

Suppression of the lipid peroxidation process in the CNS reduces neurological expression of experimentally induced autoimmune encephalomyelitis

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Abstract

Objective: Here we report the influence of malondialdehyde (MDA) as a measure of the lipid peroxidation process (LP), on multiple sclerosis (MS) pathogenesis and its neurological signs, during the treatment with aminoguanidine (AG) – a selective inducible nitric oxide synthase inhibitor and N-Acetyl cysteine (NAC) – an oxidative scavenger, in the experimental autoimmune encephalomyelitis (EAE), an animal model for studying MS.

Material and methods: Encephalomyelitis induction by the subcutaneous injection of myelin basic protein of bovine type, dissolved in phosphate buffered saline (PBS) emulsified in equal volume of the complete Freund's adjuvant (CFA), was described in detail in our earlier published papers. Each of animals was randomly assigned to seven groups – control (PBS), EAE, CFA, EAE + AG, AG, EAE + NAC and NAC group. In each animal, the development of neurological signs of EAE was scored, these results were published earlier. MDA was evaluated in the central nervous system (CNS) structure – cerebellums and spinal cords.

Results: The obtained results show that the AG and NAC treatment significantly reduces the MDA level in both examined tissues ($p < 0.05$) ameliorating at the same time EAE clinical signs ($p < 0.05$).

Conclusions: Taking together our present and earlier findings we conclude that LP may provoke and promote MS, while blocking of this process results in amelioration of the clinical onset and disease activity. These results may be useful as a new insight into mechanisms and potential targets for therapeutic strategies in MS.

Key words: malondialdehyde, lipid peroxidation, EAE, aminoguanidine, N-acetyl cysteine.

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating autoimmune central nervous system (CNS) dis-

ease with pathologically miscellaneous ground, which include demyelinating and neurodegenerative resources [10,23]. At a molecular level, the pathogenesis of MS is characterised by a series of morphological and bio-

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chemical changes which result in the loss of CNS structure and functions. Due to various pathological circumstances (reversible and irreversible), which predominate in different phases of the disease (relapsing remitting and progressive form of MS), diversity of neurological signs and symptoms leads to the MS clinical expression [29]. One of common features in the early phase of various MS clinical expressions is the nerve tissue imbalance in pro-oxidants and antioxidants processes, resulting in a high production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These include nitric oxide (NO), peroxy nitrite, superoxide ions and hydrogen peroxide. Central nervous system are constantly exposed to low levels of ROS and RNS, as a part of normal cellular physiology, which are taken under control due to coherence repair and protection by antioxidative enzyme and non-enzyme mechanisms [1, 9]. In the inflammatory condition in CNS, which prevails in the early phase of MS, these species can cause damage to nerve cells components – lipids, proteins, and nucleic acids and may lead to cell death [6]. The induction of lipid peroxidation (LP) may be a major factor in oxidative and nitrosative mediated CNS damages. It is a complex process involving the interaction of oxygen-derived free radicals with polyunsaturated fatty acids, resulting in a variety of highly reactive electrophilic aldehydes and severe nerve damages [28], potentiated by CNS particular vulnerability to oxidative damage. It is a consequence of active oxidative brain metabolism, high production of intracellular superoxides and, on the other hand, the limited ability of anaerobic respiration, resulting in superoxides accumulation. Central nervous system are consists of the cells (oligodendrocytes) whose cellular features are to be predisposed to oxidative damage due to low levels of intracellular antioxidants, high iron content and composition of myelin [3,5]. Malondialdehyde (MDA) is a small end product of the degradation of oxidized fatty acids, which can be used as a measure of the LP process. Although MDA and 4-hydroxy-2-nonenal are the major LP products, different fatty acids form specific products – other aldehydes, including oxidized phosphatidylcholine (OxPC), acrolein and isoprostanes during the LP process [7,8]. Free radical catalyzed peroxidation of the arachidonic acid, esterified in membrane phospholipids, transform it into prostaglandin-like, but cyclooxygenase-independent, isoprostanes. On the other hand, docosahexanoic acid (DHA) forms neuroprostanes, which are found also in the normal

brain tissue, regulating its other (non-neuronal) biological effects [20].

To reinforce our previous finding, here we report the influence of MDA levels as a measure of the LP process, in the different parts of CNS, on MS pathogenesis and its neurological signs, during the treatment with aminoguanidine (AG) – a selective inducible nitric oxide synthase (iNOS) inhibitor, and *N*-acetyl cysteine (NAC) – an oxidative scavenger, in the experimental autoimmune encephalomyelitis (EAE), the most frequently used animal model for studying MS.

Material and methods

Animals

The experimental protocol was reviewed and approved by the Faculty Ethical Committee. All animals included in this experiment received human care in the strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication 80-23, revised 1985). Female Sprague Dawley rats, 3 months old, weighing 300 ± 20 g, were housed in the Biomedical Research Centre animal care facility of the Medical Faculty of Nis throughout the experiment under a 12 : 12 h light-dark cycle. The rats were kept in plastic cages and fed a standard diet and water ad libitum.

Induction of encephalomyelitis

Experimental autoimmune encephalomyelitis was induced by the subcutaneous injection of myelin basic protein of bovine type (50 μ g), dissolved in phosphate buffered saline (PBS) emulsified in equal volume of the complete Freund's adjuvant, on days 0 and 7 in the hind foot pad of the animals under anaesthesia. Two intraperitoneal injections of 200 ng of Pertussis toxin were given on days 0 and 1. Each animal ($N = 49$) was randomly assigned to seven groups: control (PBS 0.3 ml/i.p./daily), EAE (PBS 0.3 ml/i.p./daily after EAE induction), CFA (0.3 ml/i.p./daily), EAE and AG (AG 100 mg/kg body weight/daily after EAE induction), AG (100 mg/kg body weight/daily), EAE and *N*-acetyl-*L*-cysteine (150 mg/kg body weight/daily after EAE induction) and NAC (150 mg/kg body weight/daily) [15].

Encephalomyelitis clinical expression was assessed daily using the following ranks 1 = healthy; 2 = loss of tail tone; 3 = hindlimb weakness; 4 = hindlimb paralysis; 5 = hindlimb paralysis plus forelimb weakness; 6 = moribund or dead (Ljubisavljevic *et al.* 2011).

The animals were sacrificed 15 days after EAE induction (under Ketalar anaesthesia) and CNS structures were dissected, washed in PBS, placed on ice and 10% homogenates of the cerebellums and spinal cords were stored at -20°C for later biochemical analysis.

Determination of malondialdehyde

The LP level was measured spectrophotometrically by the estimation of MDA concentration (nmol/mg of tissue weight) based on the reaction with the thio-barbituric acid [2].

Protein content

Protein content was measured according to the Lowry procedure using bovine serum albumin as a standard [17].

Chemicals

Chemicals were purchased from Sigma (St. Louis, MO, USA). All used chemicals were of the analytical grade. All drug solutions were prepared on the day of the experiment.

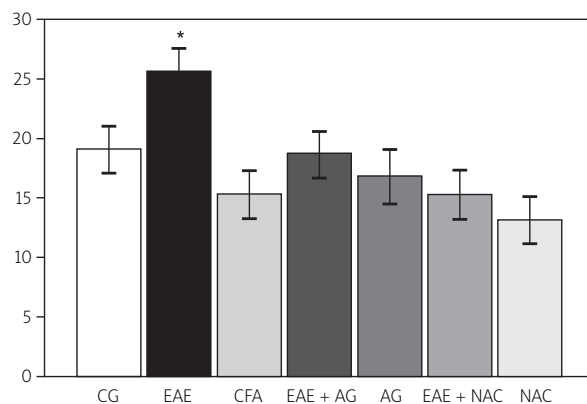


Fig. 1. Malondialdehyde concentration (nmol/mg prot.) in the rat cerebellums.

CFA – complete Freund’s adjuvant, AG – amino-guanidine, NAC – N-acetyl-L-cysteine, CG – control group, EAE – rats with experimental autoimmune encephalomyelitis, CFA – rats treated with CFA, EAE + AG – EAE rats treated with AG, AG – rats treated with AG, EAE + NAC – EAE rats treated with NAC, NAC – rats treated with NAC. Bars in the graph represent mean \pm SEM from animals for each group. AG- and NAC-treatment decrease the MDA level in the cerebellums * $p < 0.05$ vs. EAE + AG and EAE + NAC, ANOVA (Bonferroni post test)

Statistical analysis

All the data presented were mean \pm SD. Normal distribution was verified using Kolmogorov-Smirnov test. The data were analysed statistically by One Way ANOVA followed by Bonferoni post test. The correlation and clinical score differences were done by the Pearson and χ^2 test, using the statistical program SPSS version 13. Statistical significance was defined as $p < 0.05$.

Results

Previously published results [15] showed that the neurological signs of EAE-untreated animals were significantly increased compared to the control and CFA groups (between 4 and 5). Also, AG and NAC treatment ameliorate EAE neurological expression (between 2 and 3), compared to EAE-untreated animals ($p < 0.05$). It was apparent during the entire acute phase of the disease.

The obtained results showed that the level of MDA was significantly increased in EAE rats compared to the control and CFA group in examined tissues – WEM, cerebellum, spinal cord ($p < 0.05$; [15], Figs. 1 and 2, respec-

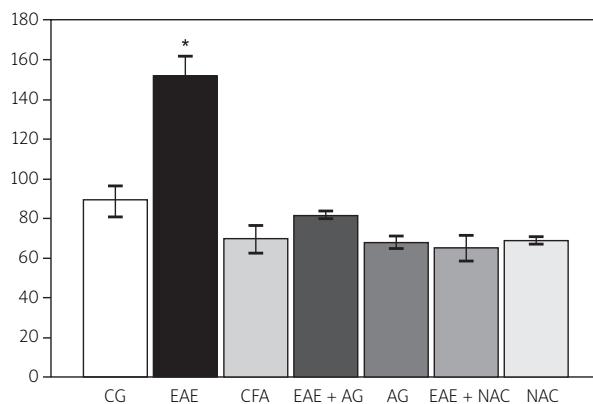


Fig. 2. Malondialdehyde concentration (nmol/mg prot.) in the rat spinal cords.

CFA – complete Freund’s adjuvant, AG – amino-guanidine, NAC – N-acetyl-L-cysteine, CG – control group, EAE – rats with experimental autoimmune encephalomyelitis, CFA – rats treated with CFA, EAE + AG – EAE rats treated with AG, AG – rats treated with AG, EAE + NAC – EAE rats treated with NAC, NAC – rats treated with NAC. Bars in the graph represent mean \pm SEM from animals for each group. AG- and NAC-treatment decrease the MDA level in the spinal cords * $p < 0.05$ vs. EAE + AG and EAE + NAC, ANOVA (Bonferroni post test)

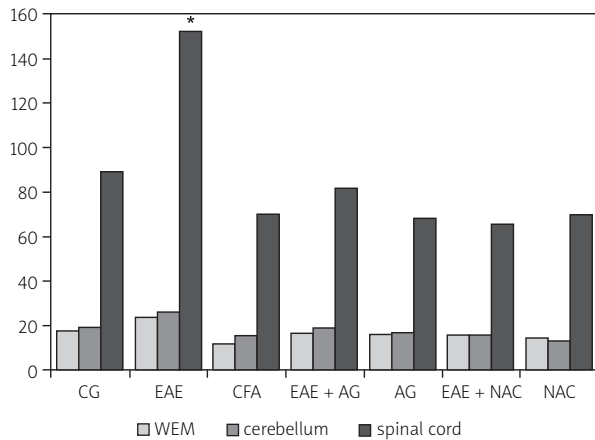


Fig. 3. Malondialdehyde concentration (nmol/mg prot.) in the rat CNS different regions. CFA – complete Freund’s adjuvant, AG – aminoguanidine, NAC – N-acetyl-L-cysteine, CG – control group, EAE – rats with experimental autoimmune encephalomyelitis, CFA – rats treated with CFA, EAE + AG – EAE rats treated with AG, AG – rats treated with AG, EAE + NAC – EAE rats treated with NAC, NAC – rats treated with NAC. Bars in the graph represent mean from 6-8 animals for each group. AG- and NAC-treatment decrease the MDA level in the WEM, cerebellum and spinal cord homogenates * $p < 0.05$ vs. EAE + AG and EAE + NAC, ANOVA (Bonferroni post test)

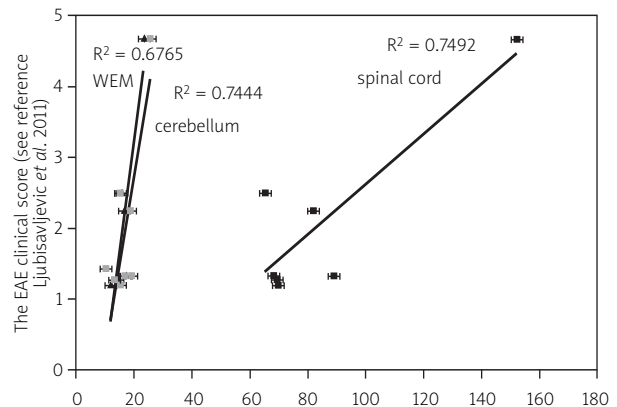


Fig. 4. The correlation of malondialdehyde concentration (nmol/mg prot.) and EAE clinical score. Encephalomyelitis (EAE) clinical score: healthy = 1; loss of tail tone = 2; hindlimb weakness = 3; hindlimb paralysis = 4; hindlimb paralysis plus forelimb weakness = 5; moribund or dead = 6. Simultaneously with the increasing malondialdehyde (MDA) level in both examined tissues (cerebellum and spinal cord) EAE clinical expression increases (worse clinical picture), $R^2 = 0.67$, $R^2 = 0.74$, $R^2 = 0.75$ for WEM, cerebellum and spinal cord, respectively. There is a positive linear relationship between these variables ($p < 0.01$)

tively). Aminoguanidine and NAC treatment decreases the MDA level in EAE-treated rats compared to the untreated EAE group in examined tissues – WEM, cerebellum, spinal cord ($p < 0.05$; [15], Figs. 1 and 2, respectively).

Malondialdehyde increase is the most pronounced in the spinal cord compared to the WEM and cerebellum ($p < 0.05$ – Fig. 3).

The clinical score significantly correlates with MDA levels in examined tissues – WEM [$R^2 = 0.67$], cerebellum ($R^2 = 0.74$) and spinal cord ($R^2 = 0.75$) ($p < 0.05$) – Fig. 4.

Discussion

Data reported in the present study absolutely confirm our previous findings that the MDA level in WEM [15], and, as we can notice here, MDA in cerebellum and spinal cords (Figs. 1 and 2), are in correlation with neurological signs of EAE, the most pronounced as hindlimb paralysis with/without forelimb weakness. Taken together, these studies suggest that in the acute

phase of the MS lipid peroxidation process predominates, due to proinflammatory reactions, leading to oxidative and nitrosative stress. That pathological condition occurs as a consequence of microglial and other immune cells infiltrating CNS tissues, resulting in high production of nitric oxide (NO) by iNOS, promoting its pathological influence in this way, as we reported in our recently published paper [4,15,16,26].

Many comprehensive insights of the underlying pathological mechanisms, leading to the development of autoimmunity in MS, propose a loss of immunological tolerance towards self-myelin basic protein and myelin oligodendrocyte glycoprotein [30]. The possible mechanism which can explain this immunological disadvantage in early development and clinical onset of MS may be the MDA-mediated covalent modification of proteins and formation of stable adducts with cysteine, lysine, and histidine amino acid residues [21]. Thus, *in vitro* MDA-mediated modification of mouse serum albumin has been reported to lead to the breaking of immunological tolerance to this biomolecule.

It is associated with the development of new epitope specificities and, at the same time, challenging immune-mediated reaction toward self protein structures [32]. Also, this condition may be useful for explanation of our findings. Namely, autoreactivity in CNS structures in MS can occur as a part of MDA affecting the three dimensional structure of the above-named protein. It induces antigen presenting cells activation and high expression of proinflammatory cytokines, as well as the activation of T cell response. These cellular and pathophysiological state befall even in the earliest phase of disease development. It seems that these may be the substantial baseline for understanding MS progression and its clinical appearance in the early phase of disease [32]. In addition to this assumption, the recently published data from Weismann *et al.* [31] identify complement factor H (CFH) as a major MDA-binding protein that can block uptake of MDA-modified proteins by macrophages and MDA-induced proinflammatory effects, promoting prevention of its worse consequences in this way [31]. On the contrary, as we report here, in the EAE-untreated animals, due to the high level of MDA in CNS structures, the clinical onset and development of neurological signs were worse than those in the other groups [15]. Therefore, the LP process provokes disease onset, but also promotes disease intensity. As we can notice, the potential oxidative and nitrosative modulators (AG, NAC) ameliorate the clinical onset of EAE, decreasing the MDA level (Figs. 1 and 2).

Myelin lipids can be oxidized by peroxynitrite, formed by overproduction of RNS and ROS, or a failure of antioxidant mechanisms. Each of them is evident in MS. Thus, peroxynitrite can lead to myelin damage through different lines, promoting myelin making cell death, affecting nerve cells signalling and through DNA breakdown. According to these facts, oligodendrocytes show particular susceptibility to NO and RNS and can be lysed by the levels of NO produced by activated microglial cells, which do not affect astrocytes or macrophages. This oligodendrocyte consumption is the explanation of the early neurological onset of MS, characterised by functional disconnection of CNS. Our study, and also studies of other authors, have shown that this lysis can be prevented by the treatment with antagonists of NO production and oxidative species scavengers [15,16,19,25]. These modulators regulated LP intensity regulate the neurological expression of EAE, although it is not a solitary protection mechanism.

So, this clinical amelioration may be explained with the prevention of lipid peroxidation, which alters the structure of biological membranes of CNS, thereby affecting their physical and chemical properties such as permeability, resorption or potential. But, on the other hand, different data show that NO and its metabolites (peroxynitrite) can have either pro-oxidant or antioxidant consequences, depending upon the concentrations of superoxide and NO. Some authors report that if the concentration of NO is high, lipid peroxidation is decreased because NO reacts directly with the alkoxy and peroxy radical intermediates formed during LP, blocking this change reaction [24]. Others, including our data, show different results. Lipid peroxidation process occurs as a consequence of high production, not only of reactive nitrogen but also oxygen species, promoting formation of peroxynitrite. Nitric oxide and peroxynitrite roles in LP have been claimed as opposing effects, depending upon the actual concentration. In the case of high level production, both of them, RNS and ROS, generate peroxynitrite most excessively. Then, peroxynitrite promotes LP, which is the case in our study [12]. Accordingly, some authors reported that clinical materials of MS patients consistently showed significantly increased LP in both blood and CSF [11,22] associated with the pathogenesis stages of the disease and clinical activity. The high level of MDA, reported in our paper, represents the LP intensity in the entire CNS, but as we can see in Fig 3., it is potently marked in the spinal cord. This evidence absolutely correlates with intensity of nitrosative disorder in this CNS structure. At the same time, it may be an explanation of characteristic neurological signs, affecting animals fore and hind limbs weakness, as we described in the previous paper [15].

Similarly, MDA may be a useful, sensitive, indirect marker of other mechanisms involving in LP. Thus, our results are consistent with the results of recent studies, which demonstrated a key role of cytosolic phospholipase A2 (cPLA2) in LP, especially during EAE [13,18]. cPLA2 can be induced by the cytokine tumour necrosis factor- α (TNF- α), highly produced in MS, and highly expressed in EAE lesions. The block of cPLA2 activity delays the onset and progression of the disease [14]. The isoprostanes, 8-epi-PGF2 α , produced by the non-enzymatic free radical-catalysed peroxidation of the arachidonic acid, may also evoke further CNS tissue damage. Prostaglandin E2 can act in conjunction with NO to disrupt the blood brain barrier, pro-

moting CNS infiltration by immune cells [27] and disease progression.

The consistent results from our lab and other authors showed that the high level of the lipid peroxidation process may be the cause which provokes and promotes MS, while blocking of these processes result in amelioration of the clinical onset and disease activity. Anyway, these results may be useful for the new insight into mechanisms and potential targets for therapeutic strategies in MS.

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References

- Acar G. Nitric oxide as an activity marker in multiple sclerosis. *J Neurol* 2003; 250: 588-592.
- Andreeva LI, Kozhemiakin LA, Kishkun AA. Modification of the method of determining lipid peroxidation in a test using thiobarbituric acid. *Lab Delo* 1988; 11: 41-43.
- Bast A, Haenen GR, Doelman CJA. Oxidants and antioxidants: state of the art. *Am J Med* 1991; 91: 2S-13S.
- Besler HT, Comoglu S. Lipoprotein oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerosis. *Nutr Neurosci* 2003; 6: 189-196.
- Bongarzone ER, Pasquini JM, Soto EF. Oxidative damage to proteins and lipids of CNS myelin produced by in vitro generated reactive oxygen species. *J Neurosci Res* 1995; 41: 213-221.
- Calabrese V, Scapagnini G, Ravagna A, Bella R, Foresti R, Bates TE, Giuffrida Stella AM, Pennisi G. Nitric oxide synthase is present in the cerebrospinal fluid of patients with active multiple sclerosis and is associated with increases in cerebrospinal fluid protein nitrotyrosine and S-nitrosothiols and with changes in glutathione levels. *J Neurosci Res* 2002; 70: 580-587.
- Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991; 11: 81-128.
- Fukuda M, Kanou F, Shimada N, Sawabe M, Saito Y, Murayama S, Hashimoto M, Maruyama N, Ishigami A. Elevated levels of 4-hydroxynonenal-histidine Michael adduct in the hippocampi of patients with Alzheimer's disease. *Biomed Res* 2009; 30: 227-233.
- Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. *J Neurol* 2004; 251: 261-268.
- Gonsette RE. Oxidative stress and excitotoxicity: a therapeutic issue in multiple sclerosis? *Mult Scler* 2008; 14: 22-34.
- Hunter MI, Nlemadim BC, Davidson DL. Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluids from multiple sclerosis patients. *Neurochem Res* 1985; 10: 1645-1652.
- Jana A, Pahan K. Oxidative stress kills human primary oligodendrocytes via neutral sphingomyelinase: implications for multiple sclerosis. *J Neuroimmune Pharmacol* 2007; 2: 184-193.
- Kalyvas A, David S. Cytosolic phospholipase A2 plays a key role in the pathogenesis of multiple sclerosis-like disease. *Neuron* 2004; 41: 323-335.
- Kronke M, Adam-Klages S. Role of caspases in TNF-mediated regulation of cPLA2. *FEBS Lett* 2002; 531: 18-22.
- Ljubisavljevic S, Stojanovic I, Pavlovic D, Sokolovic D, Stevanovic I. Aminoguanidine and N-Acetyl-Cysteine suppress oxidative and nitrosative stress in EAE rat brains. *Redox Rep* 2011; 16: 166-172.
- Ljubisavljevic S, Stojanovic I, Pavlovic D, Milojkovic M, Vojinovic S, Sokolovic D, Stevanovic I. Correlation of nitric oxide levels in the cerebellum and spinal cord of experimental autoimmune encephalomyelitis rats with clinical symptoms. *Acta Neurobiol Exp* 2012; 72: 33-39.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951; 193: 265-275.
- Marusic S, Leach MW, Pelker JW, Azoitei ML, Uozumi N, Cui J, Shen MW, DeClercq CM, Miyashiro JS, Carito BA, Thakker P, Simmons DL, Leonard JP, Shimizu T, Clark JD. Cytosolic phospholipase A2 α -deficient mice are resistant to experimental autoimmune encephalomyelitis. *J Exp Med* 2005; 202: 841-851.
- Merrill JE, Ignarro LJ, Sherman MP, Melinek J, Lane TE. Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. *J Immunol* 1993; 151: 2132-2141.
- Miller E, Mrowicka M, Saluk-Juszczak J, Majsterek I. The level of isoprostanes as a non-invasive marker for in vivo lipid peroxidation in secondary progressive multiple sclerosis. *Neurochem Res* 2011; 36: 1012-1016.
- Nadkarni DV, Sayre LM. Structural definition of early lysine and histidine adduction chemistry of 4-hydroxynonenal. *Chem Res Toxicol* 1995; 8: 284-291.
- Naidoo R, Knapp ML. Studies of lipid peroxidation products in cerebral fluid and serum in multiple sclerosis and other conditions. *Clin Chem* 1992; 38: 2449-2454.
- Ponomarev ED, Shriver LP, Maresz K, Dittel BN. Microglial cell activation and proliferation precedes the onset of CNS autoimmunity. *J Neurosci Res* 2005; 81: 374-389.
- Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 1991; 288: 481-487.
- Sajad M, Zargan J, Chawla R, Umar S, Sadaqat M, Khan H. Hippocampal neurodegeneration in experimental autoimmune encephalomyelitis (EAE): potential role of inflammation activated myeloperoxidase. *Mol Cell Biochem* 2009; 328: 183-188.
- Salemi G, Gueli MC, Vitale F et al. Blood lipids, homocysteine, stress factors, and vitamins in clinically stable multiple sclerosis patients. *Lipids Health Dis* 2010; 9: 19-21.
- Smith KJ, Kapoor R, Felts PA. Demyelination: The Role of Reactive Oxygen and Nitrogen Species. *Brain Pathol* 1999; 9: 69-92.
- Subczynski WK, Hyde JS. Concentration of oxygen in lipid bilayers using a spin-label method. *Biophys J* 1983; 41: 283-286.
- Tiberio M, Chard DT, Altmann GR, Davies G, Griffin CM, McLean MA, Rashid W, Sastre-Garriga J, Thompson AJ, Miller DH. Metabolite

- changes in early relapsing-remitting multiple sclerosis. A two year follow-up study. *J Neurol* 2006; 253: 224-230.
30. Wallberg M, Bergquist J, Achour A, Breij E, Harris RA. Malondialdehyde modification of myelin oligodendrocyte glycoprotein leads to increased immunogenicity and encephalitogenicity. *Eur J Immunol* 2007; 37: 1986-1995.
31. Weismann D, Hartvigsen K, Lauer N, Bennett KL, Scholl HPN, Issa PC, Cano M, Brandstätter H, Tsimikas S, Skerka C, Superti-Furga G, Handa JT, Zipfel PF, Witztum JL, Christoph J. Binder Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. *Nature* 2011; 478: 76-81.
32. Wuttge DM, Bruzelius M, Stemme S. T-cell recognition of lipid peroxidation products breaks tolerance to self proteins. *Immunology* 1999; 98: 273-279.