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The Effects of Neuro-metals on Prion Disease Formation

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Abstract

Prion diseases are transmissible spongiform encephalopathies that cause the neurons of the brain to become damaged and die. Prions are a unique infectious particle as they are not like bacteria or viruses since they lack nucleic acid. The PrP^{Sc} protein is responsible for the infections caused by prion diseases and accumulates in the brain. PrP^{Sc} has a beta sheet conformation and is responsible for disease in both humans and animals. Scientists have investigated prion diseases in the laboratory to try and understand how these diseases spread and can infect different species. Several of these studies have looked at the roles that essential metals play within the body and their possible contribution to prion diseases. Metals are essential for life, as they act like cofactors of many enzymes and are involved in cellular respiration and metabolism; transition metals however are potentially harmful to cells as they participate in redox reactions, producing reactive oxygen species (ROS), which can oxidize proteins, lipids, and nucleic acids. Metal homeostasis may be induced and contribute to neurodegeneration. Reducing metal homeostasis and limiting ROS and free radical production could serve as protection from the neurodegeneration seen in prion diseases. This research paper utilized scientific literature to examine the transition metals zinc, copper, iron, and manganese, pathology of prion diseases, the effects that these metals have on disease formation, and potential treatment methods.

Historical Perspective

As early as 1868, a large number of cattle fell ill to a seemingly mysterious disease unlike any infection seen before. These mysterious cattle deaths caused scientists to search for the source of this infectious agent, which was later discovered to be a prion (proteinaceous infectious particle), or simply, a misfolded protein. Although many symptoms and diseases had been documented throughout history and later recognized as being caused by prions, the discovery of Bovine Spongiform Encephalopathy (BSE), known to most as "mad cow disease," gained much public attention during the 1900s and sparked investigations into understanding the causative agent of this disease and its mechanism of transmission (Parente, 2018).

Dating back as early as the 18th century, abnormal behaviors were recorded in Merino sheep that caused the animals to excessively lick and scratch themselves against fences. Tikvah Alper concluded from radiation-inactivation data that this infectious agent does not depend on nucleic acid; this disease would come to be known as the prion disease scrapie (Parente, 2018). Over the past few decades, scientists have further begun exploring the effects of misfolded

proteins and have made numerous discoveries of types of prion diseases. Stanley Prusiner was awarded the Nobel Prize in Physiology or Medicine in 1997 for his work on the "prion hypothesis," which was a revolutionary theory about a rare type of infection that concerns an unusual protein, the prion. Unlike other infectious agents like bacteria or viruses, the prion does not contain nucleic acid but contains protein, which puzzled many scientists. Ten years after Prusiner and colleagues set out to find this agent, they were able to produce a sample preparation derived from diseased hamster brains that contained the particle. Prusiner was the first to purify this infectious agent and show that it was mostly composed of protein, and specifically glycoprotein because it has a sugar attached. Prusiner developed the term "prion" in the early 1980s as a way to describe this agent that causes fetal brain disorders known as transmissible spongiform encephalopathies (TSE); the word prion is the shorted form of "proteinaceous infectious particle." In 1984 the essential component of the prion was identified as the "prion protein," or PrP, which is encoded by the PRNP gene in the host genome (Parente, 2018).

Types of Prion Diseases and Characteristics

Prion diseases, or transmissible spongiform encephalopathies (TSEs) are rare progressive neurodegenerative disorders that can affect both humans and animals. One of the more well-known prion diseases is Bovine Spongiform Encephalopathy (BSE) also known as "mad cow disease." Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy and Ungulate Spongiform Encephalopathy are the other thus far identified animal prion diseases (Table 1).

Table 1 Animal Prion Diseases

Animal Prion Diseases	Affected Animals	Year Identified
Bovine Spongiform Encephalopathy (BSE)	-Cattle -Humans who consume meat from cows with BSE can be at risk for vCJD (human variant)	1986
Chronic Wasting Disease (CWD)	-Deer -Moose -Elk	1981
Scrapie	-Sheep -Goats	Recognized in 1772; identified in 1942
Transmissible Mink Encephalopathy	-Minks	1947
Feline Spongiform Encephalopathy	-Domestic cats & cats in captivity	1990
Ungulate Spongiform Encephalopathy	-exotic animals	Late 1980s

Additionally, the five identified human prion disease are Creutzfeldt-Jakob Disease

(CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome,

Fatal Familial Insomnia, and Kuru (Table 2) (Prion Diseases, 2018).

Table 2 Human Prion Diseases

Human Prion Diseases	Year Identified	Common Symptoms
Creutzfeldt-Jakob disease (CJD)	1920s	Personality changes, anxiety, depression, memory loss, impaired thinking, blurred vision or blindness, insomnia, difficulty in speaking & swallowing, jerking movements, delirium, coma
Variant Creutzfeldt-Jakob disease (vCJD)	1996	Similar to CJD with severe depression & intense feelings of despair
Fatal Familial insomnia (FFI)	1974	Sleep disturbance, psychiatric problems, weight loss, and balance problems
Gerstmann-Straussler-Scheinker syndrome (GSS)	1936	Progressive loss of coordination, dysarthria, loss of coordination, difficulty walking & coordinating voluntary movements (ataxia)
Kuru	Recognized in 1910s; official reports 1950s	Body tremors, random outbursts of laughter, gradual loss of coordination

Prions are a unique protein and when misfolded, form protein aggregates in brain tissue that ultimately lead to neurodegenerative diseases in species. The PrP^{Sc} is the abnormal and misfolded form of the PrP protein that builds up in diseased brains and infects both humans and animals in a variety of diseases. In common bacterial or viral infections, the spreading or transmission of a disease from one individual to another can occur through direct contact, usually in the form of person-to-person contact or droplet spread, or spread indirectly through airborne transmission, contact with contaminated objects, or through the consumption of contaminated food or water (Higuera, 2017). The causes for human prion diseases are usually sporadic (unknown) or genetic; there have also been cases where disease has been contracted by ingesting contaminated tissues, or from exposure to contaminated PrP through hormone treatments or

during surgical procedures. Transmission of diseases is usually contained to its own species because of species or transmissions barriers; such barriers limit transmission of infection from one species to another. However, there have been notable exceptions to this.

Usually, the species barrier results in a lack of propagation of the prion in a new host, but the molecular mechanism that determines the permeability of the transmission barrier to a prion in a particular host is not fully understood. Through the continuous study of prions, researchers have identified important factors that appear to influence the permeability of the barrier. The nature of the prion strain, homology (similar structure) between the primary amino acid sequences of the donor and the host PrP, and the dose/amount of exposure route in the recipient host are all factors that influence the cross-species transmission. Today, it is impossible to predict the prion's ability to propagate into a new host species and the only conclusion that can be made about the species barrier is that it is extremely unpredictable.

Prion diseases are rare, with about 300 cases reported each year. Those who have a family history of prion disease, have eaten prion infected meat, or have been exposed to contaminated corneas or medical equipment are at risk for prion diseases. Some of the common symptoms include rapidly developing dementia, difficulty walking, hallucinations, muscle stiffness, confusion, fatigue, and difficulty speaking. Prion diseases can only be confirmed by sampling brain tissue after death; however, MRI scans, samples of spinal fluid, electroencephalograms, blood tests, and neurologic and visual exams can be used to help diagnosis them (Higuera, 2017).

Prion Diseases in Other Species

Prion diseases are found in many different species such as sheep and goats, and members

of the cervid family (deer, elk, moose, caribou). There are two forms of the scrapie disease:

classical (typical) and atypical. Classical scrapie is spread within the populations of sheep and

goats. Sheep infections are detected between the ages of two and five years old, whereas in

goats, infections are detected after six years of age. Manifestations include hunched posture, hind

limb ataxia with fore limb hypermetria, scratching, and wool loss. Atypical scrapie is believed to

occur spontaneously, or randomly, due to its unique molecular and phenotype characteristic

(Houston & Andréoletti, 2019). The clinical signs of classical scrapie include normal posture,

circling, scratching, ataxia, visible head tremor, and no wool loss (Cell Press, 2008). According

to the U.S. Department of Agriculture, approximately 30 percent of US sheep are genetically

susceptible to scrapie. Scientists have claimed there is no zoonotic potential, or potential for

animal infections to spread to humans. Oral inoculation experiments have been conducted

involving chimpanzees (Pan troglodytes), capuchin monkeys (subfamily Cebinae), cynomolgus

macaques (Macaca fascicularis), and woolly monkeys (Lagothrix sp.), and all appeared not

susceptible to the scrapie disease (Scrapie, 2020).

Chronic wasting disease affects many species of free range and captive wildlife

populations of mule deer, black-tailed deer, white tailed deer, black tailed deer, elk, moose, and

6reindeer throughout 24 US states and 2 Canadian providences, South Korea, and Europe, and

will likely spread to infect more animals in other areas of the world. In 1967, the disease was

first observed in cervids (mammals of the deer family) in captivity in Colorado and Wyoming

and was first thought to have been caused by nutritional deficiency or stress due to confinement.

The disease was classified as a spongiform encephalopathy in 1980 and is the most efficiently

transmitted prion disease. To date, the origins of chronic wasting disease are still unknown, but there is a hypothesis suggesting that the disease came from sheep scrapie transmission to cervids or through spontaneous conversion of normal prion protein to the misfolded conformation. The main symptoms of chronic wasting disease are excessive weight loss and isolation for the herd population. Additional symptoms of rough hair, lower head position, and wider leg stance appear, as well as polydipsia (thirst), polyuria (excessive urine output), bruxism (teeth grinding), regurgitation, ataxia (lack of muscle control), and tremors. The behaviors of the cervids also changes hyperphagia (the visual axis of eye deviates upward), repetitive and exaggerated leg lifting, diminished alertness, and occasional aggressive behavior. The animals usually die within 18-24 months, by which time the body's muscle mass has reduced and accounts for only about 20% of its maximum body weight (Legname, & Vanni, 2017).

Chronic wasting disease is the most efficiently transmitted prion disease. According to the Centers of Disease Control and Prevention, in some locations where the disease is established in free-ranging cervid populations, infection rates may exceed 10%, and in localized populations more than 25%. In captivity infection rates are much higher with a rate of 79%, where nearly four out of five cervids are infected within one captive herd (Centers for Disease Control and Prevention, 2021). Zoonotic potential is believed to be low because experiments with Cynomolgus macaques and transgenic mice that are expressing the human prion protein, have shown low efficiency of human PrP conversion to the prion form after PrP^{CWD} exposure (Legname, & Vanni, 2017).

Human Prion Disease

Creutzfeldt-Jakob disease (CJD) is a human neurodegenerative disease that causes rapidly progressing dementia, ending in death within six to eight months of the symptom's onset, and about 70 percent of people die within one year (Creutzfeldt-Jakob Disease Fact Sheet, 2021). This disease is rare, affecting about one in every million people throughout the world, and about 250 people in the United States each year. CJD cases have been grouped into three types: familial, iatrogenic (infection through a medical procedure), and sporadic. The onset of symptoms begins with changes in sleep patterns, weight loss, loss of appetite, and visual disturbances such as double and blurry vision, or an unusual vision loss where the patient is not aware he or she cannot see before the onset of dementia. Then memory loss, disorientation, personality changes and psychosis, language disturbances, hallucinations, and muscle spasms appear as the dementia progresses (Lutwick & Odle, 2020).

CJD is found primarily in adults ages 50-75. Familial CJD is inherited autosomal dominantly, meaning either parent could pass on the disease. However, sporadic CJD is responsible for about 85% of all CJD cases, and only age and PRNP codon-129 genotype have been consistent risk factors (Lutwick & Odle, 2020). The cause or trigger of sporadic CJD is known and is regarded as a spontaneous neurodegenerative disease that is most likely due to a mutation of PRNP, the prion protein gene (Legname, & Vanni, 2017).

PrP Protein & Prion Pathology

Prion diseases are caused by one specific type of misfolded protein found in neurological tissue of mammals. Prions are unique and transmit their misfolded shape onto normal variants of the same protein, and then cause neurodegenerative disease in humans and animals. The exact

function of Prusiner's "prion protein," which is encoded by the PRNP gene in the host genome, is still unknown. There is speculation that the PrP protein plays a role in copper transport between the cells of the central nervous system, that it might be important for cell communication between nerve cells, or help protect neurons from becoming injured (Parente, 2018). It is known that PrP^C is a glycosyl-phosphatidylinositol-anchored protein, meaning it can be attached to the C-terminus of a protein during post translational modifications. PrP^C is located at both the presynaptic and postsynaptic sites throughout the central nervous system, and it is especially abundant in the hippocampus (role in learning and memory), frontal cortex (higher functions- decision making, problem solving, emotion regulations), and striatum (needed for voluntary movement control) (Watt et al., 2012).

It is known that the pathological process occurs when PrP changes its conformation. The "protein only" hypothesis also proposes that there are structural differences in PrP^{Sc} molecules depending on the species; different species have slightly different structures of PrP^{Sc}. There has also been evidence supporting that PrP^{Sc} changes conformations or changes shapes of the PrP^{Sc} due to rotational changes of the molecule (Ma & Wang, 2014). In prion diseases, the prion protein PrP^C is converted to the alternative form PrP^{Sc}. This "protein only" hypothesis proposes that the PrP^{Sc} form of the protein is the infectious agent in prion diseases, and it is able to replicate itself by converting other PrP^C to this PrP^{Sc} form (Wadsworth & Collinge, 2010). The PrP^{Sc} induces conformational transformation of PrP^C, creating a duplicate PrP^{Sc}, which causes a chain reaction inducing further transformation of PrP^C to PrP^{Sc}, and spreads to various parts of the brain. Because PrP^{Sc} is not water soluble and resistant to degradation by protease, it accumulates in the brain, leading to neuronal cell death (Gambetti, 2022). Over an extensive period of time, PrP^{Sc} can lead to aggregates of protein that cannot be degraded by normal

mechanisms and result in various diseases and affect the biological activity of organisms differently.

The difference between the PrP^C and PrP^{Sc} forms is in their monomer conformations and aggregation states. Many mammalian species are affected by prion diseases. The PrP protein is very similar in all mammals, but there are small differences in the PrP between the different species. Because of these slight differences, it can be difficult in predicting the molecular basis of prion propagation, as different strains lead to distinct phenotypes. There are a range of mammalian PrP^{Sc} conformations possible, but only a select subset will be compatible with the individual PrP; this means that an overlap of permissible PrP^{Sc} conformations between the source and recipient and the variety in cellular mechanisms affect the prion propagation (Wadsworth & Collinge, 2010).

Various clinical presentations can be observed from prion diseases in human. Progressive dementia, cerebellar ataxia (uncoordinated muscle movement due to disease in the cerebellum), pyramidal signs (spasticity, weakness, slowing of movements, hyperflexion), chorea (involuntary, irregular, and unpredictable muscle movements), and seizures can be seen in different combinations (Wadsworth & Collinge, 2010). Gliosis and histologic vacuolar changes are observed in the brain. Signs and symptoms may develop months to years after initial exposure to PrP^{Sc} (Gambetti, 2022).

Proteins PrP^C and PrP^{Sc} differ in their tertiary structure (Figure 1). The normal PrP^C form of the protein consists of an amino acid chain with a portion arranged in an alpha helical structure, which gives it a globular (bulblike) shape that is water soluble, and it can be broken down by proteases enzymes. In contrast, the PrP^{Sc} form is arranged into beta-sheets, tends to aggregate (stick together), cannot be dissolved in water, and is resistant to heat and breakdown

by proteases. The PrP^{Sc} will bind to alpha-helical PrP^{C} and convert it into the PrP^{Sc} beta-sheet

confirmation; this causes a chain reaction where large amounts of PrP^{Sc} build up resulting in

brain tissue damage and the spongy appearance of spongiform encephalopathy (Parente, 2018).
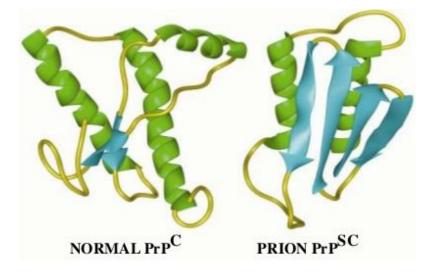

Figure 1 The normal PrP^{C} versus the abnormal PrP^{Sc} (*Molecular Structure of Normal Protein vs Prion*, 2017).

Scientists are still working to uncover information about the causes of the disease

pathology, protein misfolding, and potential therapeutic strategies of prion disease. One area of

research that has attempted to address these questions is the relationship between prions and

specific metals. In recent articles, scientists have developed the term "neuro-metals" to describe

trace elements that play a significant role in brain development and function; these metals can

exist at different concentrations and distributions throughout the various regions of the brain.

There is evidence suggesting that PrP^{C} has the ability to bind metals and plays crucial roles in the

maintenance of metal homeostasis. Zinc, copper, iron, and manganese are examples of these

neuro-metals and their effects on PrP^{C} and PrP^{Sc} are being investigated. These metals are

important for function because they help with many biological processes, and they also have the

ability to interact with oxidation-reduction reactions; continuous oxidative-reduction reaction can

create free radicals, which are harmful. With the "prion hypothesis," it is not clear whether PrP^C is losing part of its normal protective function, or if the conformational change is causing it to gain a toxic function. There is reason to believe that these neuro-metals affect the function and shape of PrP, and their creation of free radicals can also contribute to infection. Because the mechanism for PrP^C conversion to infectious PrP^{Sc} is not fully understood, there is hope that studying the way neuro-metals interact with PrP can help to understand its way of infection and create potential treatments.

Human Body Elements & Metal

There are a significant number of chemical elements that are essential for life with known functions, but there are also several elements considered 'non-essential," as their functions and relevance are uncertain. There are many important elements that the human body needs in order to survive and allow for biochemical processes to occur. Some of these important elements are metals such as Na, K, Mg, Ca, Fe, Mn, Co, Cu, Zn and Mo, which keep the body functioning, and are found in different amounts in the average person (Table 3) (Maret, 2016).

Table 3 Typical amount of each element found in the body of a 70kg person

Element	Median Amount
Ca	1000g
K	140g
Na	100g
Mg	30g
Fe	5g
Zn	2g
Cu	100mg
Mn	16mg
Mo	5mg
Co	2mg

Elements in the body can be divided into two categories: abundant and trace. Elements that are present in smaller amounts (typically these amounts are not precisely known) are called trace metals. The trace metals zinc, copper, iron, and manganese are necessary for function and growth of the brain. The brain barrier system (blood brain and blood cerebrospinal fluid barriers) is responsible for regulating the transport of these trace metals into the brain (Enelhart & Sorokin, 2009).

Zinc

Zinc is a chemical element with symbol Zn and atomic number 30 on the periodic table. With an atomic weight of 65.409 μ, zinc has a simple hexagonal structure and grey color in its solid form. Zinc has several functions as it has been shown to have catalytic, structural, and regulatory functions. In 1869, it was discovered that zinc played an important role in the growth

of fungus, and in 1961 its importance for human growth also became clear as hypogonadism and dwarfism were reported in those living off of a diet low in zinc, that consisted of mostly bread, potatoes, and milk (Roohani et al., 2013).

Zinc is the second most abundant trace metal in the body after iron. It is absorbed in the small intestine and is secreted in the gut and on average, 33% of zinc intake is absorbed in humans (Saper & Rash, 2009). There are at least 10 zinc transporters (ZnTs) and 15 zip transporters in cells whose distributions are affected by zinc distributions in the body, and they serve opposite roles in zinc homeostasis (Roohani et al., 2013).

The brain has the highest concentration of zinc relative to other organs in the body (150 μM), and it is found mainly in specific axons of the hippocampus that are mossy fibers, which suggests that it plays a role in neurotransmission (Treiber, 2005). Zinc also has antioxidant properties as it acts as a co-factor for enzymes involved in the functioning of the antioxidant defense system. Additionally, zinc protects against oxidative damage and reduces hydroxyl radicals (Marreiro et al., 2017). It is suggested that the daily intake for a human that is 50kg is 7-11mg (Wada, 2004).

Copper

Copper is another chemical element that is essential to the body. The symbol for copper is Cu, and it lies in group 11 of the periodic table, with atomic number 29. This reddish, extremely ductile metal is a great conductor of electricity and heat, and it has an atomic weight of 63.546 μ (Britannica, 2020). Copper is involved in energy production, iron metabolism, neuropeptide activation, connective tissue synthesis, and neurotransmitter synthesis. Copper also regulates gene expression, brain development, and immune system function (U.S. Department of

Health and Human Services, 2022.). In the nervous system, copper is also required for the formation of myelin (Bost et al., 2016).

Although there have been inconsistent studies to derive the suggested daily intake, it has been shown that a daily intake below 0.8 mg/day leads to a net copper loss, and net gains are observed with a daily intake of an amount above 2.4 mg/day. Dietary copper deficiencies can have adverse effects during all stages of life; in utero, deficiencies can result in impaired development of the cardiovascular system, bone malformations, as well as neurological and immunological abnormalities. In adults, deficiencies are associated with alterations in cholesterol metabolism. Additionally, copper in high level can be toxic and lead to reactions resulting in oxidative cell damage and cell death (Bost et al., 2016). The brain is one of the most energy demanding tissues of the body, so it requires a lot of energy. About 95% of the total ATP in the brain is generated in the mitochondria, so mitochondrial efficiency is important for the brain. Cytochrome c oxidase is a member of the super-family of heme-copper containing oxidase that catalyzes the last step of the mitochondrial respiratory chain, making copper an essential component of brain metabolism (Scheiber et al., 2014).

Iron

Iron is a chemical element with symbol Fe and atomic number 26. It is the first metal in group 8 of the periodic table with an atomic mass of 55.845 μ. By mass, iron is the most common element in earth and forms much of the Earth's crust. Inorganic iron plays an important role in hemoglobin synthesis and hemoglobin formation as well as oxygen transport. Although iron is an abundant element on earth, it forms oxides when in contact with oxygen, making it highly insoluble and not available for uptake by organisms. In humans, iron exists in complex

forms bound to protein (hemoprotein) as heme compounds (hemoglobin or myoglobin), heme enzymes, or nonheme compounds (flavin-iron) (Abbaspour et al., 2014).

Ranging from 5-35%, depending on circumstances, iron absorption is typically low and occurs mostly in the duodenum and upper jejunum (Abbaspour et al., 2014). It is then transferred across the duodenal mucosa into the blood, and then transported by transferrin to cell or bone marrow for the production of red blood cells. Iron balance is mainly regulated at the point of absorption since the body lacks a defined mechanism for its active excretion. Also, the circulating peptide hormone hepcidin is secreted by the liver and acts a negative regulator of iron entry in the plasma (Abbaspour et al., 2014). Iron is essential because of its role in neurological function as it contributes to oxidative metabolism, and due to the fact that it is a cofactor in the synthesis of neurotransmitters and myelin. There have been many cases studied where brain iron concentration has been seen to increase with age, and with diseases such as Alzheimer's and Parkinson's (Piñero & Conner, 2000).

Iron also plays a role in oxidative metabolism. Cellular damage can be induced through hydroxyl radical production, causing the oxidation and modification of proteins, lipids, carbohydrates, and DNA, if abnormal homeostasis of iron occurs. Additionally, iron is a cofactor in the synthesis of neurotransmitters and myelin (Ward et., 2014).

Manganese

The chemical element manganese has the symbol Mn and atomic number 25. This hard brittle, and silvery metal has an atomic mass of 54.94 μ and is found in small amounts throughout the body. Manganese is involved in development, reproduction, antioxidant defense,

energy production, immune response, and regulation of neuronal activities. It is also involved in the synthesis and activation of various enzymes (Li & Yang, 2018).

Absorption occurs by ingestion, inhalation, and dermal permeation as it is absorbed in the gastrointestinal tract and lungs, and then it is distributed to different tissues by blood circulation (Pan et al., 2018). The absorption and excretion of manganese is under constant homeostatic control to ensure a stable level is maintained within the body (Aschner & Ascher, 2005). Manganese deficiencies are rare, yet they can occur; manganese poisoning is also possible if there is overexposure to the metal. Those with iron deficiencies are at an increased risk of manganese poisoning because manganese absorption in the gastrointestinal tract increases under low iron conditions (Pan et al., 2018). Additionally, manganese deficiencies have been associated with skin lesions and bone malformations (Crossgrove & Zheng, 2004). The daily intake for manganese depends on development and lactation stages and are as follows:

Table 4 Suggested daily intake of manganese U.S. Department of Health and Human Services. (2022).

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.003 mg	0.003 mg		
7–12 months	0.6 mg	0.6 mg		
1–3 years	1.2 mg	1.2 mg		
4–8 years	1.5 mg	1.5 mg		
9–13 years	1.9 mg	1.6 mg		
14–18 years	2.2 mg	1.6 mg	2.0 mg	2.6 mg
19–50 years	2.3 mg	1.8 mg	2.0 mg	2.6 mg
51+ years	2.3 mg	1.8 mg		

Manganese is part of the antioxidant enzyme superoxide dismutase (SOD), and antioxidants help protect against free radicals that can damage cells. Accumulation of manganese in the brain can alter neurotransmitters systems and their activity in the brain (Balachandran et al., 2020).

Oxidative Stress

Oxidative stress is an important concept as it plays a role in brain aging, neurodegenerative diseases, and conditions such as ischemia. During oxidative stress, free radicals can cause damage to cells, or even cause cell death. With neuro-metals, there is potential that these transition metals can contribute to reactions causing reactive oxygen species and free radicals, which cause oxidative stress. There are many oxidative processes in the body for everyday metabolism and immune defenses; these processes are balanced by antioxidant systems (Koivula & Eeva, 2010). Many chemical reactions involve the transfer of electrons, and specifically, the oxidation-reduction reaction (known as a redox reaction). Oxidation and reduction refer to a change in oxidation number; oxidation involves an increase in oxidation number, and reduction has a decrease in oxidation number. Redox reactions are commonly defined to be a reaction in which one species loses electrons, while another one gains electrons (Redox, 2022). Redox reactions are important for biological systems because they are the principal sources of energy on this planet. Oxidation of molecules through removal of hydrogen or combing with oxygen can liberate large quantities of energy (What are redox reactions biology, 2022).

Oxidizing agents have the ability to oxidize other substances, meaning that they remove electrons from another substance; it is also known as an electron acceptor because it "accepts" electrons. The reducing agents have the ability to reduce other substances, as they transfer electrons; often called electron donors. Cellular respiration is an example of a redox reaction that

is important to the body and energy. In this process, glucose is oxidized, and oxygen is reduced (Redox, 2022). The oxidized and reduced forms of the same molecule are together called a redox couple, and redox cycling occurs when the reduction and oxidation reactions are repeatedly coupled. In theory, all redox active compounds could participate in redox cycling, but the extent of the cycling is dependent on the local conditions and state of the involved molecules (Arnér, 2012). Redox cycling can lead to disproportionate consumption of O_2 and cellular reducing substances and formation of oxygen species, which cause oxidative stress (Redox, 2022).

Studies have indicated that transition metals act as catalysts in the oxidative reactions of biological macromolecules. Oxidative stress can be caused by metals increasing the formation of reactive oxygen species (ROS). Reactive oxygen species are highly reactive forms of O_2. The ROS are byproducts of normal oxygen metabolism and are present at low level in normal cells. If these reactive oxygen species overwhelm the cells' intrinsic antioxidant defenses, it results in the condition known as "oxidative stress" (Nuran et al., 2001). Redox active and redox- inactive metals can increase the production of ROS, like hydroxyl radicals ($HO^{-)}$, superoxide (O_{2-}), or hydrogen peroxide (H_2O_2) (Nuran et al., 2001). The redox-active metals undergo redox-cycling, while redox-inactive metals deplete the cells' major antioxidants. With the production of the ROS, antioxidants are incapable of defense against the growing amounts of free radicals (Koivula & Eeva, 2010). The ROS can cause lesions in cells to lipids, proteins, and DNA, making the cells dysfunctional. This suggests that the metal induced oxidative stress in cells could be responsible for the toxic effect of heavy metals (Nuran et al., 2001). Iron and copper are two examples of metals that are redox-active metals and can form these reactive oxygen species. Zinc and manganese are examples of redox-inactive metals and can influence redox homeostasis.

Methodology

The goal of this literature review was to investigate the effects of metals in the body and their role in prion diseases. From reading published literature, it is evident that transition metals, specifically zinc, copper, iron, and manganese, have been shown to bind to PrP and affect its conformation, abilities to bind other molecules, or impact the uptake of other molecules in the brain. Some articles have created the term "neuro-metals" for these metals. Studies were examined from 2000 through 2022 and the variety of implications these metals have on PrP and aggregation formation were studied. Some newer studies on developing ideas for treatments/therapeutic techniques were also examined. The Assumption University library databases were utilized to locate information, as well as Google Scholar, and PubMed.

Effects of Neuro Metals on PrP

Although the exact function of PrP^{C} remains unclear, it possesses the ability to bind to various neuro-metals including copper, zinc, iron, and manganese. There are four complete copies of the octapeptide repeat (PHGG(G/S)WGQ), which can bind copper and zinc, on the amino terminal half of the PrP^{C}. This octapeptide repeat has been shown to play a large role in the PrP ability to bind metals and has been affected by metals as they are not in homeostasis (Watt et al., 2012).

There are two possible pathogenic pathways of prion diseases; there is the possibility that there is a loss of normal protective function of PrP, or there is the possibility that there is a gain of toxic function of PrP. Studies involving these neuro metals have looked into the ways in which the PrP and disease formation are affected by zinc, iron, copper, and manganese and the support of these two different hypotheses.

Zinc:

    A study by Watt et al. published in Nature Communications showed that the prion protein facilitates uptake of zinc into neuronal cells, which suggests that alterations in the cellular prion protein-mediated zinc uptake may contribute to neurodegeneration in prion and other neurodegenerative diseases.

    Two zinc selective fluorescent dyes, Zinpyr-1 and Newport Green, were used to show for the first time that PrP^C mediates the uptake of zinc into neuronal cells, showing that the zinc uptake is mediated by AMPA receptors containing GluA1 and lacking GluA2 subunits. When PrP^c was mutated, or cells were infected with the PrP^{Sc}, zinc uptake was disrupted, suggesting that the reduction in uptake of zinc contributes to the neurodegeneration that is commonly associated with prion diseases (Watt et al., 2012). Zinc uptake was reduced in the presence of mutated PrP^C and PrP^{Sc}, so lower levels of zinc are observed in brains when PrP^C does not function properly. This would suggest that zinc uptake distribution has some sort of negative effect on the functions of PrP^c.

    The intracellular protein tyrosine phosphatase is sensitive to zinc and has increased activity in its presence. In mouse cells expressing PrP^{Sc}, there was a significant reduction of phosphatase activity as compared with the uninfected cells; this indicates that the uptake of zinc by PrP^C has a downstream effect on cellular processes. This was confirmed by measuring tyrosine phosphatase activity in the brains of wild-type and PrP^C null mice. Higher levels of tyrosine phosphatase activity were seen in the PrP^C null mice compared to the wild-type and age matched control mice. This indicated that PrP^C is involved in the physiological homeostasis of neuronal zinc (Watt et al., 2012).

Zinc levels have been shown to be reduced in prion disease infected brains. Zinc uptake was measured in mouse ScN2a cells infected with scrapie, compared to uninfected controls following exposure to exogenous zinc. There was an increase in zinc uptake in the uninfected cells; however, there was no increase measured in the scrapie infected controls. This shows that PrP^C mediated zinc uptake is lost in cells expressing prion disease and the associated mutants of PrP^C upon conversion of PrP^C to PrP^{Sc}. Observations from this study would suggest that the loss of normal functions of PrP^C would contribute to prion disease pathogenesis (Watt et al., 2012).

Pan et al. conducted an experiment to study the effect of zinc on aggregation and conformational change of PrP. Thioflavin T binding assays, Sarkosyl-soluble SDS-PAGE, and transmission electron microscopy showed that in the absence of zinc, the aggregation of wild-type PrP undergoes the steps of amorphous aggregates, profibrils, mature fibrils, and fragment fibrils. The fibrils are formed and then resistant to degradation. When the molar ratio of Zn^{2+} to PrP was 9:1, the aggregation of wild-type PrP undergoes a different pathway in which the wild-type PrP forms oligomers quickly and then short-rod aggregates, demonstrating that this change causes the steps of aggregation to change (Pan et al., 2011). With no concrete mechanism for pathogenesis established, these results show that when zinc is in a higher ratio, it changes the pathway and formation of PrP aggregates.

Copper:

A study by Younan et al. published to the Journal of Microbiology indicated that copper ions induced secondary structural changes and reduced folding stability on the prion protein. PrP^C is concentrated at presynaptic membranes where copper ions are also highly localized and free Cu^{2+} levels at the synapse may reach levels as high as 20 µm during neuronal

depolarization (Younan et al., 2011). This study looked to investigate the direct structural effect Cu^{2+} has on the prion protein.

      To see if Cu^{2+} affected the folding stability of PrP^C, the chemical denaturant urea was used to monitor Cu^{2+} and app-PrP(23–231) levels. This app-PrP(23-231) is a section of the octarepeat region of the prion protein that binds copper. The CD (Circular Dichromism is an absorption spectroscopy method based on differential absorption of left/right circularly polarized light) signal at 225nm was used to measure the alpha-helical content over a range of urea concentrations. The urea concentration at the midpoint of folding was found to be 5.79 +/-0.04 M at pH of 7.5 for the apo-PrP(23–231), which also aligned with other studies. This destabilized the native fold of the Cu^{2+} binding to PrP(23–231). The urea unfolding was also shown to be reversible through a series of serial dilutions of copper preparations from a PrP sample with 10 M urea to 1 M urea). Additionally, using mouse PrP, it was shown that the PrP^C domain has an irregular structure in the absence of Cu^{2+}. In the absence of copper, there is a single negative CD band at 198nm, while there is a reduction in the band when Cu^{2+} was added to the mouse PrP(23-126). This suggests a loss of irregular structure. These studies have been limited due to the flexible nature of the Cu^{2+} binding site on the PrP^C which has hampered crystallographic studies, while the paramagnetic nature of Cu^{2+} has restricted NMR studies (Younan et al., 2011).

      A recent study by Chi-Fu et al. showed that copper induced structural conversion of the prion protein. This study used recombinant, full length, human PrP to perform a variety of tests to better understand the effect copper has on the prion protein (Chi-Fu et al., 2016). Proteinase K sensitive PrP and a fluorescence-based single-molecule proteinase K resistant assay was used. The PrP proteinase K sensitive PrP was biotinylated and then covalently immobilized and then incubated without Mn or copper ions. The samples were treated with proteinase K, which would

not break down the PrP if it was converted to the proteinase resistant form. The full length PrP

(23-231) incubated with the copper converted to a proteinase resistant form. Globular PrP that

lacked the unstructured N terminal region (PrP (90-231)) was still sensitive to the proteinase

digestion in both the absence and the presence of copper. This showed that the monomeric PrP

proteinase resistant formation requires both intrinsically disorder N terminal region and Cu^{2+}

ions (Chi-Fu et al., 2016).

PrP aggregation was monitored using ThT fluorescence (commonly used probe to

monitor *in vitro* amyloid fibril formation) intensity and *in vitro* seeding assay to test whether PrP

exposed to divalent metal ions forms seeds that template aggregation. There are many theories

about how PrP^C converts to PrP^{Sc}, and one of them is the seeding hypothesis. In this hypothesis,

the protein is unable to undergo structural alteration and polymerization on its own, and the

addition of a small aggregate or seed is needed for the conversion process to be initiated. Early

studies by Kocisko et al. showed that once PrP^{Sc} was added to recombinant PrP, the conversion

process was initiated (Hesketh et al., 2011). In this particular study by Chi-Fu et al., the PrP (23-

230) with copper began forming seeds (15-25hrs) and aggregates, and the PrP (23-230) in the

absence of copper only started to form seeds after 55 hours. And PrP (90-230) showed no seed

formation after 95 hours.

The cytotoxic nature of Cu^{2+} induced PrP amyloids was also demonstrated using slices of

brain tissue from young mice and PrP (23-230) pre exposed to Cu^{2+} and PrP (23-230) with no

metal exposure. Glial fibrillary acidic protein (GFAP) (indicator of astrocytic activation during

neuroinflammation) and the expression of protein kinase δ (oxidative stress sensitive and

activation shown to induce neuronal cell death) were measured. Western blot analysis indicated

that PrP Cu $^{2+}$ had higher levels of GFAP and PKC-δ (PKC-δ) than its control (Chi-Fu et al.,

2016). This indicates that oxidative stress is occurring, and astrocytes are activated because there is neuroinflammation when copper is present.

Iron:

Studies have indicated that most-regulation of iron metabolism in sporadic-Creutzefeldt-Jakob disease and scrapie infected animal brains show a redox-iron in prion disease pathogenesis. This is an important observation given its highly toxic nature and involvement in other neurodegenerative conditions of protein misfolding like in Alzheimer's and Parkinson's diseases. This has formulated the idea that brain iron gets out of the range of homeostasis as a result of massive neuronal death, but more evidence suggests that iron imbalances precede neuronal degeneration and are the trigger for neurotoxicity.

Published in the Neurobiology of Disease journal, research by Singh et al., was conducted by looking at iron imbalances in prion disease infected tissues. Frozen human brain tissues from the frontal cortex of sCJD (CJD+) and age matched cases of dementia (CJD-) ranging in age from 37-80 years old, with most in the 67-80 year range were studied. The interval from the appearance of clinical symptoms to death ranged from 1-24 months in CJD versus 2-4 months in CJD+. Control and prion infected hamster spinal cord samples were also used (Singh et al., 2012).

The tissues from the frontal cortex of the sCJD (CJD+) and non-CJD dementia (CJD-) were homogenized in a buffer containing a lysis buffer supplemented with 1% SDS and were set at room temperature or were boiled for 10 minutes, because most iron binding proteins release associated iron after boiling. The unboiled CJD+ and CJD- revealed similar iron content. The boiled samples however were quite different; the CJD- released 68% of the associated iron while CJD+ lost minimal amounts. Directly compared, there was 183% more iron in the CJD+ samples

relative to the CJD- samples. These results were similar to those with Alzheimer's disease, which is known to accumulate iron in the brain. It was demonstrated that iron is sequestered in SDS-stable protein complexes in sporadic-Creutzfeldt-Jakob disease brains, which creates a phenotype of iron deficiency. This is due to a change in the iron storage protein ferritin that becomes aggregated, detergent-insoluble, and partitions with denatured ferritin (Singh et al., 2012).

Lumbar spinal cord tissue of scrapie infected hamsters displayed a similar phenotype. The iron uptake protein transferrin (Tf) is upregulated in scrapie infected spinal cord tissue and increases with disease progression. Samples were harvested at 28, 42, 55, and 70 days post inoculation, homogenized in lysis buffer, and analyzed by Western blotting. The levels of PrP significantly increased after 55 days post inoculations and Tf also increased. After quantification by densitometry, it was revealed that PrP increased by 193%, and Tf by 385% after 70 days post inoculation relative to 28 days post inoculation. Iron deficiency continues to increase until the end stage of disease, despite minimal changes in brain iron levels. There is a direct correlation between Tf and PrP^{Sc} which suggests sequestration of iron in dysfunctional ferritin that either co-aggregates with PrP^{Sc} or is rendered dysfunctional by PrP^{Sc} through an indirect process (Singh et al., 2012).

A study conducted by Pino et al. in 2017 looked to investigate how dietary iron restriction influences brain ferritin, the main iron storage protein, and dopamine metabolism in three regions of the brain, and how altered iron and dopamine metabolism can affect the expression level and solubility of PrP^{C}.

Three-month-old male mice were studied with half receiving a normal diet containing 45ppm of iron and half with a restricted iron diet containing 3ppm of iron for four weeks. The

mice on the iron restricted diet showed reduced ferritin in serum in the liver, and hippocampus, compared to the control group. The ferritin (which is a blood protein that contains iron) level of the mice on the restricted diet was increased in the striatum compared to the control group. Thiobarbituric acid was used to evaluate the extent of lipid peroxidation and showed that although the ferritin level in the iron restricted mice was decreased, there was a higher level of lipid peroxidation compared to the control group.

These observations would suggest that there is an increase in oxidative stress in the hippocampus under iron deficient conditions. Western blots were used to investigate dietary iron deficiency on the expression of PrP^C levels. There was a significant increase in PrP^C levels in the striatum in the iron deficient group. Other regions did not produce significant effects of PrP^C levels (Pino et al., 2017).

Manganese:

Research conducted by Paul Davies and David Brown investigated sporadic forms of prion diseases and environmental risk factors by using extracts of the prion protein from soil matrices. Previous studies have suggested that environmental manganese is a possible risk factor for prion diseases, and Davies and Brown extracted the prion protein from soil matrices and showed that when exposed to manganese, there is a dramatic increase in prion protein survival (~10 fold) over a two-year period (Davies & Brown, 2009).

The two most commonly used model soil systems for protein-soil interaction studies are montmorillonite (mte) and kaolinite (kte). To investigate whether metals play a role in stabilizing PrP, copper and manganese were added to the soils along with wild-type and mutant PrP. The prion protein was extracted from the clay by electrophoretic desorption and Western blot analysis. After a 2-year incubation of the clay, PrP was found to survive better when in the

presence of metals, specifically manganese. According to the study, there is around half as much

prion protein recovered from the clay with copper (n=3, p<0.001) and around 6 times less

protein from the clay with no metals present (p<0.001) relative to the manganese condition

(Davies & Brown, 2009).

To test the infectivity, a cell cultured based assay system was utilized in which uninfected

cells (SMB-PS), were transfected with PrP. Under infected conditions, PrP infection was only

seen at the 1:10 dilution, as the higher dilutions did not result in PK resistant bands (showing PrP

infection). This was repeated with the presence of 50 µM $MnSO_4$. Results of the repeat

experiment show the PK bands indicating infection at 1:10, $1:10^2$ and $1:10^3$ dilutions. The

infectivity of the SMB extract was increased by 100 fold by the presence of manganese. These

results demonstrate that manganese enhances prion infection. Showing that manganese is a risk

factor for the survivals of PrP^{Sc} in the environment as well as aiding in its transmissibility. This

suggests that PrP and manganese separation may make the PrP less infectious (Davies & Brown,

2009).

In an experiment by Hesketh et al., an assay based on exposure of recombinant PrP to

manganese was used. A turbidity test was used to test the effect of the Mn-PrP seed in the

polymerization of PrP, and it was found that increasing the concentration of the seed increased

the rate of polymerization and the maximum amount of polymerization with manganese present.

In the absence of the seeding protein, there was no polymerization of the protein (Hesketh et al.,

2012).

A series of tests using mutants was also done: mutants included the N terminus half PrP

(23-112), the C terminus half PrP (112-231), extended mutant with N terminus PrP (23-171),

mutant lacking N terminus PrP (90-231), mutant with part of N terminus and first helical domain

PrP(90-171), mutants lacking palindromic repeat region (112-119) and mutant with histidines at positions 95 and 110 replaced with alanine residues. The two mutants PrP (23-112) and (112-231) were unable to generate seeds as well as the mutant without the manganese binding site. This showed that the minimum domain for PrP seed formation was 90-171 (Hesketh et al., 2012). As scientists still do not know the mechanism of prion pathogenesis, this experiment has helped to show that PrP (90-171) is needed and crucial for seed formation. Since seeds were not able to generate without the manganese present, it also illustrates that manganese plays a role also in the ability for seeds to form.

Treatments

Currently there is no cure for prion diseases, but various studies are being conducted targeting a variety of possibilities. Several of these treatments target the metal-prion relationship.

Clioquinol is a Cu/Zn/Fe chelator of low affinity and been shown to offer a considerable benefit in preventing Aβ containing plaques in mice infected with Alzheimer's disease. It has also been tested as a metal-attenuating therapeutic in animal models of transmissible spongiform encephalopathies. Clioquinol chelates zinc and copper and acts as an antioxidant. Unsaturated lipids are partially susceptible to oxidative modification, and lipid peroxidation is a marker of oxidative stress. Reactions of H_2O_2 with redox active metals like copper and iron, release a powerful oxidant. A study by Bareggi et al. looked at the effects of clioquinol on the changes in motor and cognitive behaviors and effects on amyloid deposition in mice infected with scrapie. Fifty 20-day old female hamsters weighing about 60 g were studied, and the treatment group was inoculated on the right hemisphere with 25µL of scrapie 263 K prions (diluted in saline). The treatment group received 0.5mg/g clioquinol by a feeding pellet incorporated into its diet. Passive avoidance behavior was tested using a series of foot shocks and showed that mice treated

with clioquinol improved passive avoidance similar to those observed by the non-infected control mice. Oxidative stress increased in the infected animals and peaked 60 days after infection. The study showed that Clioquinol counteracts massive memory deficit induced by scrapie infection, but Western blot, immunohistochemistry, histopathological and neurochemical findings showed that it did not reduce PrP deposition or synapse loss. This further indicates that the beneficia effects of clioquinol is due to its antioxidant effects (Bareggi et al., 2009).

A report by Brazier et al. detected a reduction of total superoxide dismutase (SOD) activity in mice infected with PrP, and greater Mn-SOD activity than wild type mice. Brazier et al. studied the therapeutic efficacy of EUK-189 (Eukarion salen-manganese complex) as an antioxidant compound. Daily intraperitoneal (ip) administration of EUK-189 was found to extend the incubation period, reduce nitrative and oxidative damage to proteins, and reduce brain vacuolar lesion burden, compared to untreated disease-controlled mice. Thirty-five young female mice were intra-cerebrally inoculated with a mouse adapted prion that was originally isolated from a human patient. The control infected mice survived an average of 169 dpi while the EUK-189 treated mice survived an average of 177 dpi. Lipid peroxidation, protein carbonylation, and tyrosine nitration were studied to see the effects of oxidative stress. Reduced oxidative stress was seen for all tests in the EUK-189 treated mice compared to the untreated disease control mice. This study supports the idea that oxidative stress is an integral component of pathogenesis (Brazier et al., 2008).

A 2010 study with Brazier et al. in 2010 showed that manganese chelation therapy has been seen to extend survival in mice. The di-sodium, calcium derivative of the chelator cyclohexanediaminetetraacetic acid ($Na_2CaCDTA$) was administered intraperitoneally to mice inoculated intra-cerebrally with either high or low dose inocula. This chelator has been shown to

bind and remove Mn^{2+} from the brains of experimental animals. Analysis by inductively coupled plasma-mass spectrometry revealed brain Mn^{2+} levels were reduced up to 50% in treated mice compared to the treated control (copper, iron, zinc, cobalt level remained unchanged). The mice that received a high-dose inocula did not display increased survival, but Western blot analysis of intensely treated mice showed reduced PrP^{Sc} levels. Survival time was only extended by approximately 12%, which is a modest result compared to other findings. These mice were infected with the M1000 strain, which is of human origin, and may indicate that different strains require different therapeutic strategies (Brazier et al. 2010).

Conclusion

Prions diseases include a variety of human and animal diseases that are neurodegenerative and affect the central nervous system. The infectious agent of these diseases is the prion protein (PrP^{Sc}), which begins to form aggregates in the brain when normal PrP^{C} is converted from its alpha structure to the infectious beta structure that is unable to be broken down in the brain. Although rare, there is potential for these diseases to increase in incidences, especially with forms such as sporadic CJD in humans. The mechanism for infection and the pathways in which this conversion occurs has not been well established. In attempts to understand the pathway, recent studies have suggested that metals within the body affect the PrP protein and its way of infection. Many of these experiments were conducted on animals using a form of human PrP and have showed that zinc, copper, iron, and manganese each play a role in infection. These transition metals within the body bind and interact with PrP when tested at different levels, suggesting that when metals get out of homeostasis range there is an increased risk of infection. As seen, these metals can cause PrP to affect other cell processes, metal homesostasis can affect oxidative stress, the presence/absence of metal can affect PrP structure,

and aggregate formation requires metal. Because these metals interact with PrP, it has also been suggested that targeting these metals may serve as a treatment strategy. No one metal seems to solely be responsible for the conversion of PrP, as zinc, copper, iron, or manganese, each have the ability to do so. Additionally, this area is limited in the number of publications. Taken together, these findings point to an increased need for research to fully understand the role metals play on PrP and prion diseases.

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