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Effect of whole body cryotherapy treatments on antioxidant enzyme activity and biochemical parameters in patients with multiple sclerosis

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A – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

Summary **Background.** Multiple sclerosis (MS), the most common cause of non-traumatic disability in adults, is a chronic, complex neurological disease with a variable clinical course and several pathophysiological mechanisms. Whole-body cryotherapy (WBC), due to its analgesic effects, is an increasingly popular form of rehabilitation for neurological patients, especially for those with MS.
Objectives. The following study attempted to evaluate the effect of 30 daily whole-body cryotherapy treatments (3 minutes at -130°C) on basic blood biochemical parameters and main antioxidant enzyme activity in the erythrocytes of MS patients.
Material and methods. Total protein, albumin, glucose and uric acid levels and lipid profile indicators: total cholesterol (TCh), HDL cholesterol and triacylglycerol (TAG) concentrations, were determined with the enzymatic colorimetric method. The activity of erythrocyte antioxidant enzymes: SOD1/CuZn-SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase), R-GSSG (glutathione reductase), GST (glutathione transferase), was assessed using kinetic methods before and after 30 daily WBC treatment in 30 patients.
Results. Following a series of 30 WBC treatments, no significant changes in total protein, albumin, uric acid and glucose concentrations, total cholesterol and HDL- and LDL-fraction cholesterol levels and triacylglycerol concentration, as well as a significant increase in SOD1 activity coupled with a trend for increased GST activity, were observed in the group of patients.
Conclusions. The results confirm the possibility of modulating the effect of this form of rehabilitation on the systemic antioxidant potential in multiple sclerosis patients.
Key words: multiple sclerosis, cryotherapy, enzymes.

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Background

Multiple sclerosis (MS), the most common cause of non-traumatic disability in adults, is a chronic, complex neurological disease with a variable clinical course and several pathophysiological mechanisms, such as inflammation, demyelination, axonal/neuronal damage, gliosis, oligodendrocyte loss, re-myelination and repair mechanisms and oxidative stress [1, 2]. It is well known that inflammation might raise ROS levels, leading to oxidative stress (OS). One of the most abundant sources of ROS, apart from the electron-transport chain of mitochondria, is the respiratory burst system of activated microglia. ROS have been implicated as mediators of demyelination and axonal damage; moreover, the pathogenic role of oxygen and nitrogen free radicals is postulated in MS pathology. It is suggested that antioxidants might prevent free radical-mediated tissue destruction and inhibit some of the early pro-inflammatory events, such as T cell activation and cell trafficking into the CNS, which lead to inflammation and tissue destruction in MS [3, 4]. Free radical-mediated peroxidation of biological molecules, especially of lipids, is implicated in the pathogenesis of a number of neurological diseases, such as multiple sclerosis. A low concentration of antioxidant vitamins: beta carotene, retinol, alpha tocopherol and ascorbic acid, has been observed in the serum or cerebrospinal fluid of multiple sclerosis patients [5]. Genetic and environmental factors, such as IL-1 beta, IL-1 receptor, immunoglobulin Fc receptor genes, apolipoprotein E (APO-E) gene, as well as pathogens and chemicals, have been suggested to play a role in the pathogenesis of MS [3, 6–9].

Whole-body cryotherapy (WBC), due to its analgesic effects, is an increasingly popular form of rehabilitation for neurological patients, similarly in stationary rehabilitation centres. It has been demonstrated that this therapy has beneficial effects for patients with symptoms of depression and leads to regulation of muscle tone, which affects the effectiveness of kinesiotherapy being carried out. In MS patients, whole-body cryotherapy is commonly used in order to improve the overall functionality of the patient and to reduce the fatigue level. More importantly, patients tolerate this treatment very well [10, 11]. The previously described effects of cryotherapy, involving modification of the pro-oxidant/antioxidant status [12–14], have been confirmed in studies with MS patients. Miller et al. [15] showed changes in total antioxidant status and superoxide dismutase and catalase activities in the erythrocytes of MS patients with a secondary progressive disease course in response to a series of 10 WBC exposures.

Objectives

The increasing use of whole-body cryotherapy treatments in SM patients, as well as in the series of elongated effects, prompted us to undertake a study on the effect of 30 daily WBC treatments on basic blood biochemical parameters and antioxidant enzyme activity in the erythrocytes of MS patients.

Material and methods

Subjects and WBC protocol

The research involved 30 multiple sclerosis patients, women aged 45.6 ± 12.4 years (with normal distribution of results), who had never been subjected to any form of cryotherapy. The patients did not suffer from other diseases according to the physician's qualifications. Eligibility criteria for the subjects included diagnosis of multiple sclerosis according to McDonald diagnostic criteria (in accordance with 2010 amendments). An additional inclusion criterion was a level above of 6 in EDSS (Expanded Disability Status Scale). The exclusion criteria included the presence of contraindications for whole-body cryotherapy treatments and application of immunomodulators, immunostimulators, hormones, vitamins and supplements. Each participant provided their written consent before participation in the study, and the Local Ethical Committee of the Pomeranian Medical University (Decision KB-0012/36/13) issued a formal consent according to the Helsinki Declaration. Participants were exposed to a 30-day-long series of WBC at the Department of Therapeutic Rehabilitation at the Central Clinical Hospital of the Ministry of Interior and Administration in Warsaw, Poland. Each cryotherapy session lasted 3 minutes (-130°C), preceded by a 30-second period in the vestibule at a temperature of -60°C in a two-stage cryochamber of the Wrocław-type with a nitrogen-exchanger. After the cryotherapy session, each patient participated in the group kinesitherapy (general development carried out by a physiotherapist), which was the same for all patients and lasted 30 minutes after WBC.

Blood preparation

Venous blood samples were taken twice, before and after the series of 30 WBC treatments, between 8:00 and 9:00 a.m., while fasting, using a vacuum blood collection system (Sarstedt, Germany), in order to obtain erythrocytes and blood serum. The erythrocytes were separated by centrifugation (2,600 g, 10 min, 4°C), washed three times with 0.9% NaCl and chilled to 4°C.

Biochemical analysis

The activity of erythrocyte antioxidant enzymes: SOD1/CuZn-SOD; E.C.1.15.1.1 (superoxide dismutase), CAT; E.C.1.11.1.6 (catalase), GPx; E.C.1.11.1.9 (glutathione peroxidase), R-GSSG; E.C.1.8.1.7 (glutathione reductase), GST; E.C.2.5.1.18 (glutathione transferase), was assessed using kinetic methods. All reagents used were purchased from Sigma-Aldrich Sp. z o.o. (Poznan, Poland). The analysis of enzyme activity was performed using a UV/VIS Lambda 40 spectrophotometer (Perkin-Elmer). In all the mentioned cases, haemoglobin levels were assayed using Drabkin's method [16]. Enzyme activity was calculated per 1 g of RBC haemoglobin.

Sensitivity of the SOD assay (λ 320 nm; at 30°C) was 0.1 U/mL, specificity 97%, while the coefficient of variation was lower than 4% [17]. Sensitivity of the CAT assay (λ 240 nm; at 30°C) was 1.71 U/mL, specificity 89%, while the coefficient of variation was lower than 2% [18]. Sensitivity of the GPx assay (λ 340 nm; at 30°C) was 6 U/L, specificity 94%, while the coefficient of variation was lower than 4% [19]. Sensitivity of the GST assay (λ 340 nm; at 30°C) was 1.2 U/L, specificity 97%, while the coefficient of variation was lower than 2% [20]. The activity of erythrocyte GSSG-R was determined with the spectrophotometric method by Beutler and Yeh [21]; with sensitivity 0.14 U/L, specificity 94%, while the coefficient of variation was lower than 4%. Total protein, albumin, glucose and uric acid levels and lipid profile indicators: total cholesterol (TCh), HDL cholesterol and triacylglycerol (TAG) concentrations, were determined with the enzymatic colorimetric method (BioMaxima, Poland). The LDL-cholesterol fraction was calculated using the Friedewald formula:

LDL [mg/dl] = total cholesterol (TCh) - HDL cholesterol - (TAG/5) [22].

Statistical analysis

Statistical analysis was performed with STATISTICA software (ver. 12.5 PL). In addition to descriptive statistics (median, upper and lower quartiles), the normality of distribution of the analysed parameters was determined using the Shapiro–Wilk test. For values showing a distribution that deviated from the norm, the Wilcoxon matched-pairs signed-rank test was used. To carry out the above statistical analyses, a significance level of $p < 0.05$ was adopted.

Results

During the study, no deterioration of the condition on any of the patients being exposed to whole-body cryotherapy treatments was observed. For organisational and unforeseen reasons, one of the female patients could not participate in the full series of treatments, resulting in incomplete data, which was excluded from the final analysis.

Following a series of 30 WBC treatments, no significant changes in total protein, albumin, uric acid and glucose concentrations and lipid profile were observed in the subjects. The results for the level of biochemical parameters in response to successive weeks of whole-body cryostimulation are presented in Table 1.

Table 1. Values of selected blood biochemical parameters before and after WBC treatments

Biochemical parameters	T_0 – before the 1st WBC			T_1 – after the 30th WBC		
	M	Q25	Q75	M	Q25	Q75
Protein [g/L]	63.14	60.2	65.69	64.51	62.16	68.82
Albumin [g/L]	46.96	45.68	48.19	48.32	46.67	49.92
Uric acid [mmol/L]	0.33	0.3	0.34	0.32	0.3	0.36
Glucose [mmol/L]	3.74	3.44	4.43	3.93	3.34	4.48
TCh [mmol/L]	3.81	3.16	4.43	3.88	3.36	4.39
HDL [mmol/L]	1.31	1.26	1.34	1.33	1.25	1.37
LDL [mmol/L]	2.27	1.67	2.72	2.24	1.91	2.76
TG [mmol/L]	0.7	0.42	0.82	0.64	0.53	0.8

When analysing the activity of antioxidant enzymes, a significant increase in SOD1 activity, by 3.02% ($p = 0.049$), coupled with a trend for increased GST activity, can be observed. In the case of glutathione reductase, enzyme activity decreased by 35.9% ($p = 0.000915$) after a series of 30 daily WBC treatments. No significant changes were observed in CAT, GPx and R-GSSG activity. The results of enzyme activity in response to successive weeks of whole-body cryostimulation are presented in Table 2.

Table 2. Antioxidant enzyme activities before and after WBC treatments

Antioxidant enzymes	T_0 – before the 1st WBC			T_1 – after the 30th WBC		
	M	Q25	Q75	M	Q25	Q75
SOD1 [U/g Hb]	988.16	901.6	1119.89	1019.02 *T_0	950.23	1026.73
CAT [U/g Hb]	163.73	131.25	206.14	153.27	115.63	162.15
GPx [U/g Hb]	17.51	7.56	27.45	15.96	9.78	28.19
R-GSSG [U/g Hb]	0.39	0.36	0.47	0.25 ***T_0	0.2	0.36
GST [U/g Hb]	0.38	0.26	0.71	0.51	0.36	0.6

* $p < 0.05$; *** $p < 0.001$.

Discussion

Oxidative stress (OS) is a hallmark of neurodegenerative disorders, including MS symptomatology and complications of its negative symptoms. OS is caused by prolonged altered metabolism, exposure to exogenous oxidising agents or compounds and is associated with an inflammatory response [23, 24]. Erythrocytes may contribute to the pathophysiological mechanisms of MS through impaired antioxidant capacity and altered haemorheology, leading to increased oxidative stress in the periphery and potential ischaemic tissue damage, respectively [25]. Oxidative stress in the peripheral circulation of MS patients may further impede erythrocyte deformability through erythrocyte membrane lipid peroxidation [26, 27].

The possibility of supporting the mechanisms for maintaining the pro-oxidant/antioxidant balance that limit the severity of OS in the body seems to be of particular importance in long-term chronic diseases such as MS. It seems that whole-body cryotherapy, which is an especially common form of therapy and is, at the same time, very well tolerated by patients, has such a potential. In our experiment, we paid special attention to the activity of key antioxidant enzymes, such as SOD, CAT, GPx, R-GSSG and GST, in the erythrocytes of MS patients exposed to 30 daily WBC treatments. Antioxidants, whether endogenously synthesised or exogenously administered, act as reducing agents that neutralise the oxidative compounds (ROS) before they can cause any damage to different biomolecules [28, 29]. Acute cold temperature provided on a regular basis over a period of several months represents an obvious stress that could lead to certain adaptive mechanisms. It is postulated that prolonged WBC reduces oxidative stress by increasing the activity of antioxidant enzymes, especially in immunoactive disorders, although study results are still not clear. Miller [10] observed in their study on the effect of WBC on the antioxidant status in MS patients that exposing the MS patients to 10 WBC treatments induced a significant increase in total antioxidant status (TAS), whereas SOD and CAT activity in the erythrocytes of MS patients was not changed [30]. In another experiment by these authors, an increase was observed in the activity of SOD and CAT in the erythrocytes of MS patients after exposure to WBC accompanied by melatonin supplementation [31]. It should be noted that each time this was a series of 10 treatments. In this study, with the series of treatments increased to 30, a significant increase was observed in SOD1 activity and a small upward trend in GST activity, with no significant changes for other analysed enzymes, although, interestingly, the enzyme presenting a downward trend in its activity after a series of WBC treatments turned out to be catalase. Different mechanisms have been proposed to explain how low antioxidant levels or high ROS levels might specifically mediate CNS damage in MS. Lower levels of antioxidants may promote increased activity of lipoxygenase, an enzyme which triggers the production of leukotrienes, thereby increasing the immuno-inflammatory processes in brain tissue [17]. Earlier studies on the effect of WBC on the pro-oxidant/antioxidant status and lipid metabolism have shown that an important factor that determines its effect is the number of treatments in a series [12, 32]. In the case of healthy subjects, the first changes in the lipid profile were observed after 20 daily treatments [33], whereas in the study being described here, no changes in the lipid profile of MS patients were observed.

Conclusions

Multiple sclerosis, an inflammatory demyelinating disease of unknown origin, is neither genetically fully explained, nor are all the risk factors affecting its pathogenesis known. One of the patholomechanisms in multiple sclerosis may be prolonged oxidative stress. The changes observed in the activity of antioxidant enzymes following the use of whole-body cryotherapy with prolonged exposure confirm the possibility of the modulating effect of this form of rehabilitation on the systemic antioxidant potential, which can be one of the elements improving the functional status of chronically ill patients.

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