ORIGINAL ARTICLE

Effect of Corticosteroid Therapy on Serum and CSF Malondialdehyde and Antioxidant Proteins in Multiple Sclerosis

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ABSTRACT: ***Objective:*** Multiple sclerosis (MS) is a disease characterised by perivascular infiltrates and demyelination of the white matter in the central nervous system. Although the precise cause of MS remains unknown, some investigations have been carried out on antioxidant mechanisms in these patients. ***Methods:*** In this study, malondialdehyde (MDA), as a lipid peroxidation marker, and ceruloplasmin (Cp) and transferrin (Trf), as antioxidant proteins, levels were determined in cerebrospinal fluid (CSF) and serum of 30 MS patients before and after corticosteroid therapy and in 20 control subjects. Transferrin and Cp levels were measured by the nephelometric method and MDAwas measured spectrophotometrically. ***Results:*** Mean MDA_{serum} and MDA_{CSF} levels were found to be highest in the pretreatment group and lowest in the control group. Although there was no significant difference in terms of serum Trf level, serum Cp was found higher in pre- and posttreatment groups than in the control groups. Ceruloplasmin and Trf levels of CSF were not detectable using the nephelometric method. A significant correlation was found between MDA_{CSF} and MDA_{serum} in the pretreatment group (r=0.58). ***Conclusions:*** These data revealed that lipid peroxidation was increased in serum and particulary in CSF of MS patients and was reduced with corticosteroid therapy.

RÉSUMÉ: Effet de la corticothérapie surle MDAdu sérum et du LCR et les protéines antioxydantes dans la sclérose en plaques. ***Objectif:*** La sclérose en plaques (SEP) est une maladie caractérisée par des infiltrats périvasculaires et une démyélinisation de la substance blanche dans le système nerveux central. Bien que la cause précise de la SEP demeure inconnue, des recherches ont porté sur les mécanismes antioxydants chez ces patients. ***Méthodes:*** Dans cette étude, les taux de malondialdéhyde (MDA), comme marqueur de la peroxydation lipidique, et de céruloplasmine (Cp) et de transferrine (Trf) comme protéines antioxydantes, ont été déterminés dans le liquide céphalorachidien (LCR) et le sérum de 30 patients atteints de SEP, avant et après la corticothérapie, et chez 20 sujets contrôles. Les niveaux de Trf et de Cp ont été mesurés par néphélométrie et les niveaux de MDAont été mesurés par spectrophotométrie. ***Résultats:*** On a observé les niveaux moyens de MDAdu sérum et du LCR les plus élevés chez le groupe de patients avant le traitement et les plus bas chez le groupe contrôle. Bien qu'il n'y avait pas de différence significative dans les niveaux de Trf sériques, les niveaux de Cp sériques étaient plus élevés chez le groupe SEPavant et après traitement que chez le groupe contrôle. Les niveaux de Cp et de Trf du LCR n'étaient pas détectables par néphélométrie. Il existait une corrélation significative entre les niveaux de MDAdu LCR et du sérum chez le groupe SEPavant traitement (r=0.58). ***Conclusions:*** Ces données révèlent que la peroxydation lipidique était augmentée dans le sérum et surtout dans le LCR des patients atteints de SEPet était diminuée par la corticothérapie.

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Multiple sclerosis (MS) is a disease characterized by perivascular infiltrates and demyelination of the white matter in the central nervous system.[1] Although the etiology of the disease remains unknown, studies from various animal model systems strongly support the notion that cell-mediated immune reactions against myelin antigens contribute to the disease process.[2] Several lines of evidence support the possibility that lipid peroxidation, which is one of the most important organic expressions of oxidative stress induced by the reactivity of oxygen free radicals, may be a factor in membrane damage in MS.[3] There have been reports that malondialdehyde (MDA), as a lipid peroxidation marker, is significantly raised in blood and/or cerebrospinal fluid (CSF) of MS patients.[3,4]

The primary physiologic role of ceruloplasmin (Cp) involves plasma redox reactions. Ceruloplasmin permits the incorporation

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of iron into transferrin (Trf) without the formation of toxic iron products.[5] Under physiologic conditions, Cp is also important in the control of membrane lipid oxidation, probably by direct oxidation of cations, thus preventing their catalysis of lipid peroxidation.[6,7] In the present study, serum and CSF MDA, Cp, and Trf levels were measured in 30 MS patients, before and after methylprednisolone (MP) therapy and in 20 controls in order to detect whether there is any difference between these groups with respect to the parameters mentioned above and to examine levels of these parameters in pretreatment and posttreatment periods.

Materials and methods

Serum and CSF samples were studied in 50 individuals comprising the following groups:

(1) 30 MS patients (13 men, 17 women, aged 17- 51 years, mean 30 years);

(2) 20 controls (7 men, 13 women, aged 21-39 years, mean 32 years) with back pain.

All MS patients had clinically definite disease according to the criteria of Poser et al.[8] The clinical diagnosis of MS was based on clinical signs supported by objective findings including the presence of inflammatory lesions as confirmed by magnetic resonance imaging (MRI), as well as the presence of oligoclonal bands and increased IgG level in the CSF.[9] All of the MS patients had the chronic progressive type (disease duration: 97.4±51.06; ranging from 25-240 months), and were treated with MP(0.5-1g intravenously for five to seven days). Expanded disability status scales (EDSS) were determined before and after MP therapy. Blood and CSF samples were taken within seven days of an acute exacerbation and three days after MP therapy of the disease, immediately centrifuged to remove cells and stored at -80ºC until MDA, Cp, and Trf measurements were made.

Venous blood was collected in vacutainer tubes and centrifuged at 2000xg for 10 minutes. Each sample was studied in duplicate. MDA was estimated according to the modified method of Satoh.[10] To 0.5 ml serum or CSF, 0.5 ml of 35% TCA was added. After vortex mixing, 0.5 ml Tris/HCl buffer (50 mM; pH 7.4) was added followed by further mixing and incubation at room temperature for 10 min; 1.0 ml of 0.75% thiobarbituric acid in 2 M Na_2SO_4 was added and then the mixture was heated at 100ºC for 45 minutes. After cooling, 1 ml of 70% TCA was added, the mixture was vortexed and then centrifuged at 950xg for 10 minutes. The absorbance of supernatant was determined at 530 nm. Total thiobarbituric acid-reactive materials were expressed as MDA, using a molar extinction coefficient for MDAof 1.56 x 10^5 $cm^{-1}M^{-1}$.

In the serum and CSF, both Cp and Trf levels were quantified by the nephelometric method with commercially available kits (Beckman Instruments, Inc.) in a Beckman Array® Protein System analyzer.

The results were given as mean ± SD. Statistical analysis was carried out by paired Student's t test for the comparison of the pretreatment and posttreatment groups, and unpaired Student's t test for the comparison of the patient groups with the control group. Correlations were analysed by linear regression analysis. A probability ≤0.05 was considered significant.

Results

CSF Trf and Cp levels could not be detected with the nephelometric method in a Beckman Array® 360 System. Its lower detection limit for Cp was 2.0 mg/dl and for Trf was 12.5 mg/dl. MDA in serum was found significantly increased in the pretreatment group compared to the control and the posttreatment groups but not as much increase of MDA in CSF (Table). Additionally, mean MDA_{serum} was found to be even higher in the posttreatment group than in the controls. There was no significant difference between the posttreatment and the control groups in terms of MDA_{serum} values. The mean MDA_{CSF} values were higher in the patients (both pre- and posttreatment) than that of the control group. The highest MDA_{CSF} was determined in patients of the pretreatment group. The posttreatment patient group had higher MDA_{CSF} than the controls and lower MDA_{CSF} than the pre-treatment group. Although there was no difference in Trf levels among the groups, serum Cp was found to be the highest in the pretreatment group and the lowest in the control group. There was no significant difference between the pre- and the posttreatment EDSSs (Table).

Following correlation analysis, no correlation was determined between parameters in the control group. In the pretreatment group, there was a significant correlation between MDA_{serum} and MDA_{CSF} ($r=0.58$, $p<0.001$) and a borderline correlation between Trf and Cp ($r=0.36$, $p=0.053$). In the posttreatment group, a weak correlation was found between MDA_{serum} and MDA_{CSF} ($r=0.56$, $p=0.052$). Additionally, a weak correlation was detected between EDSS and MDA_{serum} ($r=0.44$, $p<0.05$) in the pretreatment group, borderline correlation between EDSS and MDA_{serum} ($r=0.43$, $p=0.057$) and between EDSS with Cp ($r=0.46$, $p<0.05$), and with MDA_{CSF} ($r=0.39$, $p<0.05$) in the posttreatment group.

Table: MDAin serum and CSF, and antioxidant proteins (Trf and Cp) in serum from MS patients (n=30), and controls (n=20), before and after methylprednisolone therapy.

	MDA_{serum} *(nmol/mL)*	MDA_{CSF} *(nmol/mL)*	Trf *(mg/dL)*	Cp *(mg/dL)*	EDSS
Control (n=20)	7.4±0.79	1.1±0.5	237.7±45.2	38.2±10.7	-
P1 (n=30)	8.5±1.44^{*,a}	5.2±1.9^{**,b}	236.9±38.6	49.1± 9.9*	1.40±0.97
P2 (n=30)	7.8±1.11	3.3±1.9**	233.2±44.7	47.2± 9.1*	1.35±0.98

*: $p<0.01$, **: $p<0.001$ when compared to control, ^{a}: $p<0.01$ and ^{b}: $p<0.001$ P1 vs P2 .

P1: MSpatients, pretreatment group; P2: MS patients, post treatment group

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Discussion

Lipid peroxidation is one of the most organic expressions of oxidative stress induced by the reactivity of oxygen free radicals. MS is among the well-established neurological diseases and one of the most important by virtue of its frequency, chronicity and tendency to attack young adults. The chemical composition of human CSF is considered to reflect brain metabolism. In the literature, there have been several reports on the direct measurement of lipid peroxides in body fluids from MS patients.[10,11] For example, Hunter et al[10] demonstrated increases in thiobarbituric acid-reactive substances and lipid soluble fluorescent pigments in CSF samples from MS patients, compared to those from the control subjects. Newcombe et al[12] claimed that increased lipid peroxidation and oxidized LDL uptake by activated microglia and infiltrating macrophages found in MS plaques might play an important role in demyelination.

Previously, Hunter et al[10] also studied Cp and Trf in CSF of MS patients and reported that neither of these proteins was detectable in CSF using the immunodiffusion method. In this study, we also measured these parameters in CSF of MS patients, neither of which could be detectable with the nephelometric method. On the other hand, although there was no difference in serum Trf values, a statistically significant difference was present in serum Cp values in favour of the pretreatment group. This finding was consistent with the studies of Hunter et al[10] and Al-Timimi et al.[11] This increase of Cp in serum may be due to a compensatory mechanism. By keeping iron in Fe^{3+} state, Cp prevents it from undergoing the redox cycles necessary to initiate toxic effects.[7]

Furthermore, there are controversial reports about the level of MDA in serum/plasma and CSF of patients with MS. For example, while in the study of Hunter et al[10] no increases in lipid peroxidation products had been observed in the serum of patients with MS, some other investigators[3,4] had demonstrated increased concentrations of MDA in the plasma or serum of MS patients. Our study also revealed increased MDA_{CSF} in MS. While MDA_{CSF} was found increased in MS in the study of Hunter et al,[10] Calabrase et al[13] and other researchers[3,4] could not detect any difference. Prior studies have not given any information about these parameters on the comparison of the levels before and after MP therapy. We found about a four- to five-fold increase in MDA_{CSF} in the pretreatment patients and a three–fold increase in MDA_{CSF} of the posttreatment group compared to the control subjects. These data revealed that MDA_{CSF} was reduced with therapy but remained higher than the control values. A decrease of MDAin CSF was not as prominent as a decrease of MDA in serum. In addition, we found that with corticosteroid therapy, serum MDA levels decreased and there was no significant difference between the posttreatment group and the control group.

In conclusion, the findings of the present study indicate that lipid peroxidation was increased in serum, and particularly in the CSF of MS patients. Consequently, serum Cp levels might be increased as a compensation mechanism. When comparing the decrease in MDA_{serum} and MDA_{CSF} following treatment in patients, a dramatic decline is seen in the levels of MDA_{CSF}, suggesting that the MP affected predominantly the levels of MDA_{CSF}. MDAwas found to be decreased both in the serum and CSF of MS patients after corticosteroid therapy, the mechanism of which remains to be clarified.

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