Neuroscience and Behavioral Physiology, Vol. 37, No. 3, 2007

Oxidant Stress in the Pathogenesis of Multiple Sclerosis

M. A. Lutskii and I. É. Esaulenko

Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Multiple Sclerosis, Supplement, No. 3, pp. 26–30, 2006.

We report here an analysis of measures of the intensification of lipid peroxidation and the state of the non-enzymatic and enzymatic components of the antioxidant defense system in different clinical forms and stages of multiple sclerosis. The data obtained support the role of oxidant stress in the development of the pathological process in multiple sclerosis.

KEY WORDS: multiple sclerosis, oxidant stress.

Lipid peroxidation (LPO) is one of the most important regulators of the metabolism of lipids, proteins, and carbohydrates and is the process underlying plasticity and the energy support of the functions of cells and the body as a whole. LPO processes are the limiting step in controlling the morphofunctional state of biological membranes and intracellular homeostasis [3].

All cells in the body constantly experience conditions in which LPO can occur, due to the presence of substrates – polyene lipids – as well as initiators and catalysts – active oxygen species and transition metal ions. At the same time, the content of LPO products in the body is low in normal conditions. This results from the existence of a constantly operating complex of mechanisms of the antioxidant defense system (ADS). The strict control of LPO reactions results from the concordant functioning of the non-enzymatic and enzymatic components of this system, regulating the levels of active oxygen species, free radicals, and molecular LPO products in the body. This is a typical integral process with marked branching occurring by the free-radical mechanism in a number of steps. During the stepwise degradation of polyunsaturated lipids, LPO reactions form a series of primary, intermediate, and end molecular products, which play an important role in the processes of structural modification of biological membranes, with changes in their physicochemical properties. An excess of LPO products in the body leads to derangement of the processes of oxidative phosphorylation, microsomal oxidation, and determination of the process of protein molecule translation in cells. Disturbances to the structural and functional state of cell membranes as a result of the actions of excessive concentrations of active oxygen species and LPO products, as a manifestation of oxidant stress, provide the basis for the development of pathology. Oxidant stress is regarded as one of the mechanisms of the pathogenesis of a whole series of nervous system diseases, including multiple sclerosis (MS) [4, 5, 7, 8].

An important role in the pathogenesis of nervous system diseases accompanied by demyelination is played by processes of free-radical oxidation of lipids. This is primarily because myelin is a lipoprotein membrane of which more than 80% consists of phospholipids, glycolipids, and steroids, with the result that its structure is limited by LPO processes, and secondly because of the characteristics of the nervous system, namely, functional insufficiency of the enzymatic component of the ADS and high levels of unsaturated fatty acids and iron – an LPO activator – in conditions of significant intensification of oxidative metabolism, which makes the nervous system a target for oxidant stress [2, 9].

The immunopathological process in MS is accompanied by activation of phagocytosis. The ability of phagocytic cells to form active oxygen species is due to the fact that they have surface enzymes generating hydrogen peroxide, particularly NADP-I_2 oxidase, which is activated on contact of the cell membrane with immune complexes, complement components, and lymphokines, which is important in the development of the pathological process in MS, while active oxygen species are in turn initiators and activators of

N. N. Burdenko State Medical Academy, Voronezh.

0097-0549/07/3703-0209

LPO processes. Thus, the pathogenesis of MS actively involves two interrelated mechanisms operating in tandem, namely immunopathological factors and oxidant stress – the so-called immunometabolic mechanism [4, 6, 10].

With the aim of assessing the intensification of LPO and imbalance in the ADS system, we studied measures of primary, intermediate, and end products of LPO and the state of the non-enzymatic and enzymatic components of the ADS system in patients with different clinical forms and courses of MS.

The study group consisted of 120 patients with MS: 30 with primary progressive MS (PPMS), 30 with secondary progressive MS (SPMS), and 60 with recurrent MS (RMS), 30 in the remission and 30 in exacerbation.

The significance of differences in measures between the various groups was assessed by calculating group means and 95% confidence intervals for overall means.

Calculations were made using:

$$\tilde{x} - \Delta_{\bar{x}} \le \bar{x} \le \tilde{x} + \Delta_{\bar{x}};$$

$$\Delta_{\bar{x}} = t\mu_{\bar{x}};$$

$$\mu_{\bar{x}} = s/\sqrt{n},$$

where $\bar{x}$ is the overall mean, $\tilde{x}$ is the group mean, $\Delta_{\bar{x}}$ is the limiting error of the group mean, $\mu_{\bar{x}}$ is the mean square deviation of the standard error, t is the coefficient of significance (Student's t criterion for a significant probability of $p = 0.95$, $t = 1.96$), s is mean square deviation in the group, and n is the group size.

Group means were compared for all measures. The criterion for verification of the null hypothesis, that group mean values were equal (unequal) was based on the statistic:

$$t = \frac{\tilde{x}_1 - \tilde{x}_2}{s\sqrt{\frac{1}{n} + \frac{1}{m}}},$$

which has a Student distribution with $n + m - 2$ degrees of freedom.

The calculation results (95% significance intervals for group means, differences between groups, and p values) are shown in Tables 1–6.

The data provide evidence for increases in the concentrations of primary (diene conjugates), intermediate (malondialdehyde), and end (fluorescent Schiff bases) products of LPO in patients with different clinical forms of MS, as compared with the control group (see Tables 1 and 4).

The increase in LPO products characterizes intensification of these processes, which is accompanied by changes in the ADS system, the limiting component of free-radical processes. Increases in plasma antioxidant activity were accompanied by significant increases in vitamin E concentrations. This compensatory reaction of the non-enzymatic component of the ADS was accompanied by decreases in the levels of total and non-protein thiols (see Tables 2 and 5).

Intensification of free-radical processes in patients with MS was also accompanied by changes in the enzymatic component of the ADS. Superoxide dismutase activity increased because of excess concentrations of its specific substrates – active oxygen species and hydrogen peroxide – which was also supported by increases in the activity of LPO processes in different clinical forms of MS. This is reflected in the dynamics of glutathione-dependent enzymes. Thus, glutathione peroxidase activity increased, while glutathione reductase activity decreased. The decrease in glutathione reductase activity leads to decreases in blood reduced glutathione levels. This results in an increase in the oxidative degradation of glutathione, as maintenance of a sufficient level of reduced glutathione, oxidized via operation of the glutathione-dependent antiperoxide system, is mediated by the specific enzyme glutathione reductase [4]. The decrease in the level of blood reduced glutathione is indirect evidence for decreases in total and non-protein thiols, which indicates a marked imbalance in the glutathione reductase component – reduced glutathione. On this background, ceruloplasmin activation was noted, this being one of the major antioxidants in the plasma (see Tables 3 and 6).

Analysis of the clinical forms of MS demonstrated increases in the intensity of LPO processes in PPMS, particularly as increases in the concentrations of ketodiones, malondialdehyde, and Schiff bases. In SPMS, there was a tendency to activation of these processes, which was supported by high values for measures of the molecular products of LPO (see Table 1).

As regards the dynamics of the non-enzymatic component of ADS in patients with PPMS and SPMS, there were increases in the vitamin E level and plasma antioxidant activity in the presence of relatively unaltered measures of erythrocyte peroxide resistance. Analysis of measures of ADS activity in SPMS gave rather different results – there were insignificant increases in these measures. Thus, on the background of increased intensity of processes identified from the concentrations of primary, intermediate, and end products of LPO, lower values for measures of compensatory activation of the non-enzymatic component of the ADS system were recorded in SPMS. This supports the occurrence of some "fatigue" and imbalance in the non-enzymatic component of the ADS system in SPMS, which is in accord with the significance of differences in risk factors for these groups of patients (see Table 2).

Analysis of measures of the enzymatic component of the ADS system in PPMS and SPMS was variable. In PPMS, there were increases in the levels of ceruloplasmin, superoxide dismutase, catalase, and glutathione peroxidase, with significant decreases in glutathione reductase activity, while in SPMS there was a decrease in catalase activity, possibly associated with depletion of blood catalase activity. While ceruloplasmin, superoxide dismutase and glu-

TABLE 1. Primary, Intermediate, and End Products of LPO in PPMS and SPMS

	Group			Differences					
Measure	x_1	x_2	x_3	x_1-x_2	p	x_1-x_3	p	x_2-x_3	p
Ketodienes, OD units/ml	0.16±0.02	0.17±0.04	0.19±0.04	0.01±0.04	<0.26	0.03±0.04	<0.11	−0.01±0.05	<0.29
Diene conjugates, OD units/ml	0.42±0.04	0.44±0.06	0.53±0.07	0.02±0.06	<0.32	0.11±0.07	<0.01	0.25±0.07	<0.01
Blood malondialdehyde, mM	1.62±0.06	1.77±0.21	1.82±0.36	0.15±0.18	<0.04	0.20±0.29	<0.09	−0.05±0.38	<0.78
Schiff bases, OD units/ml[3]	30.01±2.87	41.13±12.5	42.62±8.72	11.03±10.4	<0.02	12.53±7.6	<0.01	−1.5±13.8	<0.82

Notes. Here and in Tables 2–6: significant intervals are at the 95% significance level.
Here and in Tables 2 and 3: x_1 corresponds to control (donors), x_2 to PPMS, and x_3 to SPMS.

TABLE 2. Non-Enzymatic Component of the ADS System in PPMS and SPMS

	Group			Differences					
Measure	x_1	x_2	x_3	x_1-x_2	p	x_1-x_3	p	x_2-x_3	p
Vitamin E, mM	28.8±3.7	40.1±7.7	35.6±8.3	11.3±7.3	<0.01	6.8±7.7	<0.05	4.5±10.3	<0.19
Plasma antioxidant activity, quanta/sec·ml·4π (bioluminescence)	9.8±2.0	11.3±1.2	10.4±2.6	1.5±2.3	<0.10	0.5±2.9	<0.72	11.3±1.2	<0.22
RBC peroxide resistance, $tg_1/tg_2 \cdot 10^3$	372.4±35.0	366.9±61.3	364.6±53.3	−5.5±61.1	<0.85	−7.8±56.2	<0.74	2.3±73.7	<0.95
Total thiols, mM	21.5±2.3	17.6±1.0	13.3±2.6	−3.9±2.0	<0.1	−8.2±3.3	<0.01	4.3±1.01	<0.01
Non-protein thiols, mM	2.09±0.32	1.63±0.12	1.53±0.68	−0.46±0.24	<0.1	−0.56±0.42	<0.05	0.10±0.29	<0.395

TABLE 3. The Enzymatic Component of the ADS System in PPMS and SPMS

	Group			Differences					
Measure	x_1	x_2	x_3	x_1-x_2	p	x_1-x_3	p	x_2-x_3	p
Ceruloplasmin, mmol benzoquinone/liter·min	277.2±30.3	335.1±37.6	308.1±37.3	57.9±43.8	<0.01	30.9±43.6	<0.08	27.0±43.4	<0.11
Superoxide dismutase, U/mg hemoglobin	1.42±0.24	1.56±0.37	1.46±0.25	0.14±0.39	<0.23	0.04±0.32	<0.78	0.1±0.33	<0.46
Catalase, mmol hydrogen peroxide/liter·min × 10^3	23.5±2.0	25.3±4.9	22.0±4.7	0.8±4.5	<0.21	−1.5±2.3	<0.24	3.3±3.4	<0.09
Glutathione peroxidase, mmol/G-SH/liter·min·10^3	13.1±1.9	16.4±4.2	18.3±5.1	3.3±3.9	<0.05	5.2±4.5	<0.02	1.9±4.6	<0.11
Glutathione reductase, mmol/G-SS-G/liter·min	337.5±35.1	275.6±22.5	246.3±27.1	−79.9±41.3	<0.01	−91.2±43.0	<0.01	29.3±42.2	<0.04

tathione peroxidase levels were relatively similar, glutathione peroxidase levels showed more significant decreases. Thus, in SPMS, depletion of blood catalase activity was observed on the background of more intense LPO processes, along with a more marked imbalance in the glutathione reductase/reduced glutathione compartment (see Table 3).

We will now consider the intensity of LPO processes and the state of the ADS system in patients of RMS, divided into two essentially equal randomized groups – those in remission and those in exacerbation. Increases in the intensity of these processes were seen in both groups, though exacerbation was associated with a tendency to more marked increases in the intermediate LPO product malondialdehyde (see Table 4).

Compensatory increases in the vitamin E concentration were seen in RMS in remission and exacerbation, while measures of plasma antioxidant activity and RBC peroxide resistance showed a tendency to increases only in exacerbation. Decreases in the levels of total and non-protein thiols were seen both in remission and in exacerbation (see Table 5).

In exacerbation of RMS, changes in the enzymatic component of the ADS system were more marked than in remission, with greater decreases in glutathione reductase activity. This shows that this stage of the process is associ-

TABLE 4. Primary, Intermediate, and End Products of LPO in RMS in Remission and Exacerbation

	Group			Differences					
Measure	x_1	x_2	x_3	x_1-x_2	p	x_1-x_3	p	x_2-x_3	p
Ketodienes, OD units/ml	0.16±0.24	0.16±0.05	0.17±0.01	0.004±0.048	<0.84	0.01±0.03	<0.18	0.01±0.03	<0.18
Diene conjugates, OD units/ml	0.42±0.04	0.42±0.08	0.49±0.03	0.0±0.08	<0.97	0.07±0.05	<0.01	0.07±0.05	<0.01
Blood malondialdehyde, mM	1.62±0.06	1.72±0.36	2.08±0.16	0.10±0.32	<0.27	0.46±0.26	<0.01	0.36±0.18	<0.05
Schiff bases, OD units/ml^3	30.1±2.8	34.2±7.7	51.4±5.3	4.12±7.29	<0.13	21.3±8.6	<0.01	19.2±6.5	<0.05

Note. Here and in Tables 5 and 6: x_1 indicates donors, x_2 indicates remission, and x_3 indicates exacerbation.

TABLE 5. Comparison of Measures of the Non-Enzymatic Component of the ADS System in RMS in Remission and Exacerbation

	Group			Differences					
Measure	x_1	x_2	x_3	x_1-x_2	p	x_1-x_3	p	x_2-x_3	p
Vitamin E, mM	28.8±3.8	35.5±4.8	36.3±3.3	6.6±5.6	<0.02	7.5±5.7	<0.01	0.8±4.1	<0.25
Plasma antioxidant activity, quanta/sec·ml·4π (bioluminescence)	9.8±2.0	9.7±2.6	12.5±0.6	−0.2±3.0	<0.09	2.7±1.4	<0.01	2.8±1.8	<0.01
RBC peroxide resistance, $tg_1/tg_2 \times 10^3$	372.4±35.0	329.4±49.2	405.9±15.6	−42.9±54.8	<0.06	33.5±31.4	<0.02	76.5±42.2	<0.01
Total thiols, mM	21.5±2.3	14.7±3.1	18.8±1.1	−6.8±3.9	<0.01	−2.7±2.2	<0.1	4.1±1.07	<0.01
Non-protein thiols, mM	2.09±0.32	1.72±0.78	1.79±0.13	−0.37±0.52	<0.08	−0.30±0.28	<0.2	0.09±0.33	<0.45

TABLE 6. The Enzymatic Component of the ADS System in RMS in Remission and Exacerbation

	Group			Differences					
Measure	x_1	x_2	x_3	x_1-x_2	p	x_1-x_3	p	x_2-x_3	p
Ceruloplasmin, mmol benzoquinone/liter·min	277.2±30.3	311.7±41.4	283.0±17.9	34.5±46.7	<0.07	5.8±33.1	<0.72	−28.7±29.2	<0.10
Superoxide dismutase, U/mg hemoglobin	1.2±0.4	1.5±0.2	1.52±0.1	0.8±0.3	<0.14	0.1±0.2	<0.27	0.02±0.2	<0.06
Catalase, mmol hydrogen peroxide/liter·min $\times 10^3$	23.5±2.0	18.6±3.2	29.1±1.0	−4.9±3.4	<0.01	5.6±1.9	<0.01	10.5±2.1	<0.01
Glutathione peroxidase, mmol/G-SH/liter·min $\times 10^3$	13.1±1.9	17.0±2.9	10.9±2.3	3.9±3.2	<0.02	−2.1±3.8	<0.14	−6.1±4.6	<0.01
Glutathione reductase, mmol/G-SS-G/liter·min	337.5±35.3	241.2±49.9	322.9±22.1	−96.2±54.5	<0.01	−14.5±50.5	<0.29	81.7±45.5	<0.01

ated with a more marked imbalance in the glutathione reductase/reduced glutathione system (see Table 6).

Thus, the results obtained here show a significant increase in the intensity of LPO processes in patients with MS regardless of form and stage. There was some compensatory activity of the non-enzymatic component and an imbalance in the enzymatic component of the ADS system, which was most marked in the glutathione reductase/reduced glutathione system. All these points provide support for a role for oxidant stress in the development of MS.

Intensification of LPO processes, the compensatory activity of the non-enzymatic component, and the imbalance of the enzymatic component of the ADS system in all forms and stages of MS demonstrate the continuous nature of the pathological process.

In SPMS, on the background of more marked intensification of LPO processes, compensatory activity of the non-enzymatic component of the ADS system was less marked than in PPMS, which was interpreted as "fatigue" and imbalance of the non-enzymatic component of the ADS system. In the enzymatic component of the ADS system, there was "exhaustion" of blood catalase activity in SPMS, along with a more marked imbalance in the glutathione reductase/reduced glutathione system. All these points indicate that oxidative stress develops with particular characteristics in each of the clinical forms of MS.

REFERENCES

1. S. Glants, *Medical Biological Statistics* [Russian translation from English], N. E. Buzinkashvili and D. V. Samoilov (eds.), Praktika, Moscow (1999).
2. I. A. Zavalishin and M. N. Zakharova, "Multiple sclerosis: current aspects of etiology and pathogenesis," *Zh. Nevrol. Psikhiat.*, **2**, Special Issue, 10–17 (2003).
3. M. N. Zakharova, A. V. Peresedova, O. S. Brusova, et al., "Free-radical mechanisms in the pathogenesis of multiple sclerosis," in: *Proceedings of the Conference on Current Questions in Neurology, Neurosurgery, and Medical Genetics* [in Russian], Ufa (1998), pp. 91–92.
4. M. A. Lutskii, *Systems Analysis and Multiphasic Modeling of the Components of Laboratory Status in Multiple Sclerosis* [in Russian], Novyi Vzglyad, Voronezh (2002).
5. *Multiple Sclerosis: Selected Questions in Theory and Practice* [in Russian], I. A. Zavalishin and V. I. Golovkin (eds.), Moscow (2000).
6. L. Diemel, "Macrophages in CNS remyelination: on friends or foe?" *Neurochem. Res.*, **23**, 341–347 (1998).
7. S. Li, "Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na^+-dependent glutamate transport," *J. Neurosci.*, **19**, 16 (1999).
8. J. Noseworthy, "Progress in determining the causes and treatment of multiple sclerosis," *Nature*, **399**, 40–48 (1999).
9. M. Renganathan, T. Cummins, W. Hormuzdiar, "Nitric oxide is an autocrine regulator of Na^+ currents in axotomized C-type DRG neurons," *J. Neurophysiol.*, **83**, 2431–2443 (2000).
10. K. Smith, "Demyelination: the role of reactive oxygen and nitrogen species," *Brain Pathol.*, **9**, 9–92 (1999).