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Oxidative modification of blood serum proteins in multiple sclerosis after interferon or mitoxantrone treatment

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ABSTRACT

This study was aimed at (i) comparison of the usefulness of serum protein oxidation parameters for assessment of oxidative stress (OS) in multiple sclerosis (MS), and (ii) comparison of OS in MS patients subject to various therapies. Elevated glycophore level was noted in relapsing–remitting (RRMS) patients without treatment and patients treated with interferons β1a and β1b (10.33 ± 3.27, 8.02 ± 2.22 and 8.56 ± 2.45 vs control 5.27 ± 0.73 fluorescence units (FU)/mg protein). Advanced oxidation protein products (295 ± 135 vs 83 ± 65 nmol/mg protein), carbonyl groups (3.68 ± 1.44 nmol/mg protein vs 2.03 ± 0.23 nmol/mg protein), kynurenine (7.71 ± 0.1.67 vs 5.5 ± 0.63 FU/mg protein) and N′-formylkynurenine (7.69 ± 0.7 vs 4.97 ± 0.59 FU/mg protein) levels were increased, while thioredoxin level was decreased in RRMS patients without treatment (5.03 ± 2.18 vs 10.83 ± 2.75 ng/ml) with respect to control. The level of OS was higher in untreated RRMS patients and in SPMS patients treated with mitoxantrone than in patients treated with interferon.

1. Introduction

Multiple sclerosis (MS) is the most common autoimmune disease of the central nervous system (CNS) leading to severe disability in young adults, with female predominance (Sellner et al., 2011). The most typical disease course is relapsing–remitting multiple sclerosis (RRMS) characterized by periods of clinical stability, punctuated by subacute attacks of clinical worsening after complete or partial recovery (Seven et al., 2013). Most patients initially displaying a relapsing–remitting course eventually convert to a secondary progressive disease course (SPMS) after 10–25 years of disease (Chanvillard et al., 2012). Recently, significant advances in the understanding of MS have resulted in the suggestion that excessive generation of reactive oxygen species (ROS) leads to oxidative stress, inducing myelin loss and nerve tissue degeneration. While occurrence of oxidative stress has been examined in MS, relatively few studies have been devoted to oxidative protein damage in this disease (Ljubisavljevic et al., 2013).

Protein carbonyls are widely used as chemically stable biomarkers of oxidative stress. Protein carbonyls may be generated by the oxidation of several amino acid side chains (e.g., Lys, Arg, Pro, and Thr). They may be also formed through oxidative cleavage of proteins by either the α-amidation pathway or by oxidation of glutamyl side chains, leading to formation of a peptide in which the N-terminal amino acid is blocked by an α-ketoacyl derivative. In addition, carbonyl groups may be introduced into proteins by secondary reaction of the nucleophilic side chains of Cys, His, and Lys residues, with aldehydes (4-hydroxy-2-nonenal, malondialdehyde and acrolein) produced during lipid peroxidation or with reactive carbonyl derivatives (ketoamines, ketoaldehydes, deoxyosones) generated as a consequence of the reaction of reducing sugars or their oxidation products with lysine residues of proteins (glycation and glycoxidation reactions). Such reactions lead to eventual formation of the advanced glycation/lipoxidation end products (AGEs/ALEs) (Dalle-Donne et al., 2003). AGEs are widely studied as reliable biomarkers of oxidative and glycooxidative damage. More recently, AGEs have also been recognized as important pathogenetic factors of some oxidative-stress based diseases (Kalousová et al., 2005; Aldini et al., 2013).

Advanced oxidation protein products (AOPP) were defined as a reliable marker to estimate the degree of oxidant-mediated protein damage (Ramasamy et al., 2005). AOPP are produced mainly by the action of chlorinated oxidants (mainly chloramines and hypochlorous acid). Like AGE products, AOPP can participate in the initiation and promotion of inflammatory processes, increasing cytokine production and expression of many adhesive molecules (Ljubisavljevic et al., 2013). Other markers of oxidative protein damage include the loss of tryptophan, formation of dityrosines, kynurenine and N′-formylkynurenine, which can be assessed fluorimetrically (Rice-Evans et al., 1991; Robaszkiewicz et al., 2008).

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Table 1
Characteristics of the patients studied.

	Control group (n = 18)	RRMS without immunomodifying treatment (n = 13)	RRMS (clinically stable) interferon β 1a (INF-β1a) (n = 18)	RRMS (clinically stable) interferon β1b (INF-β1b) (n = 20)	SPMS (clinically stable) mitoxantrone (MX) (n = 9)
Female/male ratio	1.1	0.8	1.83	3.75	1.4
Age (years) ± SD	35.4 ± 9.1	43.4 ± 10.5	40.3 ± 9.6	42.5 ± 10.5	53.6 ± 6.8
Disease duration (months) ± SD	NA	50.4 ± 21.2	63.1 ± 52.3	101.2 ± 77.93	222 ± 6.8
Treatment duration (months) ± SD	NA	NA	26.3 ± 16	32.05 ± 27.7	21 ± 14.01
EDSS	NA	3.8	2.81	3.11	5.54

Human cytosolic thioredoxin (hTrx1) is an important factor in the protection against cytotoxicity mediated by ROS (Wu et al., 2010). Its release into the serum is another marker of oxidative stress.

Current therapies for MS focus mainly on immune aspects of the disease and benefit principally patients with RRMS, while their efficacy is minimal or even lacking in patients with primary/secondary progressive disease (Chanvillard et al., 2012). Human IFN-β proved to be efficient for the treatment of RRMS. There are 2 forms of recombinant human IFN-β on the market, IFN-β1a (Avonex [Biogen Idec Inc., Weston, Mass] and Rebif [Merck Serono S.A., Geneva, Switzerland]) and IFN-β1b (Betaferon and Betaseron [Bayer HealthCare Pharmaceuticals Inc., Berlin, Germany]).

Mitoxantrone hydrochloride (Novantrone®, MX) is an anticancer drug synthesized in the end of 1970s as an alternative to anthracycline therapy. It has been largely used in the treatment of solid tumors, acute leukemia, lymphoma, prostate, and breast cancers (Rossato et al., 2013). Evidence shows that MX could be a first-line treatment for malignant forms of MS, and a second-line drug in relapsing–remitting or secondary progressive MS that is unresponsive to IFN-β1a, and -1b or glatiramer acetate (Morrissey et al., 2005).

MX inhibits DNA replication and induces single and double strand breaks by intercalating DNA through hydrogen bonding. The mechanisms of action of MX are still not fully understood, and clear data on its effects on the immune system are limited (Chanvillard et al., 2012).

MX acts as an immunosuppressive agent which alters B and T lymphocytes responses to CNS antigens, and seems to prevent axonal lesion and macrophage-mediated demyelination in this way (Montú et al., 2005). The administration of higher MX doses increases the risk of the heart damage due to the high cardiotoxicity. MX, besides cardiotoxicity, can cause a number of side effects, including acute leukemia, leukopenia, nausea, vomiting, hair loss, urinary tract infections, kidney failure and bone pain (Stankiewicz et al., 2013).

There are no data available on the comparison of effects of MS therapy with interferones and mitoxantrone on the severity of oxidative stress in this disease.

We hypothesized that type of therapy (interferones or MX) can influences on oxidative/antioxidative status of blood serum in MS patients. In particular, taking into account the prooxidant effects of mitoxantrone reported by us previously (Adamczyk-Sowa et al., 2012), we wanted to check whether oxidative protein modifications are more extensive in patients treated with this drug in comparison to interferons. To tests this hypothesis, we aimed at quantification of markers of oxidative protein oxidation in MS patients without treatment and in MS patients treated with interferons and with MX using a range of assays easily applicable to blood serum in a diagnostic laboratory and compare their usefulness for this purpose.

2. Materials and methods

2.1. Ethical permission

The study was approved by the local Ethical Committee of the Medical University of Silesia and informed consent was obtained from each patient prior to entry into the study, according to the declaration of Helsinki.

2.2. Control patients

18 patients, nonsmokers, aged 26–45 years, were admitted at Clinic for Neurology in Zabrze and underwent the complete diagnostic procedure due to suspected demyelinating disease. Laboratory and neuroimaging tests were normal. Therefore the final diagnosis was mainly tension headache or conversion disorder. All control patients were screened to be free from any neurological or other major medical illnesses.

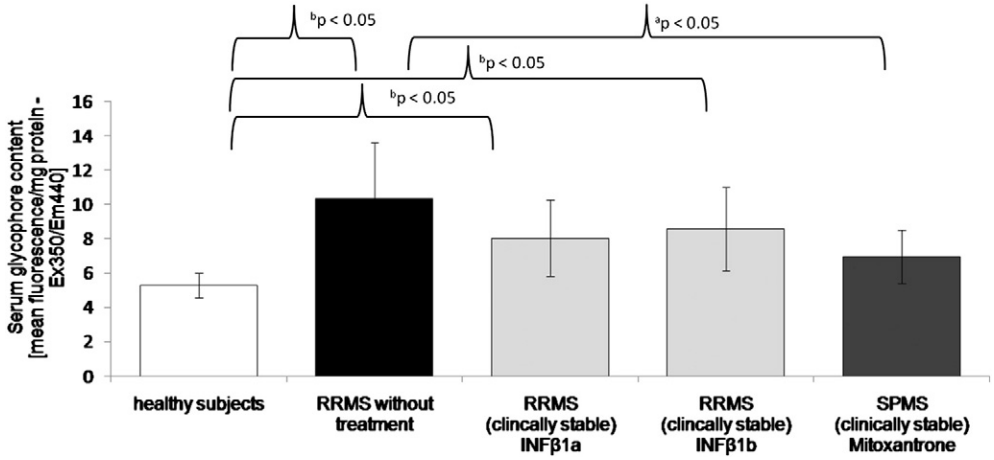

Fig. 1. Comparison of the glycophore content of blood serum proteins in control group, in RRMS patients without treatment, in clinically stable patients RRMS treated with interferons β1a and β1b, and in patients SPMS treated with mitoxantrone. Statistically significant differences (Kruskal–Wallis test): $\Delta p \leq 0.05$ SPMS MX vs RRMS without treatment, $^{*}p \leq 0.05$ RRMS (clinically stable), INF-β1a and RRMS (clinically stable) INF-β1b vs control group, $^{\bullet}p \leq 0.05$ RRMS without treatment vs control group and SPMS MX. Data are presented as mean ± S.D.