Therapeutic potential and biological role of antioxidants and antioxidant enzymes in multiple sclerosis pathology

G. Schreibelt¹, J. van Horssen¹, S. van Rossum¹, C.D. Dijkstra¹, B. Drukarch², and H.E. de Vries¹

Departments of ¹Molecular Cell Biology and Immunology, and ²Anatomy and Neurosciences, VU University Medical Center, Amsterdam, The Netherlands

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Summary

Reactive oxygen species (ROS) contribute to the formation and persistence of multiple sclerosis (MS) lesions by acting on distinct pathological processes. In the initial phase of MS lesion development, locally produced ROS may induce blood-brain barrier (BBB) disruption and enhance leukocyte migration. Macrophages are the most prominent cell type present in active demyelinating MS lesions and produce large amounts of ROS, which contribute to lesion persistence by mediating oligodendrogial damage and axonal injury. To counteract the detrimental effects of ROS the central nervous system is endowed with a protective mechanism consisting of enzymatic and non-enzymatic antioxidants. Expression of most antioxidants is regulated through the transcription factor nuclear factor-E2 related factor (Nrf2) and antioxidant response elements (ARE) in the genes encoding enzymatic antioxidants and is induced by oxidative stress. In brain tissue of MS patients, enhanced expression of Nrf2/ARE regulated antioxidants is suggestive of the occurrence of oxidative stress in these lesions. Antioxidant therapy may therefore represent an attractive treatment of MS. Several studies have shown that antioxidant therapy is beneficial \textit{in vitro} and \textit{in vivo} in animal models for MS. However, the use of exogenous antioxidants for MS treatment has drawbacks, as large amounts of antioxidants are required to achieve functional antioxidant levels in the CNS. Therefore, the induction of endogenous antioxidant enzymes by activators of the Nrf2/ARE pathway may be an interesting approach to obtain sufficient levels of antioxidants to interfere with pathological processes underlying MS lesion formation. In this review we provide an overview on the role of ROS in various processes underlying MS lesion formation and progression. Next, we will summarize and discuss the biological role, regulation and potential therapeutic effects of endogenous antioxidants in MS. We propose that antioxidants may inhibit the development and progression of MS lesions and may therefore represent an attractive therapeutic target for the treatment of MS and other oxidative stress-related neurological diseases.
1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). MS is considered a multifactorial disease and MS patients may suffer from a variety of clinical symptoms, including changes in sensation, visual problems, muscle weakness and difficulties with coordination and speech. Macroscopical examination of CNS tissue of individuals with MS reveals multiple sharply demarcated plaques in the white matter, with preference for the optic nerves and white matter tracts of the periventricular regions, brain stem, and spinal cord. Pathological features of MS plaques are blood-brain barrier leakage, destruction of myelin sheaths, oligodendrocyte damage and cell death, axonal damage and axonal loss, glial scar formation and the presence of inflammatory infiltrates that mainly consist of lymphocytes and macrophages. In particular monocyte-derived macrophages are thought to play a central role in MS pathology. Monocyte-derived macrophages phagocytose myelin, which causes damage and breakdown of myelin sheaths, oligodendrocytes and axons. In addition, during inflammation, macrophages produce a variety of inflammatory mediators, including cytokines, chemokines, nitric oxide, and reactive oxygen species (ROS), which contribute to the development and progression of the disease. In recent years, it has become apparent that ROS and oxidative stress contribute to the formation and persistence of MS lesions. This review addresses the role of ROS in MS pathology. In addition, we discuss the possible contribution of antioxidants and antioxidant enzymes to MS treatment.

2. Reactive oxygen species and MS pathology

ROS play a physiological role in numerous cellular regulatory processes. An overview of ROS production and scavenging by antioxidants is given in Figure 1 and described in more detail in section 4.1. When the rate of free radical generation exceeds the capacity of antioxidant defense, oxidative stress occurs, which leads to subsequent damage to macromolecules, such as proteins, lipids and nucleic acids. ROS-induced damage of lipid membranes by lipid peroxidation has been demonstrated in MS patients and markers of oxidative stress, such as thiobarbituric acid reacting substances (TBARS), are increased in serum of MS patients. Additionally, ROS production is increased in activated peripheral blood mononuclear cells of MS patients during a relapse. In the CNS of MS patients, free radical-induced damage is detected in active demyelinating MS lesions as demonstrated by the presence of increased nuclear and mitochondrial DNA oxidation. In addition, enhanced protein carboxylation has been detected in lesion areas and normal appearing white matter of MS patients.

Nitric oxide, an inflammatory mediator produced by macrophages, endothelial cells and neurons, is also implicated in MS pathology. The expression of inducible nitric oxide synthase (iNOS) is increased in the CNS of MS patients and, in addition, enhanced levels of end products of nitric oxide, nitrate and nitrite, have been found in the serum and cerebrospinal fluid of patients suffering from MS. Svenningsson and colleagues...
showed that enhanced serum levels of nitrite were linked to disease activity\textsuperscript{22}, while others, however, found no correlation with clinical features of MS\textsuperscript{21,23}.

Superoxide reacts with nitric oxide to form highly reactive peroxynitrite, which can damage axonal organelles, membranes and enzymes and is particularly toxic to oligodendrocytes\textsuperscript{24,25}. The presence of nitrotyrosine, an end product and biochemical marker for peroxynitrite formation, has been demonstrated in active MS lesions\textsuperscript{19,20}, indicating the presence of ongoing oxidative stress in these lesions.

ROS are implicated in various pathological processes underlying the formation and persistence of MS lesions. To enter the CNS, monocytes need to cross the blood-brain barrier, which consists of highly specialized brain endothelial cells and their tight junction complexes. Recently, we demonstrated that ROS are produced within minutes upon the interaction of monocytes with brain endothelium\textsuperscript{26}. Subsequently, exposure of brain ECs to ROS enhances monocyte migration\textsuperscript{27}. ROS were shown to affect brain endothelial tight junction integrity and to rearrange the brain endothelial actin cytoskeleton\textsuperscript{26-31}, thus enhancing blood-brain barrier permeability\textsuperscript{28,29,31-37}. In addition, it has been shown that ROS induce phagocytosis and degradation of myelin by macrophages, oligodendrocyte damage, and neuronal and axonal degeneration\textsuperscript{14,38-43}. Taken together, there is substantial evidence that oxidative damage is an important pathological feature of MS and that ROS are involved in a variety of pathological processes underlying MS lesion formation. As scavengers of ROS may thus interfere at multiple levels during the formation of MS lesions, antioxidant therapy may be expected to be beneficial for the treatment of MS.

3. Antioxidant treatment of MS

The CNS is equipped with a powerful antioxidant defense mechanism to scavenge ROS. The antioxidant system can be divided into two major groups: enzymatic antioxidants (discussed in section 4) and non-enzymatic or low-molecular-weight antioxidants. The latter consist of both endogenous (e.g. glutathione and NADPH) and exogenous molecules. The majority of exogenous antioxidants are derived from dietary products, including ascorbic acid (vitamin C), α-tocopherol (vitamin E), α-lipoic acid, flavonoids, polyphenols, and carotenoids\textsuperscript{44-46}.

Altered concentrations of antioxidants have been observed in sera of MS patients. Serum levels of β-carotene, retinol, α-tocopherol (vitamin E), and ascorbic acid (vitamin C), are reduced in MS patients during disease exacerbation\textsuperscript{11,47}. In addition, decreased levels of reduced glutathione and α-tocopherol were measured in MS lesions compared to normal appearing white matter\textsuperscript{48}, suggesting that high levels of ROS may have resulted in the depletion of cellular antioxidants in MS patients.

In vitro, exogenous antioxidants have been shown to affect various processes that underlie MS lesion formation and persistence. Our group demonstrated that α-lipoic acid and the flavonoid luteolin reduce monocyte migration across the blood-brain barrier\textsuperscript{26,49} and myelin phagocytosis by macrophages\textsuperscript{39,42}. In addition, α-lipoic acid reduces monocyte-induced blood-brain barrier permeability\textsuperscript{26} and affects T cell migration across a
Fibronectin barrier. Furthermore, α-lipoic acid, coenzyme Q10, and the flavonoids luteolin and quercetin protect OLN-93 oligodendrocytes from hydrogen peroxide-induced oxidative damage. In addition, catalase prevents ROS-induced axonal damage in retinal ganglion cells. Together, these data imply that antioxidants interfere with multiple processes underlying MS lesion formation.

Experimental allergic encephalomyelitis (EAE) is a widely used animal model to study underlying mechanisms of MS pathology. EAE shares a number of clinical features with MS, including optic neuritis, paralysis and ataxia. In most models EAE is induced in rodents or non-human primates by active immunization with whole myelin or myelin components together with a strong adjuvant, such as complete Freund's adjuvant. Depending on the immunization protocol, EAE can be either monophasic (acute EAE) or biphasic (chronic EAE). Histopathologically, EAE is marked by the presence of high numbers of infiltrated T cells and monocyte-derived macrophages in the CNS and, depending on the model, demyelination and axonal damage. In EAE protective effects of antioxidants, such as flavonoids, N-acetyl-L-cysteine, N-acetylcysteine amide (AD4), and α-lipoic acid have been described. These ROS scavengers affect cellular infiltration into the CNS, the activity of matrix metalloproteinases (which may contribute to cellular migration), demyelination, and axonal damage, thereby decreasing the severity of disease. Surprisingly, vitamin C, a powerful water-soluble antioxidant, did not influence the course of EAE. Several studies have shown that the peroxynitrite scavenger uric acid ameliorates EAE by reducing cellular infiltration into the CNS and increasing blood-brain barrier integrity. However, inhibitors of nitric oxide synthase, such as nitro-L-arginine methyl ester (L-NAME) or NG-L-monomethyl-arginine (L-NMMA), have had variable, and sometimes deteriorating effects on the course of EAE, suggesting a complex role of nitric oxide in the development of EAE.

Despite promising results in vitro and in vivo, only a few studies have been published on successful antioxidant therapy in MS patients. A study on the relation between nutrition and the risk of developing MS revealed a lower risk with higher vitamin C intake, while there was no relation with intake of carotenoids or vitamin E. An other epidemiological study found no association between the intake of vitamins or vitamin-rich food products, such as fruits and vegetables, and the incidence of MS. These data suggest that vitamin intake via diet is not sufficient to treat or prevent MS. Higher doses of antioxidants or other routes of administration may be needed to affect the course of MS. A small clinical trial demonstrated that oral administration of inosine, a precursor of the peroxynitrite scavenger uric acid, raises serum levels of uric acid without causing serious side effects. MS patients treated with inosine did not show disease progression, and in two patients a reduction of MRI lesions was observed. Recently, a pilot study in MS patients demonstrated that daily oral administration of the antioxidant α-lipoic acid for two weeks was well tolerated and resulted in decreased serum levels of MMP-9 and soluble intercellular adhesion molecule-1, markers of inflammatory activity in MS. However, the duration of this study was too short to demonstrate significant effects on clinical symptoms. Further clinical trials are needed to study the potential therapeutic effects of antioxidants for the treatment of MS.
4. Endogenous antioxidant enzymes

Although it has been shown that exogenous antioxidants are beneficial in the course of EAE there is a number of drawbacks to the use of exogenous antioxidants for MS treatment, as most antioxidants do not efficiently cross the blood-brain barrier, are rather unstable in the body, and high doses are generally required to achieve protective effects in EAE. Hence, alternative strategies to inhibit the detrimental effects of ROS, for instance through the induction of endogenous enzymatic antioxidants are desirable. Gene transcription of most antioxidant enzymes is regulated through the transcription factor nuclear factor-E2-related factor (Nrf2) and antioxidant response elements (ARE) in the genes encoding enzymatic antioxidants. Under physiological conditions, Nrf2 is linked to the actin-bound Kelch-like ECH-associated protein 1 (Keap1) and located in the cytoplasm. However, upon oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it activates ARE-mediated gene transcription and induces the coordinate transcription of ARE-regulated genes (Figure 1)\(^{70,71}\). To date, over 200 Nrf2-ARE-driven genes involved in detoxification and antioxidant defense have been identified, including superoxide dismutases (SODs)\(^{72}\), peroxiredoxins (Prxs)\(^{73}\), heme oxygenases (HOs)\(^{74}\), glutathione peroxidases (GPxs)\(^{75}\), catalase\(^{73}\), NAD(P)H:quinone oxidoreductase 1 (NQO1) and NHR:quinone oxidoreductase 2 (NQO2)\(^{76,77}\), and metallothioneins (MTs)\(^{78}\).

Figure 1. Schematic overview of Nrf2/ARE activation by ROS. Nrf2 is retained in the cytoplasm by actin-bound Keap1. Upon oxidative stress the Keap1-Nrf2 complex is disrupted and Nrf2 is translocated to the nucleus, where it activates ARE-mediated gene transcription of antioxidant enzymes.
4.1 Superoxide dismutases

The first line of defense against oxidative stress is provided by SODs, a group of metal-containing enzymes that catalyze the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide\(^{79}\). SODs exist in several forms, differing in structure, active metal center, and number of subunits\(^{80}\). In humans, three different forms of SODs are expressed: copper and zinc-containing cytosolic SOD (Cu/ZnSOD, SOD1), which is a homodimer of 32 kDa\(^{81}\), manganese-containing mitochondrial SOD (MnSOD, SOD2), a 89 kDa homotetramer\(^{82}\), and extracellular Cu/ZnSOD (SOD3), a tetrameric glycoprotein of 135 kDa\(^{83}\). A minor fraction of SOD1 is found in the mitochondrial intermembrane space\(^{84}\). SOD1 and SOD2 are abundantly expressed in the CNS. SOD1 is primarily expressed in astrocytes, and to a lesser extent in neurons, whereas SOD2 is mainly found in neurons, and less in astroglial cells. In microglia, oligodendrocytes, and brain endothelial cells basal expression of both SOD1 and SOD2 is low\(^{85}\). SOD3 is also produced in the CNS, albeit in considerably lower concentrations than SOD1 and SOD2\(^{86}\).

Various observations suggest involvement of SOD in neurodegeneration and neuroinflammation. Increased expression of SOD has been described for various oxidative stress-associated neurodegenerative and neuroinflammatory diseases, such as Alzheimer’s disease\(^{87;88}\), and stroke\(^{89;90}\). Familiar forms of the neurodegenerative disease amyotrophic lateral sclerosis (ALS), a degenerative motoneuron disease characterized by the presence of oxidative stress markers in post-mortem CNS tissue, are linked to dominant missense mutations in the gene encoding SOD1\(^{91;92}\). In MS, significantly enhanced gene expression of SOD1 has been observed in active demyelinating lesions\(^{93}\). Enhanced expression of SOD2, but not SOD1, was found in guinea pigs suffering from EAE\(^{94}\). Several research groups studied therapeutic effects of SOD administration in EAE, but without success. Intrapertoneal and intrathecal administration of exogenous SOD did not affect the development of EAE in a rat model\(^{3;95}\) and SOD treatment had only minor effect on EAE and experimental allergic optic neuritis in guinea pigs\(^{96;97}\). Recently, synthetic SOD mimetics have been developed that were effective in rodent models of ischemia and Parkinson’s disease\(^{98-100}\). Thus far the effect of such compounds on EAE has not been studied and future studies should reveal whether SOD mimetics have potency for the treatment of MS.

Superoxide is the predominant ROS produced by mitochondrial respiration and enzymatic oxidoreductases. The removal of superoxide anion by SODs leads to the production of hydrogen peroxide, which is more stable than superoxide and can diffuse across membranes. In the presence of transition metals superoxide and hydrogen peroxide can form highly reactive hydroxyl radicals. Therefore, SODs act in conjunction with hydrogen peroxide removing enzymes catalase, GPx, and Prxs to protect cells against oxidative damage (Figure 2). Consequently, (co-)treatment with hydrogen peroxide-removing enzymes may be more effective in the treatment of neuroinflammatory diseases than superoxide scavengers alone.
4.2 Catalase

Catalase is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes and to some extent in the cytosol of mammalian cells. It is a tetrameric enzyme consisting of four identical, tetrahedrally arranged subunits of 60 kDa, each containing a heme group and NADPH in its active center. Catalase has two enzymatic activities depending on the concentration of hydrogen peroxide, a powerful oxidizing agent. If the concentration of hydrogen peroxide is high, catalase catalyzes the conversion of hydrogen peroxide into water and molecular oxygen. However, at a low concentration of hydrogen peroxide the enzyme also has peroxidase activity and reacts with organic peroxides and hydrogen donors to water and organic alcohols. Catalase is particularly important in the case of limited glutathione availability and plays a significant role in the development of tolerance to cellular oxidative stress. In the CNS, expression of catalase has been demonstrated for all cell types, both in vitro and in vivo. In brain homogenates of rats suffering from EAE, decreased peroxisomal function was demonstrated, which was accompanied by reduced gene expression and activity of catalase. A number of studies showed beneficial effects of catalase treatment on neuroinflammation in vivo. Catalase treatment of guinea pigs suffering from EAE significantly reduced demyelination of the optic nerves, increased blood-brain barrier integrity, and suppressed neurologic manifestation of EAE. In addition, intraperitoneal and intrathecal administration of catalase reduced the severity of clinical signs in a rat model for EAE. Furthermore, upregulation of endogenous catalase expression via viral vectors ameliorates EAE. Collectively, the above-described studies demonstrate that various catalase treatment protocols have beneficial effects on the development of EAE.
4.3 Glutathione peroxidases
GPxs constitute a family of selenium-containing enzymes that detoxify cellular organic peroxides and hydrogen peroxide by oxidizing two molecules of glutathione. Thus far, four types of GPxs have been identified in mammalian cells. GPx1 is generally expressed in the cytosol and mitochondrial matrix of virtually all cell types, while GPx2, GPx4, and GPx5 are found in specific organs and tissues and GPx3 is an extracellular glycoprotein. GPx1, GPx2, and GPx3 are expressed as homotetramers, whereas GPx4 is a monomer. In the brain, the activity of GPx1 is higher than that of catalase. In addition, since catalase is predominantly expressed in peroxisomes, while GPx1 is found in the cytosol and mitochondria, where large amounts of superoxide are generated, GPx may be more important than in removing hydrogen peroxide in the central nervous system. Tajouri and coworkers demonstrated that GPx gene expression is significantly increased in active demyelinating MS lesions. In addition, already in 1989, Guy et al. demonstrated that treatment with GPx reduces loss of blood-brain barrier integrity in chronic EAE, suggesting that treatment with GPx is a beneficial therapy for neuroinflammatory diseases.

4.4 Peroxiredoxins
In recent years it has become clear that Prxs may be the major hydrogen peroxide removing enzymes in mammals. The family of Prxs consists of six distinct groups (Prx1-6) of thio-specific antioxidant proteins that are involved in the enzymatic degradation of hydrogen peroxide, organic hydroperoxides and peroxynitrite. They also play a role in the modulation of cytokine-induced hydrogen peroxide levels, redox signaling, cell proliferation, differentiation and gene expression. Prxs can be divided into three major subclasses: typical 2-cysteine Prxs (Prx1-4), atypical 2-cysteine Prxs (Prx5) and 1-cysteine Prxs (Prx6), based on the number and position of cysteine residues involved in catalytic function. The presence of Prxs is most abundant in the cytosol, but Prxs are also found in mitochondria, peroxisomes, plasma, nuclei and associated with membranes, while Prx4 is secreted from cells. In the human brain, Prx1 is primarily expressed in astrocytes, while Prx2 is predominantly localized to neurons. Krapfenbauer and colleagues studied the expression of Prxs in a number of neurodegenerative diseases that are associated with oxidative stress, including Down syndrome, Alzheimer’s disease and Pick’s disease. This study revealed that Prx1 expression was not significantly different from control brains, whereas expression of Prx2 and Prx6 was significantly increased in brains of patients suffering from neurodegenerative disease. In contrast, Prx3 expression was reduced, indicating that expression of Prxs is differentially regulated in neurodegenerative diseases. Thus far, the distribution and involvement of Prxs in neuroinflammatory diseases, such as MS, has not been investigated. Therefore, studies on the functional role of Prxs in MS pathogenesis are warranted.
4.5 Heme oxygenase
The heme oxygenases (HO), which consist of constitutive (HO-2) and inducible (HO-1) isozymes, catalyze the rate-limiting step in the catabolism of heme and break down the porphyrin ring to yield equimolar amounts of biliverdin, free iron ($\text{Fe}^{2+}$) and the vasodilator carbon monoxide (CO). HO-1 has both anti-oxidative and anti-inflammatory properties and is highly inducible by a variety of stimuli, including its substrate heme and oxidative stress. HO-2 is predominantly expressed constitutively and is thought to function in normal heme capturing and metabolism$^{117}$. It has been shown that hemin-induced HO-1 upregulation reduced the clinical severity of EAE in rats, whereas tin mesoporphyrin, a well-known inhibitor of HO-1 markedly exacerbated EAE$^{118}$. Surprisingly, Chakrabarty and coworkers demonstrated that administration of the HO-1 inhibitor tin-protoporphyrin attenuated clinical scores in murine EAE$^{119}$. These discrepancies may be due to differences in HO-1 inhibitors used and species. In MS spinal cord tissue, HO-1-immunoreactivity was significantly enhanced in hypertrophic astrocytes compared to the spinal cord white matter of control patients$^{120}$. Also in the CNS of animals suffering from EAE, enhanced HO-1 expression was observed in astrocytes, reactive microglia, and infiltrated macrophages$^{121}$.

In mammalian cells, biliverdin is rapidly converted by biliverdin reductase into bilirubin, while the pro-oxidant iron is directly sequestered and inactivated by co-induced ferritin. Bilirubin exerts potent antioxidant activity and suppresses ongoing EAE and halted EAE progression when administered after disease onset, likely by reducing blood-brain barrier permeability and oxidative damage$^{122}$. Interestingly, bilirubin protected primary rat oligodendrocytes against hydrogen peroxide-mediated cell death. Treatment with biliverdin reductase, like bilirubin, reduced oxidative injury in EAE lesions and significantly suppressed clinical symptoms of EAE$^{95}$. Together, these studies show that HO-1 or downstream products of heme metabolism may be interesting targets for MS treatment.

4.6 Quinone oxidoreductases
NQO1 and NQO2 are cytosolic flavoproteins that catalyze the two-electron reduction of quinones and derivatives, preventing their participation in redox cycling. In addition, NQO1 and NQO2 have broad-spectrum antioxidant properties$^{123-126}$. Besides their function as antioxidants, NQO1 and NQO2 function to maintain both $\alpha$-tocopherol and coenzyme Q10 in their reduced antioxidant state. NQO1 is expressed in tissues that require high levels of antioxidant protection, like lung respiratory epithelium and the CNS$^{127}$. Immunohistochemical analysis of healthy human brain tissue showed that NQO1 immunoreactivity was predominantly found in astrocytes and brain endothelial cells$^{128,129}$. In Alzheimer’s disease and Parkinson’s disease brains, astrocytes are highly NQO1-immunoreactive in areas of ongoing disease activity, indicating the occurrence of oxidative stress$^{128,130}$. We recently showed that NQO1 is markedly upregulated in inflammatory MS lesions, particularly in hypertrophic astrocytes and myelin-laden macrophages$^{129}$. In human tissue, NQO2 gene expression has been observed in kidney,
liver, lung and heart, while very low expression was found in the brain\textsuperscript{131}. To date, little is known about the regulation of NQO2 expression in neurodegenerative or neuroinflammatory diseases.

4.7 Metallothioneins

Metallothioneins (MTs) constitute a family of small cysteine-rich proteins that bind heavy metals through thiol groups of their cysteine residues. MTs play a role in cellular zinc metabolism and have antioxidant properties. Thus far, four distinct MT isoforms have been identified, of which MT-I and MT-II are expressed in the brain and peripheral tissues. MT-III is mainly found in the brain and to a lesser extent in the intestine and pancreas\textsuperscript{132,133}, whereas MT-IV is exclusively expressed in stratified squamous epithelia\textsuperscript{134}. In the human CNS, MT expression is mainly found in astrocytes\textsuperscript{132}. It has been described that MT-I and MT-II expression can be induced by oxidative stress\textsuperscript{135,136}. Enhanced expression of MT-I and MT-II has been observed in MS lesions\textsuperscript{137} and in CNS tissue of EAE animals\textsuperscript{138-140}, particularly in infiltrated monocytes and reactive astrocytes. In contrast, MT-III expression is unaltered during EAE\textsuperscript{138}. A number of studies demonstrated that treatment with Zn-MT-II significantly reduces clinical signs and cellular infiltration in EAE\textsuperscript{137,141}. In addition, MT-I+II deficient mice develop more severe EAE than wild-type mice\textsuperscript{142}, suggesting that MT-I and MT-II have a protective role in EAE.

5. Therapeutical potential of antioxidant enzyme upregulation

Endogenous antioxidant enzymes have been implicated in ROS-associated neurodegenerative disorders, such as Alzheimer’s disease\textsuperscript{87,88,130,143,144}, ALS\textsuperscript{145-148}, stroke\textsuperscript{89,90}, and Parkinson’s disease\textsuperscript{128,149,150}. In addition, enhanced expression of various Nrf2/ARE regulated antioxidant enzymes has been found in CNS tissue of EAE animals and MS patients. Recent data revealed that the Nrf2/ARE pathway is involved in the regulation of monocyte chemoattractant protein-1 and vascular adhesion molecule-1, proteins both implicated in the pathogenesis of MS\textsuperscript{151}. Increased expression of endogenous antioxidant enzymes may reflect ongoing oxidative stress and activation of the Nrf2/ARE pathway in MS and EAE lesions and function as a protective mechanism against ROS-mediated cellular toxicity. Hence, targeting the Nrf2/ARE pathway may represent a novel therapeutic approach for the treatment of neuroinflammatory diseases, such as MS. Interestingly, upregulation of HO-1, SOD and catalase via specific enzyme inducers or viral vectors has been shown to ameliorate EAE\textsuperscript{104,105,118}. Various compounds, including tert-butylhydroquinone (tBHQ), dimethylfumarate, sulforaphane and 3-hydroxycoumarin, are well-tolerated, have the ability to cross the BBB and promote transcription of endogenous antioxidant enzymes, making these compounds interesting candidates\textsuperscript{152,153}. Recently, Shih and coworkers demonstrated the beneficial effects of dietary tBHQ in animal models for neurodegeneration and cerebral ischemia\textsuperscript{154,155}. Interestingly, sulforaphane reduces the severity of traumatic brain injury and cerebral ischemia \textit{in vivo}\textsuperscript{156,157}. We speculate that enhanced antioxidant enzyme
activity by specific enzyme inducers may play a protective role in the pathogenesis of MS by operating on distinct levels; 1) antioxidant enzymes can directly scavenge free radical products, thereby restoring BBB integrity and subsequently reducing transendothelial leukocyte migration, 2) increased levels of redox enzymes may inhibit myelin phagocytosis and breakdown, and 3) upregulation of antioxidant enzymes may prevent ROS-induced oligodendrocyte and axonal damage (Figure 3).

In conclusion, ROS play a central role in various pathological processes underlying MS lesion formation and persistence. Enhanced expression of Nrf2/ARE-regulated antioxidants in EAE and MS tissue is suggestive of the occurrence of ongoing oxidative stress. Antioxidant therapy may therefore represent an attractive treatment of MS. Several studies have shown that antioxidant therapy is beneficial in vitro and in vivo in animal models for MS. However, the use of exogenous antioxidants for MS treatment has drawbacks, as large amounts of antioxidants are required to achieve functional antioxidant levels in the CNS. Therefore, the induction of endogenous antioxidant enzymes by activators of the Nrf2/ARE pathway may be an interesting approach to obtain sufficient levels of antioxidants to interfere with pathological processes underlying MS lesion formation, such as leukocyte migration into the CNS, demyelination, and oligodendrocyte and axonal damage. Future studies should provide insight into the value of Nrf2/ARE enzyme inducers for the treatment of neuroinflammatory diseases, such as MS.
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