Iron in the brain (with apologies to Jean-Paul Sartre): Heavy metal mismanagement

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Abstract

Iron's activity in the body can be two-faced. On the one hand it is integral to many enzymatic reactions; on the other hand it is toxic, with a great capacity for cellular damage. This review examines iron in the brain through the lens of multiple sclerosis (MS), reviewing the functions of intracellular and extracellular iron and their impact on the disease, as well as highlighting the focus of new research and controversial therapies. The primary cause of MS has remained enigmatic, ever since its first clinical documentation. Several studies have suggested a link between MS and iron. Abnormal iron accumulation has been found as deposits in MS lesions around cerebral veins, in the macrophages surrounding MS lesions, and also in deep brain structures. There are features of MS, such as the inflammatory environment and altered vasculature, which are important in highlighting mechanisms of how iron can accumulate, and also how iron dysregulation can create a positive feedback cycle that further promotes neurodegeneration and increased iron accumulation. This potential link between iron and MS has gained widespread attention, in part due to the controversial cerebrospinal venous insufficiency (CCSVI) hypothesis and "Liberation Therapy" first introduced by Dr. Zamboni in 2008. Determining the role of iron in MS will help provide a better insight to the different factions of scientists who disagree on whether MS is primarily an autoimmune disorder, or whether a neurodegenerative mechanism is the instigator.

Keywords: multiple sclerosis (MS) and therapies; iron (regulation in the brain, link to disease); CCSVI hypothesis; Liberation Therapy (Zamboni, 2008); review

Introduction

Canada has one of the highest incidences of multiple sclerosis (MS) in the world (*Figure 1*) [1]. The Multiple Sclerosis Society of Canada states that three people are diagnosed with this chronic disease every day. MS tends to be clustered geographically, with higher incidences in temperate regions amongst Caucasians, and in women [2]. The symptoms of MS are complex and unpredictable, but all individuals suffer from progressive muscle weakness and fatigue [2]. Ultimately balance, memory, vision, and hearing may be impacted, depending on what region(s) of the brain are affected by the disease [2].

There has historically been a general reluctance to diagnose patients with MS. Even as recently as the mid-1900s, neurologists viewed the diagnosis as a drawn-out death sentence. Foster Kennedy exemplified this sentiment when he said in 1950 that "one should no more tell our patients they have multiple sclerosis than we should tell them

they have inoperable cancer. Hope is an emotion in its own right, and the physician may be wrong" [3].

While there have been improvements in the treatments of MS, there is still no cure or efficient way to halt the progression of the disease, and the causes and etiology of MS have remained elusive.

Figure 1. Prevalence of MS in the world [1].

CCSVI and the Internet

Due to its uncertain nature, there are many debates surrounding MS. While scientists still disagree about the cause of MS and whether it is autoimmune or neurodegenerative in origin, this is not the issue that has gained widespread public interest. The sudden increase in interest of MS began in 2008, with a hypothesis called Chronic Cerebrospinal Venous Insufficiency (CCSVI) [4]. Differing views on support for the hypothesis resulted in public outcries from MS patients and their families against the government and the scientific community.

Chronic cerebrospinal venous insufficiency (CCSVI)

The CCSVI hypothesis, first published online in late 2008 by Dr. Zamboni, was based on an observation of iron deposits surrounding veins in MS. In this hypothesis, iron took a lead role; it purported MS to be a neurodegenerative disease occurring as a result of venous pathology. Zamboni based his theory on his opinion that iron deposits in the MS brain had some similarities to the iron pathology around veins in legs in a chronic venous disorder [4]. Therefore, he hypothesized that MS was due to poor drainage of venous blood from the brain. The congestion of blood would cause increased pressure across the vascular wall and promote the release of hemoglobin to the area surrounding the vein. As will be reviewed in more detail later, hemoglobin and its breakdown products are able to damage endothelial cells and contribute to oxidative stress. Macrophages that break down hemoglobin also release iron [5].

Zamboni's publications, detailing the findings of vascular anomalies in MS patients and purporting CCSVI as a cause of MS, however alluring, were treated with skepticism in the scientific community due to the lack of a double-blind randomized control normally required for clinical trials. In surveys, such as the one conducted by Zamboni, the Hill Criteria can be used to infer causality [6]. Criteria on the Hill checklist include temporality and consistency. As Awad et al. deftly review, Zamboni's hypothesis as CCSVI being a cause of MS fails a number of the criteria [6]. In the case of temporality, it requires that if CCSVI is indeed a cause of MS, then it should always be present before the development of the disease [6]. It was observed that CCSVI is rare at clinical onset of MS, but its occurrence increases as the disease progresses [5,7]. This fact already suggests that CCSVI is, at best, a secondary phenomenon. There has also been a problem with consistency. Studies conducted afterwards failed to reproduce the results found by Zamboni [6]. A potential explanation of this lack of consistency could be due to inadequate methods. As noted by Chambers et al., neither Zamboni nor others who attempted to replicate his results state how their control subjects were selected [8].

However, the hypothesis of CCSVI conjured up a whirlwind of interest in the public MS community. One

aspect that might have carried the greatest weight was that sufferers of what had been hitherto regarded as an untreatable disease were now promised a "cure". The remedy that Zamboni proposed was simple: his method was to open congested veins with an inflatable balloon, a procedure known as a balloon-angioplasty. Zamboni's surgical angioplasty intervention then gained a very provocatively chosen name: 'Liberation Therapy'. Due to the lack of alternative treatments that promised such an easy cure, MS sufferers looked for anything to free them from this debilitating disease.

The divide

The public awareness and interest in medical research at this level has created an interesting dichotomy between the rigors of the scientific process and the demands of MS sufferers.

While the medical community and government are following well-established protocols for determining the viability of this procedure and the validity of Zamboni's results, MS sufferers have been outraged at being denied access to this treatment by the government. In response, thousands of patients have paid large sums of money and travelled to countries that conduct the procedure for the promise of this miracle cure [9].

The media has taken advantage of the general public interest and has responded with numerous reports. Since CTV carried out an exclusive interview with Dr. Zamboni in 2009, there have been 22 follow-up CTV news stories on Liberation Therapy in the past two years alone [10]. In February 2012, the Canadian Broadcasting Corporation (CBC) aired *MS Wars: Hope, Science, and the Internet* on "The Nature of Things" television program [9].

The impact of social media has been astounding. There are thousands of blogs, websites, and YouTube videos promoting a "miracle cure" for MS (Figure 2). There is even a directory encompassing all of the social network sites for CCSVI [11]. These have allowed MS patients to focus on the reportedly positive results from this treatment. As the positive affirmations spread with the Internet as their primary medium, they provided what every MS sufferer and their families so desperately needed: hope for a cure.

Figure 2. A selection of CCSVI logos found readily on the Internet.

Recently, a case-control study published in 2012 demonstrated that CCSVI does not have a causal role in MS, as it was not present in those with early stages of MS disability [8]. Another 2012 study indicated that there was no relationship between cerebral vein abnormalities and disease progression in patients [12]. As CCSVI is likely soon to be retired as another false theory in the history of MS, balloon-angioplasty will become just another example of the miracle of faith-healing, also known as the placebo effect.

The attention that the Zamboni article gained from the general public brings up significant questions about the traditional practices of how scientific research is communicated and refuted. It also raises the question of whether patient pressure and social media should affect the timing of clinical studies.

It has also shown the divide that exists between scientists and the public. While releasing the results of scientific research widely on the Internet will potentially help advance scientific discovery at a faster pace, there is still a disconnect between the discoveries of science and public knowledge. When patients and the public are using social media to advocate and mobilize, scientists who want their findings to reach the public and make an impact must do the same. A long term goal of science should be to provide more resources to educate and engage with the public, politicians, and the media. Improved scientific literacy would help ensure that resources are focused on effective scientific research, and not on ineffective therapies.

As the theory of CCSVI is increasingly being refuted by objective clinical data suggesting that, at most, it is a secondary phenomenon, the greatest question on the lips of both the scientific and public community remains: what is causing MS? To investigate this question further, aspects of potential links between MS pathology and iron will be examined. This review will address how iron may play a role in this disease, either initially or during its progression, and aims to emphasize the need for more research in this field.

Myelin and Multiple Sclerosis

Myelin in the central nervous system

Throughout the brain and spinal cord, together referred to as the central nervous system (CNS), nerves function like electrical wires, transmitting information in the form of electrochemical impulses. An insulating membrane wraps itself around these nerves, and is responsible for protecting the nerves and promoting the transmission of electrical information. Known as myelin, this lipid-rich sheath has been termed 'white matter', referring to its white glossy appearance due to its lipid composition [13].

In MS there is a profound and preferential destruction of the myelin sheath. In the process of demyelination, the myelin sheath is stripped off neurons in a pattern that follows the venous system [13]. The loss of myelin insulation and plaque formation not only interferes

with the transmission of the nerve impulse, but also means loss of support for the neuron, potentially leading to cell death [14]. Eventually the neurodegeneration spreads, encompassing the entire CNS [15].

Multiple sclerosis

MS is now accepted as being a multifactorial disease; it is grouped into a variety of different forms on the basis of the pattern of its progression. While it is known that MS destroys the protective myelin sheath of neurons, it is not known what initiates this destruction.

Relapsing-remitting is the most common form of MS in which periods of clinical episodes are followed by a partial recovery in symptoms [2]. In approximately 15% to 20% of cases, the pathology gradually worsens over time with no improvement, and is referred to as primary progressive [2]. The disease can take this course from the onset, but relapsing-remitting cases can also eventually convert to this progressive course [2]. This latter form is called secondary progressive. In the progressive form of the disease, primary or secondary lesions do not heal; the degeneration is continuous and irreversible [15]. There are currently no effective treatments available to halt the progressive course of MS.

Iron in the Cells and CNS

Despite the scientific evidence accumulating against the CCSVI hypothesis, there are other mechanisms that could lead to iron injury playing a role in MS. Iron's activity in the body can be two-faced; on one hand it is integral as a cofactor in many enzymatic reactions but on the other, when it is unbalanced or unregulated, it has the potential to be toxic and cause extensive cellular damage.

The physiology of intracellular iron

Iron is integral to nervous system function. It plays a role in neurotransmitter synthesis, DNA synthesis, and energy production via the electron transport chain [16]. It also plays a role in biogenesis which occurs when nutrients or oxygen are in short supply, and involves an increase in the number of mitochondria to meet energy demands [15].

Iron is also essential in myelin formation, as well as the maturation of the cells that synthesize myelin, known as oligodendrocytes, which are the main iron-containing cells in the adult CNS [16]. Iron is especially prevalent in the loops of myelin due to the high metabolic demands of myelin synthesis [16].

Toxic iron

Free or unbound iron is available to interact with reactive oxygen species, thereby producing free radicals. This process can be especially damaging if it occurs in the

brain because neuronal membranes are high in polyunsaturated fatty acids, making them extremely vulnerable to free radicals [17]. Lipid peroxidation products from this damage can be a source of pathogenesis by their ability to modify proteins, as well as become a target for an immune attack [18].

One mechanism of free radical production is through reaction of redox-active iron and the reactive oxygen species, hydrogen peroxide, which is a natural by-product of aerobic metabolism and is relatively non-toxic. However, hydrogen peroxide and free ferrous iron (Fe^{2+}) can react via the Fenton reaction, producing the extremely reactive hydroxyl radical [17]. Whereas the hydrogen peroxide molecule has limited reactivity with biological molecules, the hydroxyl radical's reactivity is only limited by its rate of diffusion [19]. The hydroxyl radical potently reacts with membrane lipids via a free radical mechanism, and thus propagates membrane damage [20]. The outcome of the Fenton reaction is the conversion of ferrous iron to ferric iron (Fe^{3+}). The superoxide anion is another reactive oxygen species produced as a by-product from normal aerobic metabolism [21]. Superoxide can react with ferric iron to reform ferrous iron (Figure 3) [22]. This allows the newly formed ferrous iron to produce hydroxyl radicals, and thereby facilitate inflammation and damage [21]. Another way that iron can cycle back to its redox-active state is through the high levels of ascorbate in the brain [23]. In the absence of transition metals, ascorbate has important antioxidant properties. However, in the presence of iron or other transition-metals, such as copper, ascorbate creates free radicals by reducing ferric iron and recycling iron back to its redox-active state [17,23]. This ability of iron to recycle back to its redox-active state is one way that damage by iron can become self-sustainable.

Figure 3. Cyclic of iron between ferrous and ferric states can promote the continual production of hydroxyl radical [22].

Iron also has a close link to the immune system. It acts as a chemoattractant to attract immune cells [17]. It can stimulate changes in vasculature, promoting the expression of adhesion molecules [17]. Iron can promote the release of certain proteinases that degrade the cells surrounding the vascular system, thereby promoting adhesion and migration of autoimmune cells into the surrounding tissue, which become activated and can then release pro-inflammatory molecules [17]. Activated immune cells, such as microglia, can undergo oxidative bursts which involve the release of reactive oxygen species; activated microglia have been

identified in MS as mediators of demyelination [17]. An oxidative burst not only increases the concentration of free radicals, but can also liberate iron from ferritin [15]. The free iron, converted into its reactive ferrous form, is then able to react and produce hydroxyl radicals. Thus, this mechanism results in the amplification of oxidative damage by promoting iron's ability to create toxic hydroxyl radicals [7].

Iron promotes oxidative damage when it facilitates mitochondrial stress [17]. The demyelination that occurs in MS creates a greater energy demand for the cell's mitochondria. Moreover, iron accumulation promotes inflammation, which further disturbs the already stressed mitochondria. Iron can also inhibit base repair of DNA, contributing in yet another way to oxidative stress [17].

An extracellular source of iron

Iron's properties make it a useful cofactor in important enzymes. It exists as part of a heme component in enzymes such as catalase and peroxidases, which are important in protection against oxidative damage. Heme consists of rings of carbon and nitrogen with iron at the core. By far the most abundant source of heme is hemoglobin, and there is no doubt that hemoglobin plays a vital role in sustaining mammalian life. Despite its usefulness, hemoglobin is neurotoxic and has an immense capacity to cause damage to cells [17]. The endothelium is protected from this damage by erythrocytes which transport hemoglobin in an environment containing a lot of antioxidant enzymes [24].

However, vasculature problems promote erythrocyte rupture, and hemoglobin can be released either intravascularly or extravascularly. When released from erythrocytes, hemoglobin is very reactive. Once hemoglobin is oxidized into its methemoglobin form, it can release its heme moieties readily [25].

Due to its hydrophobic nature, heme can cross lipid membranes. This property is an integral aspect of its ability to cause cellular damage [25]. Once inside the plasma membrane, heme can react directly with peroxides, promoting oxidative stress. This reaction breaks down heme, and releases free iron, which is then able to participate in other reactions [26]. In this manner, heme acts as a Trojan horse to transport hydrophobic iron into the interior of cells. Iron itself is then able to cause damage. Damage due to hemoglobin release can be measured by the presence of lipid peroxide breakdown products [27]. The lipid peroxide products can activate the immune system as well as react with hemoglobin, promoting the conversion of the protein to its methemoglobin form, and thereby facilitating heme release [25].

Iron regulatory proteins

Due to iron's integral but damaging nature, iron regulatory proteins are key to managing the delicate balance between the body's need for iron, as well as limiting iron's

damage. One protective mechanism that the body has developed is to sequester iron in a non-reactive form in the cell. The protein ferritin is responsible for sequestering iron, and is conserved amongst diverse life forms, from bacteria to humans [23]. Due to iron's importance in cell function, neurons usually contain an endogenous supply of iron bound to ferritin [16]. There is also an exogenous delivery supply of iron – transferrin is the major iron delivery protein for neurons [28].

As mentioned earlier, as a source of iron, heme can promote oxidative damage by producing radicals, but also by releasing free iron to react further. A protective mechanism is in place to prevent this damaging degradation of heme. This enzyme complex consists of the enzyme heme oxygenase (HO), as well a source of electrons for the reaction, derived from NADPH cytochrome P450 reductase (referred to as NADPH henceforth) [29]. The mechanism consumes oxygen and seven electrons supplied by NADPH to cleave the heme ring, releasing carbon monoxide, biliverdin, and free ferrous iron [29]. HO protects against damage from heme by safely breaking down heme without production of free radicals [23]. Biliverdin, converted to bilirubin, also has antioxidant effects [23]. For the protective mechanism of HO to be effective, the redox-active iron must be then bound to ferritin [23,28-30].

Dysfunction of iron regulatory proteins

Iron regulatory protein levels are controlled by their responsiveness to iron levels, and dysfunction in these proteins can be a source of iron mismanagement. A dysfunction in transferrin, leading to an alteration in iron transport, can lead to a multitude of neurological impairments, and is one of the symptoms in MS patients; it also occurs in iron deficiency, inflammation, cancer, Alzheimer's disease, Parkinson's disease, and Restless Legs Syndrome [28].

HO acts as an antioxidant during short-term instances of heme overload. However, chronic activation of this system can have deleterious effects. One of the reasons that chronic activation of HO can have damaging effects is the requirement of NADPH for the breakdown of heme. NADPH is also required for the production of glutathione, an important antioxidant for the cell [30]. Therefore, chronic depletion of NADPH due to HO hyperactivity can slow the recovery of glutathione. Depletion of glutathione and NADPH increases the availability of hydrogen peroxide to react and produce radicals and cell death [30]. Another cause of damage by overexpression of HO lies in the free iron produced from heme breakdown. Normally, this iron is quickly sequestered with ferritin. In times of iron overload, however, ferritin binding sites can become saturated, leading to increased levels of free ferrous iron to react.

In summary, inadequate storage of iron allows the redox active iron to produce reactive hydroxyl radicals that can lead to oxidation of lipid membranes, denatured proteins, and damaged DNA [3]. Iron can cause oxidative damage and

promote inflammation, thereby promoting further release of iron. This process can then become self-sustaining.

Linking Iron to Multiple Sclerosis

Neurodegeneration and iron

Iron dysregulation is not unique to MS. Iron's mismanagement occurs in numerous disease states and can occur through more than one mechanism. However, there are features of MS, such as the inflammatory environment and altered vasculature, which are important in highlighting mechanisms of how iron can accumulate, and also how iron dysregulation can create a positive feedback cycle that further promotes neurodegeneration.

Studies of iron in multiple sclerosis

There are a number of studies linking iron to MS, ranging from large-scale analyses studying the brain as a whole, to small-scale analyses involving the measurement of molecular interactions.

Whole brain scans, such as magnetic resonance imaging (MRI), analyze iron content in areas of the brain. Another technique, called susceptibility-weighted neuroimaging (SWI), measures iron concentration by assessing magnetic field susceptibility differences in different tissues [31]. The disadvantage of these studies is that they cannot differentiate whether iron deposits in MS are a benign result of the disease progression, or a key promoting factor of neurodegeneration.

What these studies emphasize is that the importance lies not in total brain iron levels, but in the areas of the brain with abnormally accumulated iron and iron in a redox-active form [17]. It is important to note that iron accumulation occurs as a natural process of aging. Under pathological conditions, iron levels may accumulate to the point where they overcome the capacity of the normal mechanisms in place to properly manage iron [32]. Abnormal iron deposition has been observed in a number of neurodegenerative diseases, and may contribute to neuronal damage and progress degeneration [32]. This situation could occur in MS, since there is evidence of abnormal iron accumulation [16]. In white matter, there is accumulation in oligodendrocytes, macrophages, and microglia around sites of inflammation associated with veins [16,33,34].

Higher than normal levels of iron are also found in deep gray matter structures in the brain. The significance of this finding is that some of these structures are involved in message relay. For example, the thalamus, one structure that is affected, is a diffuse relay center important for the control of sensory and motor functions [35]. If iron accumulation is responsible for damage to these structures, it could have far-reaching effects on the many brain regions that are connected by these relay stations [35,36]. In general, the extent of iron accumulation in gray matter structures and lesions has been

shown to be a good predictor of disability progression in MS, as well as the extent of lesion accumulation and level of cell death [16,33,37]. Histological studies have analyzed the lesions in the brains of MS patients, as well as accumulation of molecular players in lesion sites. They have shown that lesions are surrounded by iron-loaded macrophages. A likely cause of iron accumulation in these cells may be macrophage uptake of cell debris from degeneration, such as the remnants of oligodendrocytes or blood extravasation.

Measuring levels of iron-regulatory proteins in the blood or cerebrospinal fluid yields a general idea of brain iron levels. Evidence of abnormal iron homeostasis can come from analysis of iron and iron-related proteins in cerebrospinal fluid, blood, and post-mortem analysis [36]. Levels of ferritin have been found to be elevated in patients with some forms of MS, suggesting local iron overload [4]. In a situation like this, there is potential for medical research; however, what is currently missing is the ability to follow the disease progression before the clinical onset.

The problem of causality

Studies documenting the occurrence of iron accumulation and MS show that there is a connection between the occurrence of MS and iron mismanagement. What they do not show is whether iron is a cause of the degeneration in MS, or whether iron accumulation in plaques is a protective mechanism, a degenerative mechanism, or merely a by-product from the degeneration. To determine causality, there must be a way to identify the beginning of the degeneration. This point is not easily identifiable, as it occurs when there are no outward signs. Clinical trials are further complicated by the multifactorial nature of MS, as well as uncertainty of where the disease is in its progression when a diagnosis is made [3].

Work to be Done

There still remain dichotomous views of whether MS is a neurodegenerative disorder or primarily an autoimmune disorder. However, evidence is accumulating suggesting that MS might have a neurodegenerative rather than autoimmune origin. Demyelination and death of myelin-producing oligodendrocytes can occur before inflammation or the activation of immune cells [38,39]. Inflammation due to the presence of immune cells in the brain could occur as a secondary response to a neurodegenerative process [39].

Vasculature anomalies in multiple sclerosis

Even as Zamboni's CCSVI theory is turning out to be false, the evidence of a vascular pathology in MS on which the theory was founded remains valid. Studies have found dilated cerebral veins in the brain of individuals with MS as well as fibrin cuffs found around the veins [4].

It is important to investigate further the venous abnormalities in MS patients in order to understand the physiological mechanisms. Vasculature problems can cause nutrient deficiency and hypoxia, which is a lower oxygen concentration than normal [22]. The brain is especially susceptible to hypoxic damage due to its high metabolic rate and therefore steep oxygen requirements [22]. The hypoxia not only causes the death of neurons and glia directly affected, but also increases the levels of reactive oxygen species and raises the level of glutamate, a prevalent excitatory neurotransmitter, to damaging levels [22].

This process can become cyclic, as the vasculature damage due to hemoglobin release can promote further damage to the vasculature and surrounding tissue. However, what remains to be determined is whether the greatest potentiator of vasculature damage is from hemoglobin, heme, iron, or another unknown player. The oxidative stress due to venous haemorrhage and iron deposits can have real implications on the process in demyelination. Aside from MS, iron deposition has been observed in other demyelinating diseases, such as in Hurst acute haemorrhagic leukoencephalitis [14].

Future research into the vascular pathology in MS will be of benefit. This avenue of inquiry will hopefully lend new insight into the role of iron deposits in MS, the potential source of hemoglobin as a source of iron overload, and the role of hemoglobin and its breakdown products in venous pathology.

Conclusion

The question still remains regarding iron's role in the pathogenesis of MS. Is iron an instigator of the disease, a by-product of an inherent neurodegenerative process, or merely an epiphenomenon and not a real player in this illness? What is certain, however, is that iron dysregulation is a potent source of damage, and should be focused on as a future source of research and therapies. Determining iron's role in MS will hopefully move the scientific world one step closer to elucidating a mechanism for this disease.

Another significant note is the divide that still exists between scientific research and the public. While patients are using social media to mobilize, scientists in this aspect are falling short. A statement of this is in the impact that CCSVI has made in the public sector. A quick Internet search reveals a plethora of CCSVI organizations (some examples are shown in *Figure 2*). To search for the articles that disprove CCSVI as a cause of MS, one must search harder. Scientists need to become more effective in public communication, not only on this issue, but in general.

Science will never be independent of society. The discoveries of science will impact the lives of the public, and funding from the public and government impact the type and amount of research that can be conducted. Therefore, improving scientific literacy will create a better appreciation for the current scientific discoveries that are occurring, and

hopefully bridge the divide that has emerged between science and society.

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