the hypothesis that changes in metal exposure can indeed change the rates of prion disease pathogenesis. The results of the literature reviews generally supported the claim that reducing/increasing metal intake to evolutionarily required concentrations and limiting aqueous metal ion exposure will aid in the reduction of disease development and prion aggregation, which allowed the researcher to come to the conclusion that manipulating the influx and exposure to metal is an epidemiologically viable option for reducing the rampant rates of prion related death, ultimately reducing clinical and financial stress on the global healthcare setting. The aforementioned literature review was then used to design and outline a possible experimental paradigm to test the manipulation of metal levels in mouse brain homogenate in vivo.

Introduction

The purpose of this paper is to analyze the effects of metallic alteration on neurotoxicity, create applicable guidelines that will aid in reducing case severity, and to better enable the medical industry to cope with patients diagnosed with prion disease. In the current medical landscape, nearly 120,000 people die each year due to neurodegenerative disease in the United States, while worldwide, a new person is diagnosed with prion disease every three seconds, which bears a heavy toll on both associated persons as well as the already strained medical infrastructure. The rampant development of prion disease in modern society has led to aided living capabilities stretching too thin, causing a surplus of uncared for patients, making it of utmost importance to understand the role of metals in prion disease, and how they can be manipulated to reduce the effects of said disease. Till date there has not been a set of solidified and methodical guidelines in order to reduce the copious amount of cases being diagnosed; however, multiple clinical trials and case studies have yielded an imperative piece of information, which is that metal intake is a key factor in the development of prion disease (18). Metal intake primarily focuses on copper, zinc, and manganese, all of which can be readily oxidized, playing an important role in the development of prion disease; however, through manipulating these concentrations, the possibility of manipulating the development of prion disease becomes a reality. Reducing the amount of cases through metal concentration alteration and health & safety procedure implementation can reduce the strain on global medical infrastructure, as well as reallocating costs to other needed sectors such as the research of other rampant diseases.

Background

A Prion is essentially a misfolded protein, which is made up of *a-synuclein* and *b-synuclein*. The uncoiled shape of the protein allows it to bind to octapeptides and spread throughout the brain. Prions–in their stable state–exist in all mammalians; however, chemical imbalances in the brain can cause them to misfold, which indicates that instead of amino acids being in a sheet form, they will look similar to the coil of a spring. The mentioned coil allows for them to bind to other prions and begin a chain reaction which can cause rapid neurodegeneration (see Figure 1). The mechanism for why a prion is so malevolent is split into two aspects; neurotoxicity, and infectivity. Infectivity is its namesake, which is the ability for the prion to spread through a "domino effect," on neighboring proteins. Neurotoxicity on the other hand, is a complex process which allows prions to degrade other cells, hence neurodegeneration. It is imperative to understand what influences neurotoxicity in a prion, and how it can be manipulated to reduce the spread of said diseases. A keystone benchmark in conquering the spread is to determine the role of metals in the development of the disease. The role of metals in prion disease have not been much explored till date, as the prion itself has only been studied very recently, and metal cofactors have been among the newest addition to the information on prions. Although a relatively unexplored topic, many metals such as copper, zinc, aluminum, and magnesium have been known to act as cofactors in a prion's development, and ultimately spurring on neurotoxicity. The essential question that needs to be asked is, is it possible to manipulate the concentration of metals in a patient to successfully reduce the onset of neurodegenerative diseases? Based on current anecdotal evidence, it can be claimed that reducing overexposure to metal levels through diet and environmental exposure will be able to reduce the effect of neurodegenerative diseases.

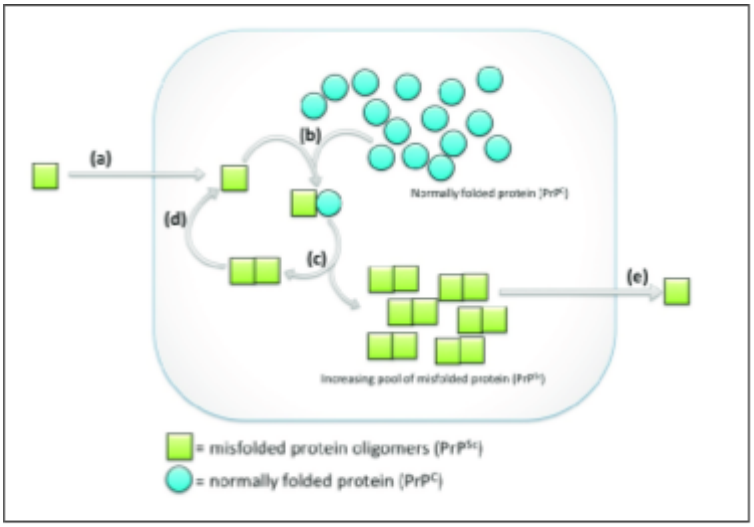

Figure 1, https://creativecommons.org/licenses/by/4.0/

Risk Factors & Aqueous Metal Ions

Metal overexposure has been a common trend starting from the industrial revolution up until the twenty-first century, and its effect on the human brain has just started to come to light. Biometals, such as Copper and Zinc are extremely common metals found in the human brain and are often found to be oversaturated within the average person (4). These metals are found in quantities of micro-molars (μm) where in such miniscule quantities, metals have the ability to bind to octapeptides. Binding to the octapeptides allows them to directly access the synaptic cleft and alter the neurotoxicity of a potentially benign prion protein also known as PrP (16). Neurotoxicity may increase based on the redox reactions that occur when biometals are oxidized/reduced in the synaptic cleft. The neurotoxicity of PrP is what creates the degenerative effect common in prion disease (2). Metal ions do not always have the ability to turn PrP

neurotoxic and the aforementioned ability is highly dependent on the role of cofactors. Cofactors are essentially the environmental surroundings in the octapeptide, and may significantly influence neurotoxicity. Examples include chemical makeup, temperature, pressure, and stressors like concussions (2). Although there are varying cofactors, the vast majority of the time neurotoxicity increases with the addition of biometals (18). Inevitably, the potency of prion disease increases alongside neurotoxicity further strengthening said disease.

Metal overexposure is extremely common in occupations such as construction and welding, where gaseous fumes from metal soldering can be inhaled, reaching the brain's neural fluids, and eventually the synaptic cleft. Neurodegenerative diseases were found to be rampant in those who work in a construction oriented profession (16). As such, general guidelines for construction oriented professions would be to integrate large fume hoods and mask mandates while being in an enclosed area where soldering is taking place (4). Work hours should also be strictly enforced, as prolonged exposure even with guidelines may prove to be risky. The risk associated with prolonged exposure even with guidelines, would be the reduced effectiveness of equipment. Masks and fume hoods require filtration capacity and energy respectively, and exhausting these elements would be unsafe for the workers themselves as the ability for fumes to penetrate the brain increases (5). Fundamentally, reducing direct exposure to metals will likely be the strongest option in combating metal induced prion disease at the source.

The chemical aspect of metal overexposure cannot be understood without the method in which it occurs. Most commonly, contaminated food leads to excess ion accumulation in the brain (18). An example of this would be a crop such as potatoes, grown with water that is saturated with high concentrations of mercury or copper. This can occur in a multitude of ways, such as faulty pipeline transmission, and corporate runoff (14). An important factor to note would also be common drinking water. Pipelines with excessive corrosion will lead to large amounts of heavy metals leaching into the water supply, such as lead, mercury, copper, zinc, and iron (20).

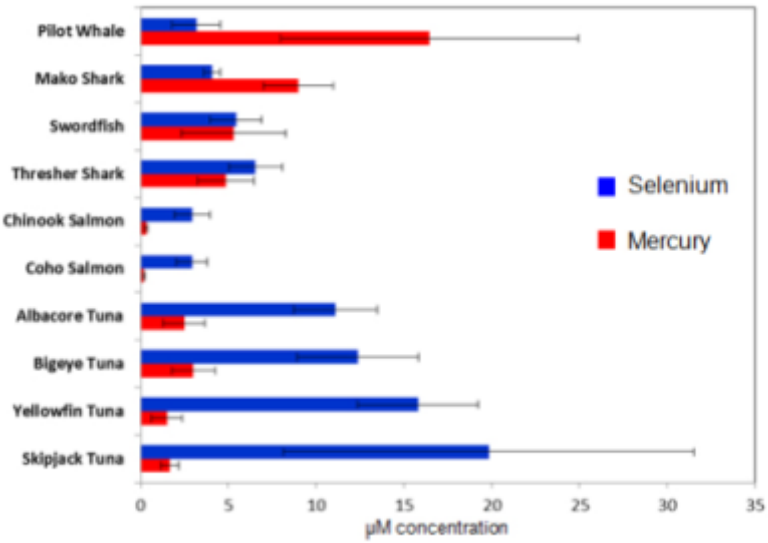

Figure 2, https://atuna.com/pages/mercury

This amount of ion accumulation over a period of numerous years can oversaturate the brain and affect the spontaneity of redox reactions by increasing the frequency of which metals are oxidized. In addition, rates of prion disease have been found to be significant in communities that heavily depend on large fish (9).This is not as common as with general food grade fish such as salmon; however, large species such as shark, skate, ray, and tuna are extremely saturated with heavy metals through bioaccumulation and magnification. Due to their larger size, these species bioaccumulate methylmercury (see Figure 2), which has the ability to act as a cofactor in an oxidation-reduction reaction. The aforementioned chemical reaction directly ties into the development of prion disease, as redox reactions supercharge the toxicity of the PrP protein (7).

On the other hand, diet can also aid in prevention of neurodegenerative diseases. Based on current recorded metal levels done through a subjective blood test, foods lower in metal, and sourcing food from farms without contamination can reduce chances of spontaneous redox reactions (6). However, it is important to note that insufficient amounts of metal, which would entail a range of 15-20 mg of metals such as zinc and copper; however, this range will shift per person depending on age, ethnicity, and pre-existing health factors (21). Metal deficiency can lead to a variety of problems, in which case having appropriate metal levels for subjective biological characteristics should be the main goal, rather than a simple reduction in concentration.

Apart from the consumption of food, prescription medication with a large metal content in the micro-molar quantity (μm), can have a large impact on the pathogenesis of prion diseases. For those who suffer from extreme metal deficiencies, and those who require metal prescription to combat another issue, there is a risk of ion accumulation within the synaptic cleft of a neuron. In direct quantities such as prescription tablets, the danger of overexposure rises greatly, as the concentration of the metal is not diluted by other organic compounds and will not be buffered. Due to the lack of buffering, the margin of error when prescribing quantities greatly decreases, leading to potential metal contamination (18). When the metal is not diluted, chemical processes that would have otherwise occurred with an organic compound, will cease to happen, leading to a more potent redox reaction. As mentioned earlier, a more potent combustion will increase the neurotoxicity of the prion, and encourage the pathogenesis of PrP (2). The PrP protein is quite sensitive to changes in neurochemistry, as such, different metals consistently accumulating within the synaptic cleft have been known to produce multiple strains of the PrP protein. These strains are responsible for the variations of neurodegenerative diseases seen today, such as Parkinsons, Alzheimers, and Cerebral Palsy (15).

Novel Treatments in Vitro

The multitude of strains also have different levels of infectivity and neurotoxicity which is attributed to the main species of ion that has reacted. In a recorded study by the University College London, it was found that THC prions in mice, which have an increased level of infectivity, did not have the same level of neurotoxicity due to the injection of the detergent Sarkosyl, as well as a deficiency in vital minerals. Sarkosyl is an extremely basic detergent which can render the neurotoxicity of a benign in sufficient quantities (23). This allows for the neurotoxicity to be reduced and possibly nullified, which is the most important function to address when analyzing the mechanism of prion disease. The general consensus has been reached that metal overexposure will often lead to the increase of prion pathogenesis, due to an increase in spontaneity of redox reactions found in the synaptic cleft; however through the addition of calculated quantities of Sarkosyl, this neurotoxic prion can be nullified (10). Regarding the crux of the matter, metals act as cofactors, and as such, will be a major deciding factor in the continued pathogenesis of the PrP protein alongside both the neurotoxicity and infectivity (16). Metal overexposure in correlation with disease rates can be reduced through the already established guidelines, along with the addition of sodium sarkosyl treatments to combat neurotoxicity.

Neurotoxicity and infectivity are the two main factors that tie into the severity and spread of prion diseases respectively. Infectivity is the ability of a prion to coil and begin a chain reaction, while neurotoxicity is the ability to continuously degenerate the brain (6). Due to the

established potency of Sarkosyl, it can be deduced that if the neurotoxicity can be managed by detergent based treatments, the infectivity of the prion can become negligible (23). It is also important to note that metal cofactors play a major role in the neurotoxicity of the prion, so the addition of metal ions and detergents is a large unknown factor, as there is a possibility for ionic bonds to form. Having unstable ionic bonds in the synaptic cleft may result in the death of the neuron, which would be counterproductive; however, recent preclinical studies by the University College London have displayed that the use of sarkosyl is proving to be rather effective.

Infectivity on the other hand, also poses a large dilemma. Regardless of neurotoxicity, if the potency of infection rises, this can give way to new strains of prion based neurodegenerative disorders, similar to the spread of COVID-19 (11). Infectivity as well as neurotoxicity play a role in the pathogenesis of prion disease; however, the potency of one factor cannot be solidified without the other. If the infectivity of a prion can be reduced to a negligible level, the general development of neurotoxic PrP would be reduced. On the other hand, with the use of Sarkosyl based treatments, if neurotoxicity is reduced, then the infectivity serves to provide no impact towards pathogenesis. One major factor that comes into play regarding infectivity, is the medium through which it spreads. Prions have been known to spread through blood, feces, urine, and bodily fluids (19). The logical pathway to be taken in order to reduce spread, would be to reduce bodily fluid cross contamination between mammals, alluding to regulations within the farming industry, as well as the medical industry.

When the prion is exposed to a pH far outside the required realm of relative habitation, the acidity of whatever compound will corrode and denature the structure of the prion, thus eliminating continued pathogenesis (22). The issue arises however, in the efficacy of said denaturation. It is important to note that pH is derived from the equation; $pH = -log_{10}[H_3O+]$. Due to the scale of the equation, it is apparent that pH is calculated logarithmically. This means that every point will be multiplied by a factor of ten. The aforementioned pH derivation clearly displays the fortitude of the prion as an organic entity, which helps to establish why it is imperative that proper sterilization procedures be used in order to eliminate and possible cross contamination. In the event an acid does not entirely denature a prion, there is a significant possibility of the protein relapsing to its neurotoxic form, which would render the vast majority of acid based sterilization techniques low in efficacy, and posing great risk if utilized in surgical sterilization paradigms.

The mediums through which prions spread is directly tied into placing regulations as covered earlier, in order to prevent breakthroughs. Prions have been known to survive without a host body for up to two years, which is an unusually long time for a mainstream biological entity to survive without its life support system (19). The aforementioned lifespan requires an analysis of current sterilization procedures, especially regarding medical equipment. To destroy/denature prions, an agent must be used that will hydrolyze its peptide bonds and destroy its tertiary structure. The continued issue that arises is that if the prion is not denatured in totality, they can simply fold back into their neurotoxic state and renature. The aforementioned use of sarkosyl, though a novel in-vitro treatment, may be able to fill the role of complete denaturation and hydrolyzation. In theory, this will kill off an active prion rather than simply make it dormant which is what high temperatures, radiation, and acid treatments will accomplish. These procedures regarding the use of detergents like sarkosyl would be applied to the medical industry, especially in the context of surgical sterility, which would reduce the chances of prion cross contamination and continued propagation.

Societal Impacts

Apart from the scientific aspect of prion disease, there is also a rampant societal one. Patients diagnosed with Alzheimers, Cerebral Palsy, and Parkinson's Disease all require avid care and monitoring depending on the level of progression of each respective disease (3). This leads to a depletion of funds for healthcare facilities, which can be reallocated for better medical infrastructure and grant based disease research. Neurodegenerative diseases take a major toll on physical and mental health as well, because the central nervous system is in control of functions such as coherence, motor skills, emotion, sensory developments, and judgment (8). Without developed control of basic sensory functions, life within society becomes difficult and taxing on both the patient and affiliated peoples and the healthcare system. This raises another dilemma, which is cost. Primary care, and emergency services, as well as experimental procedures will have a detrimental financial effect on middle class citizens diagnosed with a prion based disease; however, through the use of general guidelines and sarkosyl based treatments, the frequency and severity of occurrence will be reduced, thus reducing the general cost. With growing concerns of prion diseases, the alteration of metals in conjunction with the use of detergents can reduce neurotoxicity and possibly reduce the sheer amount of neurodegenerative diseases. As with occupational overexposure, the addition of regulations can prove beneficial to the rates of diseases present. Prions have solidified themselves as a truly complex and malevolent protein, however with the use of countermeasures, they can be combated and nullified.

Proposed Research Methods & Data Collection

The methodology for an experiment to test and/or consolidate the premise would include the use of groups of three samples of mouse brain homogenate infected with the same prion based disease up to the researchers discretion (ie; Mad Cow Disease). It should be noted that mouse brain homogenate will be used because neural functioning can be extrapolated to human neurobiology with relative accuracy. The vehicle to administer an aqueous metal ion solution would likely be water, ingested orally, however it is up to the researchers discretion on what vehicle would yield the greatest efficacy, and what method of ingestion, whether it be aerosol transmission, oral, or through intramuscular injection (see Figure 3). All collected samples must be at the same temperature and have the same environmental factors in order to reduce possibilities of error and prevalence of any confounding variables. One sample will be metal deficient and will serve as a negative experimental group, while the other is oversaturated with metal and will serve as a positive experimental group. The last sample will be at metal concentrations appropriate for mice at the respective age, sex, and weight stage. The researcher will gradually increase metal concentrations at a natural pace in order to observe the results of said increase in correlation to cell death. During the execution of the experiment, the

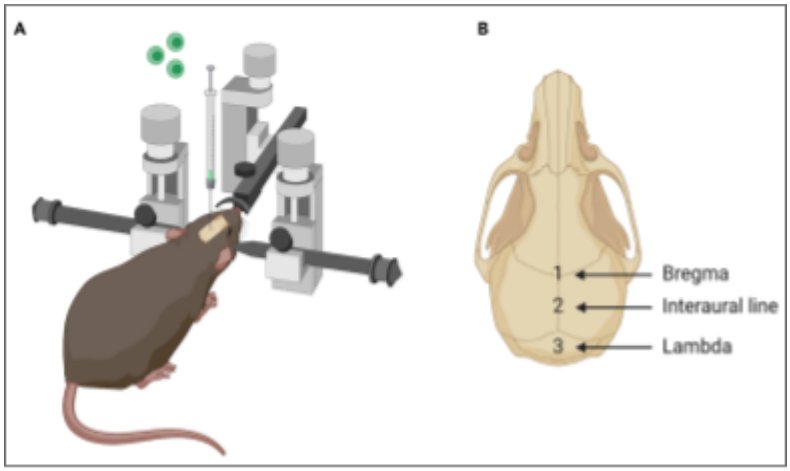

Figure 3, https://creativecommons.org/licenses/by-nc-nd/4.0/

researcher must keep in mind the appropriate blood/brain metal concentration for the gender and size of the mouse, as well as using metals that are common cofactors in prion disease pathogenesis (ie; aluminum, copper, nickel, etc). Using immunohistochemistry and protein identification techniques, homogenate cells as well as the prions-infected cells will be stained and expressed using western blotting, and the researcher would have to track the rate and severity of cell death at different intervals over the course of a set amount of time. The researcher would then have to repeat the exact same procedure, but they must now decrease metal concentrations and record the rate of cell death. The use of immunohistochemistry allows for a visual of the cell degradation at different intervals while increasing and decreasing metal concentrations. In addition, the researcher must take into account the concentration at which reduced metal levels impact other aspects of the mouse body and record the baseline concentration as not to hypothetically disturb other important processes. Following the completion of the procedures, the researcher will have to stain and use western blot for all samples, and graph/represent the results accordingly. Based on the recorded results, the experiment will likely support the hypothesis and be able to give the scientific community an insight into how reducing metal exposure can slow the onset of prion disease. For the western blot and protein expression results, hypothesized genes of interest include PRNP, APP, PSEN1, and PSEN2. This hypothesis would have to be confirmed or refuted by the researcher following the completion of the data analysis. If the manipulation of metal concentration reducing the rate of cell death is supported by the experiment, hundreds of thousands of prion disease cases can be prevented through natural methods without significant cost. In regards to the medical industry, resources can be put to better use and aided care will be able to better manage the number of cases.

Discussion & Conclusion

Based on the outline of the literature review, it is apparent that biometals can act as significant cofactors in the pathogenesis of prion related disease, and as such, manually manipulating exposure to such cofactors can help to slow and reduce the onset of such diseases. In a more social approach, facilitating education regarding appropriate foods with adequate mineral content can both offer part of a healthy diet, as well as a slight buffer against the onset of neurodegenerative disease. Furthermore, lobbying local and federal governments, and unionizing can help to establish more adequate safety measures when in the presence of freely floating metallic debris. Such measures would include gas-masks, restrictions on work hours, and integrated fume hoods for small scale industrial work. Moving away from the public health aspect, there can be some large sources of error regarding the understanding of the experiment. Mainly, rodent population results can indeed be extrapolated to humans; however, this extrapolation would not take into account variations in age, sex, weight, income level, daily stress, race, and other such variables. The experimental paradigm would serve as a baseline preclinical test for the validity of the hypothesis in metal level manipulation. Despite the possible sources of error, novel research and literature, as well as academic theory point towards a balance between manual health management (ie; metal intake and exposure) and biomolecular agent use as the future of mitigating widespread prion related neurodegenerative disease.

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