Glutathione in multiple sclerosis

B. FERREIRA, F. MENDES, N. OSÓRIO, A. CASEIRO, A. GABRIEL and A. VALADO
Department of Clinical Analysis and Public Health, Coimbra Health School, Polytechnic Institute of Coimbra, Portugal

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Introduction

Multiple sclerosis
Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterised mainly as an autoimmune neurodegenerative disorder that affects the white matter with infiltration of macrophages and T cells. It causes demyelination, focal lesions with inflammation in various areas of brain and spinal cord, loss of oligodendrocytes, axonal damage and loss of neuronal function, although these processes are not present uniformly in all patients. It manifests clinically with impaired vision, dizziness, muscle fatigue, pain and sensory deficit. It is estimated that about two million people suffer from MS. It usually begins in those in the 20–40 age group, becoming one of the main causes of disability in young adults, with women at two-fold higher risk.

The geographical distribution of MS reveals predominance in temperate climates, and its prevalence is 4–248 per 100,000, depending on the country. It is estimated that about two million people suffer from MS. It usually begins in those in the 20–40 age group, becoming one of the main causes of disability in young adults, with women at two-fold higher risk.

It is a multifactorial disease of unknown cause; however, some studies point to viruses (eg Epstein–Barr virus), toxins or other infectious agents, sun exposure, oxidative stress (OS), proinflammatory cytokines and/or autoimmune response. It is also defined by a strong genetic and environmental predisposition, already known to have about 60 risk loci that predispose to disease susceptibility. It has been shown that prognosis and degeneration in the long-term MS patient are genetically influenced by an inability to remove the toxic products of OS.

The diagnosis of MS is clinical and there are several forms of the disease. The most common is the relapsing remitting type (RR, 85%), which is considered a primary early-stage inflammatory disease. The most common symptoms include changes in vision, motor dysfunction and disturbances of urinary and bowel function. Symptoms improve after a few days or weeks, which is called remission. Over time, the recovery from symptoms occurs less frequently, and sometimes changes become permanent, with progression to the secondary progressive (SP) type of the disease. This is characterised by a decrease in inflammatory activity and subsequent brain atrophy due to progressive neuronal loss, seen on magnetic resonance imaging. It is estimated that 90% of people with RR disease converted to the SP type after 20 years.

A more aggressive form of MS is the primary progressive (PP) type, which occurs in about 10% of patients. This is characterised by a progressive disability, without relapse or remission. Finally, there is the progressive relapsing (PR) type of MS, which shows continuous progression of disability, with occasional periods of relapse. Thus, MS is characterised not only by inflammatory reactions mediated by the immune system but also by neurodegeneration. It is believed that the inflammatory focus is involved in the RR phase, whereas axonal loss and neurodegeneration are responsible for the progressive symptoms that are the leading cause of disability.
Oxidative stress and reactive species

Currently, the oxidative stress has been related to degenerative diseases such as autoimmune disease, cancer and diabetes, and demonstrated also to have a crucial role in neurodegenerative diseases such as Alzheimer’s, Parkinson’s, Huntington’s, amyotrophic lateral sclerosis, MS and others. In neurodegenerative diseases there is a lack of obvious physical trauma (e.g., obstruction) and oxidative stress is considered to be one of the mechanisms leading to functional and/or structural deficits.

Oxidative stress is a biological condition resulting from an imbalance between the production of free radicals (i.e., reactive oxygen species [ROS] and reactive nitrogen species [RNS]) and their elimination, using biological antioxidant systems to remove or repair the damage caused. The production of free radicals is mainly attributed to normal oxidative metabolism, activated macrophages and monocyte-endothelium interactions responsible for immune mechanisms (e.g., phagocytosis) and cellular respiration, but the over-production of free radicals or a decrease in the antioxidant mechanism (or overload) can lead to changes in lipids, proteins and nucleic acids, causing injury or cell death.

Several studies have shown that they are involved in phagocytosis of myelin, in cytoskeletal rearrangements, loss of blood-brain barrier integrity, alterations in tight junctions and extravasation of leucocytes into the CNS, as well as mitochondrial injury and consequent failure to produce adenosine triphosphate (ATP), which contribute to various processes underlying the pathogenesis of MS. The CNS is particularly vulnerable and liable to damage caused by oxidative stress due to the high oxygen demand in the brain, the low concentration of endogenous antioxidants and high concentrations of polyunsaturated fatty acids. Thus, in inflammatory conditions these defences can be overloaded, leading to oxidative stress and resultant damage to structural and functional constituents of cells.

In an oxidation-reduction balance, the oxidant and antioxidant molecules are in equilibrium. An increase in free radicals results in increased activity of the antioxidant systems, resulting in redox homeostasis and subsequent oxidative stress. The mechanism of production and action of ROS/RNS has been an area of intense research in order to prevent, reduce or even reverse the processes of various diseases such as MS.

Antioxidants and glutathione

Free radicals are constantly produced in the CNS, where there are endogenous and exogenous antioxidant systems comprising antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase (GPx), glutathione reductase (GR), coenzyme Q and uric acid, and non-enzymatic antioxidants such as vitamins and other micro- and macroelements.

Reduced glutathione (GSH-γ-glutamyl-cysteinyl-glycine) is one of the most important agents of the endogenous antioxidant defence system, and protects cells against damage resulting from exposure to agents such as iron, radiation and free radicals, providing protection against oxidative stress. In particular, GSH provides the first line of defence against singlet oxygen and hydroxyl radicals, which are known to cause damage and cell death by apoptosis and necrosis. When exposed to oxidising agents it forms oxidised glutathione (GSSG). Glutathione peroxidase catalyses the reduction of hydroperoxides, including H₂O₂ with GSH, forming GSSG, and also protects cells against oxidative damage.

With the exception of phospholipid-hydroperoxide GPx, glutathione peroxidases are tetramers in which each subunit contains selenocysteine in the active site, and this participates directly in the reduction of two electrons in the substrate peroxide, using GSH as final donor of electrons to regenerate the reduced form of selenocysteine. The recovery of GSH is facilitated by GR enzyme which is essential to keep the cell protection system intact. Glutathione reductase is an NADPH-dependent flavoprotein that plays an important role in the redox processes, in the detoxification of H₂O₂ and organic peroxides.

Under normal physiological conditions, GR maintains more than 98% of intracellular glutathione in a reduced form, helping to maintain the intracellular environment in the reduced state. In situations where the redox system is intact, there is recovery of GSH, but under conditions of excess oxidising agents and/or failure of the protective system, it is assumed that there is an imbalance between GSH consumption and GSSG production (i.e., deficit in GPx) or an imbalance between GSSG consumption and GSH production (i.e., deficit in GR). Some studies have already shown that as GSH is consumed in the protection process, it is expected to decline in tissues affected by oxidative stress, and has been shown that it could be a sensitive marker; however, other studies disagree and point to the instability of these compounds.

Treatment perspectives

Oxidative stress and antioxidants have been the subject of intense research in order to prevent, reduce or even reverse the manifestations of MS. Shi et al. demonstrated that acrolein (2-propanal) plays an important role in oxidative stress in many diseases including neurological disorders. It is an α,β-unsaturated aldehyde found in various endogenous and exogenous sources. It commonly appears as an environmental pollutant released from the cigarette manufacturing process, exhausts from combustion fumes and superheated cooking oil. It is also produced endogenously by oxidation of various compounds. This substance is toxic to neuronal tissue, catalysing the production of ROS, and is found in high concentrations in patients with MS. This study also demonstrated that acrolein is the most reactive lipid peroxidation (LPO) product, consuming GSH 100–150 times faster than 4-hydroxynonenal, another unsaturated aldehyde, thereby stimulating the production of ROS. In addition, acrolein readily forms conjugates with proteins and GSH (GS-propionaldehyde) that remain highly reactive and have a half life much longer than free acrolein. This reaction is essential for their elimination; however, it decreases GSH reserves, limiting the ability of the organism to react against additional oxidative stress. Furthermore, it is thought that the GR is inactivated directly through toxic intermediates, preventing the decrease of glutathione and causing further damage to the system.

Thus, there is strong evidence that this substance plays a critical role in oxidative stress, as demonstrated by its long half life, its ability to generate ROS and its potential...
cytotoxicity, and elimination of acrolein could prevent the cycle that perpetuates oxidative stress. Substances that bind and eliminate acrolein offer a powerful tool to evaluate the usefulness of anti-acrolein treatment in MS. Most research in this area has examined an anti-hypertensive agent containing hydralazine, which binds and neutralises acrolein and acrolein-binding protein, forming a compound that reduces the cellular toxicity of acrolein and permits safe excretion. Owing to the many pathological processes associated with acrolein, research on anti-acrolein therapy may be beneficial in the treatment of MS and also patients with other diseases related to oxidative stress.

Salinthone et al. studied the occurrence of the natural antioxidant lipoic acid (LA), an essential co-factor for the conversion of pyruvate to acetyl-CoA, a critical step in cellular respiration. Together with its reduced form, dihydrolipoic acid (DHLA), LA reduces and recycles cellular antioxidants such as glutathione and acts as an eliminator of ROS and RNS, and modulates signal transduction pathways. These functions suggest that LA can be therapeutically effective in the treatment of diseases associated with oxidative stress, such as MS.

The direct potential neuroprotective effects of dimethyl fumarate (DMF) was investigated by Scannevin et al. in work on cellular resistance to oxidative damage in primary cultures of CNS cells, which showed that treatment with DMF or MMF increased the redox potential cellular levels of glutathione, ATP and mitochondrial membrane potential. These data suggest that DMF and MMF provide protection of neurons and astrocytes from cellular injury induced by oxidative stress. The clinical usefulness of DMF as oral therapy (BG-12) in MS is being explored through phase III trials.

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### Table 1. Summary of the main information in the literature.

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<td>Fasting</td>
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<td>Number of patients (M/F)</td>
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<td>17 (3/14)</td>
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<td>12 (5/7)</td>
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<tr>
<td>Subtype of MS and EDSS</td>
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<td>SP – 6</td>
<td>RR and SP at various stages – Information not available</td>
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<td>RR – Not referred</td>
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<td>History with recurrent gradual worsening of their function for at least the previous year</td>
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<td>No – Without relapse for at least two months</td>
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<td>19.7</td>
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<td>Not referred</td>
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<td>Mean age (years)</td>
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<td>Parameter analysed</td>
<td>Total glutathione, GSH, GSSG, GPx, GR</td>
<td>GSH and GR</td>
<td>GSH and GSSG</td>
<td>GSH</td>
<td>GPx</td>
<td>GR</td>
<td></td>
</tr>
</tbody>
</table>

RR: Relapsing remitting form; SP: Secondary progressive form; GSH: Glutathione; GSSG: Oxidised glutathione; GPx: Glutathione peroxidase; GR: Glutathione reductase; HPLC: High-performance liquid chromatography; EDSS: Expanded disability severity scale.

### Literature search

Information obtain from the literature is compiled in Table 1 and takes account of the different characteristics of the studies (e.g., number of patients, age, disease duration) as well as references to the parameters analysed and the type of sample examined.

Tasset et al. measured total glutathione (GSH+GSSG) and GSH levels using the Bioxytech GSH and GSH 420-400 kits, respectively (Oxis International, Portland, OR, USA). Determination of total glutathione was based on the formation of a chromophoricthione and absorbance at 420 nm, and the concentration of GSH was based on a reaction
which leads to the formation of a chromophore and absorbance at 400 nm. Levels of GSSG were calculated by subtracting the concentration of GSH from the total glutathione. The GSH:GSSG ratio was also calculated. Glutathione peroxidase and GR activities were evaluated by the method of Flohé and Günzler, which is based on the oxidation of NADPH to NADP⁺ with maximum absorbance at 340 nm. The authors concluded that there was no correlation between patient age and the levels of markers of oxidative stress. The redox balance GSH in erythrocytes in RR-MS patients was characterised by higher values of total glutathione, GSH and GSH:GSSG ratio, and a decrease in the level of GSSG. Also observed were differences in the levels of erythrocyte antioxidants, with a significant increase in GR activity and a decrease in GPx activity. The authors found no correlation between the extent of oxidative damage or antioxidant system and disease severity, and also suggested that oxidative changes are not accompanied by inflammatory activity.⁷

Choi et al. detected GSH in the human brain using various filtering techniques including magnetic resonance spectroscopy. Levels of GSH were determined using an internal reference method which uses a creatine signal obtained simultaneously with the GSH signal. The authors concluded that GSH levels were lower in MS patients than in controls at 12.5% in the frontoparietal region, 18.5% in the frontal region and 7.7% in the parietal region. In the control group, GSH concentration was lower in the parietal region than in the frontal region. There was no significant difference in the concentration of GSH in MS patients with region. Levels of GSH did not differ according to gender; and there was no correlation between age and GSH concentration in the frontoparietal region in MS patients. The concentration of GSH in the frontoparietal region tended to be lower in patients with higher Expanded Disability Severity Scale (EDSS) score, although the correlation was not significant. Disease duration was indirectly proportional to the concentration of GSH in the frontoparietal region, but the correlation was not significant. Age at diagnosis directly correlated with GSH levels in the frontoparietal region and most of the frontal area.⁸

Krotenko et al. analysed peripheral blood and showed glutathione levels were lower in RR disease compared with controls, and also found very low erythrocyte GR activity. However, compensatory activation of catalase was observed only in patients with RR disease.⁹

Di Giuseppe et al. measured plasma glutathione by high-performance liquid chromatography (HPLC) and concluded that there were no significant differences in fasting concentrations of GSH and GSSG between groups.

Srinivasan et al., using brain tissue, determined GSH level by magnetic resonance spectroscopy. The authors concluded that GSH concentration in the grey matter was significantly higher in relation to the white matter in controls, and MS patients had reduced GSH levels compared with controls. They observed a decrease in GSH concentration in lesions in the white matter in MS patients, and showed a statistically significant decrease in GSH concentration in the grey matter of MS patients compared to controls, but no significant difference in the white matter between the two groups.

Glutathione peroxidase activity was determined according to a method based on the oxidation of GSH in the presence of cumene, GR and NADPH by Ortiz et al. and they concluded that the activity of GPx is 1.3 times higher in MS patients than in controls. Paschina et al., who provided no information about their method, showed that GR was increased in patients compared to controls.¹⁰

Tasset et al. demonstrated that GSH and GR concentration was higher in the blood of patients than in controls, although this contradicts the findings of Krotenko et al.¹¹ Also in disagreement are the results obtained by Choi et al. and Srinivasan et al., who showed a decrease in GSH, albeit using different methodologies. In the work of Di Giuseppe et al., on patients’ plasma, no statistically significant differences in the levels of GSH in RR MS patients and controls were found.¹²

Tajouri et al. obtained results that showed that GPx gene expression is increased,¹³ and this finding is supported in a review by Mirshafiey et al., who postulated that selenium-independent GPx is increased in MS lesions and observed that GSH is increased in blood samples of patients with progressive forms of the disease, but a reduction of GPx and an increase of GR is seen in the patients’ cerebrospinal fluid.¹⁴ Work undertaken by Calabrese et al.¹⁵ agreed with these findings.

Clearly, there is no consensus in the scientific community on the role of glutathione in MS. There is no correlation between age, gender, disease severity and oxidative stress markers,¹⁶ although the concentration of GSH in the frontoparietal region in MS shows a tendency to decrease in patients with greater disability, as evaluated by EDSS. However, the correlation is not significant and the decrease in GSH is indirectly proportional to the period of disease progression.¹⁷

Accordingly, it is expected that the antioxidant activity system in these patients is diminished, independently of the form of MS (i.e., RR or progressive) and the stage of disease (i.e., remission or relapse);¹⁸ however, the increase of GSH and enzymes involved in protection against oxidative stress appears to be a compensatory mechanism that protects cells from subsequent oxidative damage.¹⁹

One of the causes of increased GSH was demonstrated by stimulation of extracellular glutamate, indicating that GSH can have a neuroprotective role to minimise glutamate toxicity and an effect on neurodegeneration. However, GPx and GR activities in MS remain unclear, as some studies show decreased activity in blood cells, while others indicate normal or increased activity in lymphocytes, monocytes and demyelinating lesions of MS patients.²⁰ An increase in GR activity was observed by Paschina et al., which may explain in part the increase in concentration of GSH.²¹ Also, decreased GSH level in the brain, primarily in the white matter, should be considered, as it can serve to identify patients who have a higher risk of disease progression, and should be investigated in terms of how this decrease affects functional and cognitive activity.²²

The apparent contradictions may be due to the nature of the samples examined (e.g., brain tissue, cerebrospinal fluid, plasma, erythrocytes), differences in the study methodology, and each patient’s intrinsic characteristics (e.g., other endogenous antioxidants, exogenous antioxidants in food, physical exercise). However, the increase in antioxidants supports the hypothesis that the response to oxidative damage in an attempt to protect cells is likely to occur before the inflammatory response, and when this response is insufficient then a relapse in MS occurs.²³
Conclusions

The aetiology of MS remains unclear, although it has been shown that the genome, the environment, the production of ROS/RNS, as well as other compounds and immune cells, contribute to the development and progression of this disease. However, the production of ROS/RNS is also an innate property of the immune system to certain processes (e.g., phagocytosis) and to fight inflammation.

These uncertainties lead the authors to conclude that oxidative stress plays an important role in MS. Clearly, however, further studies should be performed on larger numbers of patients with different forms of the disease, and with many types of biological samples, in order to find a consensus and clarify the pathogenesis of this multifactorial disease that is responsible for severe disability.

Identifying biomarkers of prognosis and oxidative stress is crucial in the evaluation of MS. It is also important to promote the development of new treatments because antioxidant therapy does not appear to be effective, with more studies required on substances such as acrolein, lipoic acid and DMF.

Clarification of the mechanisms involved in oxidative stress, in different forms of multiple sclerosis, could result in improvements in the monitoring and prognosis of the disease, with subsequent increases in a patient’s quality of life.

References


6 Choi IV, Lee SE, Denney DR, Lynch SG. Lower levels of glutathione in the brains of secondary progressive multiple sclerosis patients measured by 1H magnetic resonance chemical shift imaging at 3 T. Mult Scler 2011; 17 (3): 289–96.


