



ORIGINAL INVESTIGATION

Effects of whole-body cryotherapy on a total antioxidative status and activities of antioxidative enzymes in blood of depressive multiple sclerosis patients

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Abstract

Objectives. Oxidative stress (OS) plays an important role in the pathogenesis of multiple sclerosis (MS). In MS patients depression is often observed. Cryotherapy might have an effect on OS. The aim of this study was to compare the effects of whole body cryotherapy (WBCT) on changes in total antioxidative status (TAS) of plasma and activities of antioxidative enzymes in erythrocytes from depressive and non depressive MS patients. **Methods.** Twenty-two MS patients with secondary progressive disease course (12 depressive and 10 non depressive) were treated with 10 exposures in a cryochamber. Before and after WBCT the plasma TAS and the activities of superoxide dismutase (SOD) and catalase (CAT) in the erythrocytes were measured. **Results.** The level of TAS in depressive MS group was significantly lower than in non depressive MS ($P < 0.0003$). WBCT increased the level of TAS in depressive ($P < 0.002$) more than in non depressive MS patients ($P < 0.01$). WBCT treatment of MS patients resulted in the significant increase of TAS level in plasma but had no effects on activities of SOD and CAT. **Conclusions.** Our results indicate that WBCT suppresses OS in MS patients, especially in depressive patients.

Key words: Depression, multiple sclerosis, antioxidative systems, cryotherapy, oxidative stress

Introduction

Multiple sclerosis (MS) is a chronic and inflammatory demyelinating disease of central nervous system (CNS) with axonal degeneration and astrogliosis. It affects women 2–3 times more often than man (Gilgun-Sherki et al. 2004). Depression is frequently observed in persons with MS (Sollom and Kneebone 2007). A higher incidence of depressive symptoms and major depressive disorder in patients with MS is well documented, and reported in both large community surveys and studies of persons with MS. Depressive symptoms are associated with reduced quality of their life. Accumulating data indicate that oxidative stress (OS) plays a major role in the pathogenesis of multiple sclerosis. Reactive oxygen and nitrogen species (ROS/RNS), leading to OS, generated in excess primarily by macrophages, have been

implicated as mediators of demyelization and axonal damage in MS. ROS cause damage to main cellular structures and components such as lipids, proteins and nucleic acids (e.g., RNA, DNA) and might be responsible for the neuronal dysfunction, and result in cell death by necrosis or apoptosis contributing to the pathogenesis of this disorder. In addition, weakened cellular antioxidant defense systems in the CNS in MS, and its vulnerability to ROS effects may augment damage (Gilgun-Sherki et al. 2004). Thus, treatment with antioxidants might theoretically prevent propagation of tissue damage and improve both survival and neurological outcome. Whole body cryotherapy (WBCT) has been found useful in neurological diseases including MS (Miller et al. 2010) and depressive and anxiety disorders (Rymaszewska et al. 2008). Treatment with the total immersion of the

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body at extremely low temperatures was first introduced in Japan towards the end of the 1970s by Thosiro Yamauchi (1989) who constructed the first cryogenic chamber and successfully used cryotherapy to treat rheumatism. Rymaszewska et al. (2008) suggest a possible role for WBCT as a short-term adjuvant treatment for mood and anxiety disorders. In MS patients with neurological deficits after WBCT the increased muscle strength, decreased spastics and disability reduction in EDSS (Expanded Disability Status scale) were observed [in press]. Reduced demand for oxygen during hypothermia slows the rate of lipid peroxidation and protects ischemic cell membranes by stabilizing potassium efflux (Gordon 2001). Cryotherapy may partly inhibit oxidative stress and reactive species generation (Miller et al. 2010).

The aim of this study was to compare the effects of WBCT on changes in total antioxidative status (TAS) of plasma and activities of antioxidative enzymes superoxide dismutase (SOD) and catalase (CAT) in the erythrocytes of depressive MS patients and non depressive MS patients.

Materials and methods

Twenty-two MS patients with secondary progressive disease course (12 depressive and 10 non depressive) and 20 healthy controls (Hcs) participated in the study. Subjects with clinically definite MS according to McDonald criteria were included. Concurrent disease-modifying therapies or other medications were permitted as long as the dosage had been constant for at least 3 months before the evaluation. To determine MS depressive group ($n = 12$), the Beck Depression Inventory (BDI) [1], a 21-item self-report rating scale was used. Depression was defined as a score of ≤ 13 on the BDI. According to the BDI scale the standard cut-offs were as follows: 0–12 indicates that a person is not depressed. In the depressive group, six patients with mild-moderate depression (13–18 BDI) and six patients with moderate-severe depression (19–25 BDI) were studied. They received standard psychopharmacotherapy as prescribed by their psychiatrists: fluoxetine, four; sertraline, six; mianserine, two. This treatment was not modified during the evaluation period.

The MS group consisted of 15 females and seven males. Mean age was 42.2 (SD = 15.2) years, mean EDSS score was 4.5 (SD = 1.93), range 4.0–5.5, and mean disease duration was 9 (SD = 6.5) years.

Inclusion/exclusion criteria for this study were a diagnosis of MS and the ability to ambulate independently. Patients suffering due to circulatory or breathing insufficiency, clotting, embolism, inflammation of blood vessels, open wounds, ulcers, serious cognitive

disturbances, fever, addictions, claustrophobia, and over-sensitivity to cold were excluded from the study.

The MS subjects ($n = 22$) received no immunomodulators, immunostimulators, hormones, vitamins, minerals or any other substitutions with antioxidative properties. Prior to the study, all the subjects had undergone medical check-ups including neurological and internal examinations. All 20 healthy volunteers (mean age 28 years) were chosen to the study as a control to MS.

The protocol and procedures were done according to the Helsinki Declaration and were approved by Ethics Committee of the Medical University of Lodz, Poland. The study was performed at the Neurorehabilitation Division, III General Hospital Lodz, Department of Biochemistry Medical University of Lodz, Department of General Biochemistry and Department of Chemistry and Clinical Biochemistry, University of Bydgoszcz, Poland.

Experimental design

Whole body cryotherapy was applied to MS patients that were treated with a cycle of 10 exposures in a cryogenic chamber carried out daily from Monday to Friday. The cryogenic chamber has two rooms: the vestibule, with a temperature of -60°C , and the main chamber, with temperatures between -110°C and -160°C and with liquid nitrogen as the coolant. Sessions in the chamber lasted 2–3 min according to guidance of Gregorowicz and Zagrobelny (2007) on the appropriate duration of exposure and temperature for adult patients.

The study was carried out from March to November 2009. WBCT was applied to 22 MS patients: MS D (depression), 12 patients, and MS non-D (non depressive), 10 patients.

Biochemical studies were performed before and after WBCT therapy. Blood samples from MS patients and healthy controls were collected into cooled EDTA-containing tubes and were centrifuged to isolate plasma and erythrocytes. In both MS groups the samples of blood were taken 1 h before the first 10-day cycle of therapy and 1 h after the last immersion.

Biochemical investigations

TAS was measured in plasma samples (healthy group and MS patients) using the kit by Randox Laboratories Ltd. (Cat. No. NX 2332). The plasma volume taken to estimation was 5 μl , the total assay volume was 305 μl . The reaction was carried out for 3 min and the absorbance measured spectrophotometrically at 600 nm.

The activities of antioxidative enzymes SOD and CAT were determined in erythrocytes obtained from blood of MS patients and control subjects.

Superoxide dismutase activity in erythrocytes was measured according to the method of Misra and Fridovich (1972). The absorbance of the examined samples was estimated at 380 nm (using a Beckman spectrophotometer) at 37°C. The activity was expressed as U/gHb.

Catalase activity in erythrocytes was determined according to Beers and Sizer method (1952). Absorbance was measured at 240 nm using a Beckman spectrophotometer. Enzymatic activity was expressed as Berg Mayer units U/gHb.

Statistical analysis

Results were statistically elaborated and were compared with healthy subjects. Differences were considered significant when the significance was $P < 0.05$. The statistically significant differences were assessed by applying the paired Student's *t*-test.

Results

Our studies have shown that the activity of CAT was distinctly (2-fold) higher in erythrocytes of MS patients than in erythrocytes of healthy volunteers (Table I, Figure 1). The activity of CAT was lower in erythrocytes of depressive MS patients than in MS without depression ($P < 0.02$), whereas the activity of SOD was higher ($P < 0.05$) only in non depressive MS patients (Table I, Figure 2). We have observed that the level of TAS in depressive MS group was significantly lower than in non depressive MS ($P < 0.0003$). After WBCT the level of plasma TAS distinctly increased as well in MS depressive (from 0.21 to 0.65) ($P < 0.002$) as in non depressive MS groups (from 0.51 to 0.78; $P < 0.01$) (Table I, Figure 3). The activities of SOD and CAT in erythrocytes from MS patients after treatment with WBCT were not changed ($P > 0.05$).

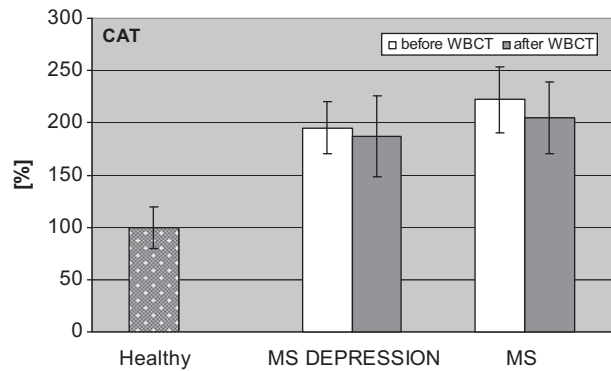


Figure 1. The effect of whole body cryotherapy (WBCT) on catalase (CAT) activity in erythrocytes from multiple sclerosis (MS) patients with and without depression. The CAT values for healthy controls are expressed as 100%. The changes of the CAT activity induced by WBCT are presented as % (means \pm SD) of control value.

Discussion

Numerous studies indicate disturbed equilibrium between oxidative processes and antioxidative defense in patients with depression (Cumurcu et al. 2009; Kodydkvo et al. 2009). Oxidative stress 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 oxidizing agents or compounds and it is associated with inflammatory response (Miller et al. 2010).

The antioxidant defence system in the organism comprises enzymatic and non enzymatic components. The enzymatic antioxidant system includes a variety of antioxidant enzymes such as SOD, CAT, glutathione peroxidase. The measurement of TAS in plasma represents the body redox status better than measurement of the single circulating antioxidant. Therefore, in our study to establish the effects of WBCT on oxidative stress we estimated the level of TAS in plasma (Beiske et al. 2008).

We observed that in depressive MS patients the level of TAS is distinctly reduced ($P < 0.0003$) in comparison with healthy subjects and MS patients without depression.

Table I. Effects of whole body cryotherapy (WBCT) on total antioxidative status (TAS) of plasma and activities of superoxide dismutase (CuZnSOD) and catalase (CAT) in the erythrocytes multiple sclerosis (MS) patients with depression and without depression.

	MS depression WBCT ($n = 12$)				MS non depression WBCT ($n = 10$)				Healthy ($n = 20$)	
	Before		After		Before		After		Mean	SD
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
TAS [mM]	0.26	0.3	0.79	0.3	0.57	0.3	0.78	0.35	0.65	0.4
CuZnSOD [U/gHb]	1351.7	310	1358.4	328	1640.4	450	1634.7	505	1412	511
CAT [10^4 IU/gHb]	17.7	2.7	17	2.7	20.15	3.1	18.6	3.2	9.05	1.8

MS, multiple sclerosis; WBCT, whole body cryotherapy.

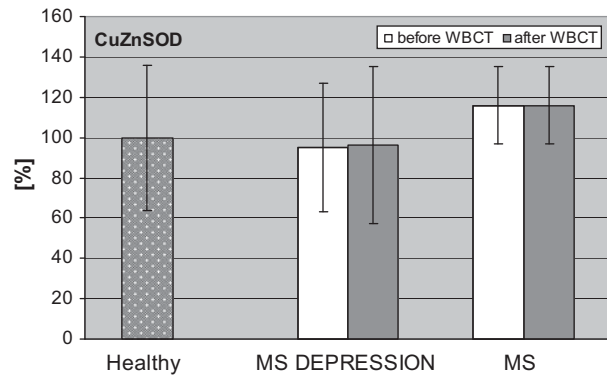


Figure 2. The effect of whole body cryotherapy (WBCT) on superoxide dismutase (CuZnSOD) activity in erythrocytes from multiple sclerosis (MS) patients with and without depression. The CuZnSOD values for healthy controls are expressed as 100%. The changes of the CuZnSOD activity induced by WBCT are presented as % (means \pm SD) of control value.

In our study we noticed that after WBCT treatment of depressive MS patients TAS level in plasma was markedly increased compared to untreated patients and reached the values of healthy controls. It is unclear exactly how WBCT might improve depression in non MS populations; however, several theories have been proposed to suggest a possible role for WBCT treatment for mood and anxiety disorders including: regulation of the hypothalamic-pituitary axis, increased β -endorphin levels, normalisation of hippocampal brain-derived neurotrophic factor, regulation of monoamines and improved perceptions of self efficacy. The hypothalamic-pituitary axis, brain-derived neurotrophic factor and serotonin have all been implicated in MS pathology (Rymaszewska et al. 2008). If WBCT like

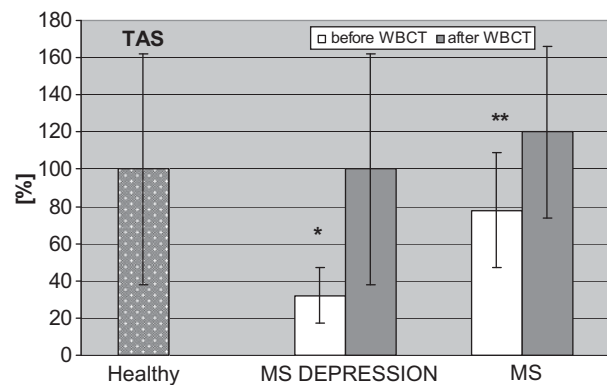


Figure 3. The effect of whole body cryotherapy (WBCT) on total antioxidative status (TAS) level in plasma from multiple sclerosis (MS) patients with and without depression. The TAS values for healthy controls are expressed as 100%. The changes of the TAS level induced by WBCT are presented as % (means \pm SD) of control value; * $P < 0.002$ after WBCT versus before WBCT in MS depressive patients; ** $P < 0.01$ after WBCT versus before WBCT in non MS depressive patients.

exercise influences hypothalamic-pituitary axis function, brain-derived neurotrophic factor concentration or serotonin concentration in persons with MS, this provides a possible explanation for the decreased incidence of depression observed in persons with MS who regularly participate in physical activity. Alternatively, depression aetiology in MS may have a psychological rather than neurobiological explanation. Due to the relatively high incidence of depression in MS, both the aetiology and the influence of WBCT on depression are areas that warrant further investigation (Stroud and Minahan 2009). This study aimed to investigate whether WBCT could be an effective aid to psychopharmaceutical treatment of MS patients. The results suggest that cryotherapy may play an important role by suppressing oxidative stress and ROS production, especially in MS patients with depression. Our data demonstrate that plasma TAS level was found to be significantly lower in depressive MS patients than in the healthy group (Table I). These findings indicate that in MS patients an impaired antioxidant defence system takes place. Treatment of MS patients with WBCT induced a significant increase of TAS, whereas SOD and CAT activities in erythrocytes of MS patients were not changed. Our earlier study revealed that supplementation of melatonin as antioxidant during treatment of MS patients with WBCT increases the activities of SOD and CAT [in press]. Hypothermia has long been known as a potent putative neuroprotectant. It delays energy depletion, reduces intracellular acidosis and ischaemia, related to the accumulation of excitotoxic neurotransmitters, and attenuates the influx of intracellular calcium. Additionally, hypothermia inhibits generation of oxygen free radicals involved in the secondary damage, associated with reperfusion. It also suppresses mechanisms of blood-brain barrier degeneration and post-ischaemic remodelling (Yamauchi 1989; Liu and Yenari 2007; Gonsette 2008; Miller et al. 2010). Generation of reactive oxygen and nitrogen species may be partly inhibited by hypothermia. The strongest inhibitory effects on oxidative stress were observed when hypothermia together with supplementation of melatonin were used [in press]. Our present results demonstrate that WBCT significantly increased the level of TAS ($P < 0.002$) in MS patients (Table I, Figure 3). It seems that the lower level of TAS observed in plasma of MS patients is dependent on the low concentrations of endogenous antioxidants, mainly uric acid. Antioxidants, synthesized endogenously as well as exogenously administered are reducing agents and neutralize the oxidative compounds (ROS) before they can cause the damage to different biomolecules.

In humans, over half the antioxidant capacity of blood plasma comes from uric acid. It is known that the lower values of uric acid in plasma ($\sim 194 \mu\text{mol/l}$)

have been associated with MS, whereas serum uric acid in healthy subjects is higher and reaches ~290 $\mu\text{mol/l}$. MS patients in remission reach the level of uric acid about 230 $\mu\text{mol/l}$. Uric acid like ascorbic acid is a strong reducing agent and a potent antioxidant responsible for TAS level in plasma (Siems et al. 1994; Massa et al. 2009).

Our results indicate that WBCT distinctly increases TAS level in plasma of MS patients. The increase of TAS was extremely high after 10 days of WBCT exposure in depressive MS patients. Our observations showed that WBCT treatment of depressive MS patients resulted in a significant increase of TAS level in plasma but had no effects on activities of antioxidative enzymes SOD and CAT. This indicates that WBCT may suppress oxygen free radical generation.

The mechanisms of action of hypothermic protection are not entirely understood and require further studies.

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Statement of interest

None to declare.

References

- Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. 1961. An inventory for measuring depression. *Arch Gen Psychiatry* 4:561–571.
- Beers R, Sizer T. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 195:133–140.
- Beiske AG, Svensson E, Sandanger I, Czujko B, Pedersen ED, Aarseth JH. 2008. Depression and anxiety amongst multiple sclerosis patients. *Eur J Neurol* 15:239–245.
- Cumurcu BE, Ozycurt H, Etikan I, Demir S, Karlidag R. 2009. Total antioxidant capacity and total antioxidative status in patients with major depression: impact of antidepressant treatment. *Psychiatry Clin Neurosci* 63:639–645.
- Gilgun-Sherki Y, Melamed E, Offen D. 2004. The role of oxidative stress in the pathogenesis of multiple sclerosis. The need for the effective antioxidant therapy. *J Neurol* 251:261–268.
- Gonsette R. 2008. Oxidative stress and excitotoxicity: a therapeutic issue in multiple sclerosis? *Mult Scler* 14:22–34.
- Gordon ChJ. 2001. The therapeutic potential of regulated hypothermia. *Emerg Med J* 18:81–89.
- Gregorowicz H, Zagrobelny Z. 2007. Systemic cryotherapy. Indications and contraindications, its course, and physiological and clinical results. In: Podbielska H, Stręk W, Miller G, editors. *Whole body cryotherapy*. Acta Biomedical Engineering: Indygo Zahir Media, 4–16.
- Kodydkvo J, Vavvrova L, Zeman M, Irak R, Macasek I, Stankova B. 2009. Antioxidative enzymes and increased oxidative stress in depressive women *Clin Biochem* 42:1368–1374.
- Liu L, Yenari M. 2007. Therapeutic hypothermia: neuroprotective mechanisms. *Front Biosci* 12:816–825.
- Massa J, O'Reilly E, Munger KL, Delorenze GN, Ascherio A. 2009. Serum uric acid and risk of multiple sclerosis. *J Neurol* 256:1643–1648.
- Miller E, Mrowicka M, Malinowska Zolynski K, Kedziora J. 2010. Effects of whole body cryotherapy on total antioxidative status and activities of antioxidative enzymes in blood of patients with multiple sclerosis. *J Med Invest* 57:168–173.
- Mirsa HP, Fridovich J. 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay superoxide dismutase. *J Biol Chem* 247:3170–3175.
- Rymaszewska J, Ramsey D, Chladzinska-Kiejna S. 2008. Whole body cryotherapy as adjunct treatment of depressive and anxiety disorders. *Arch Immun Therap Exp* 56:63–68.
- Siems W, Van Kuuk F, Maas R, Brenke R. 1994. Uric acid and glutathione levels during short-term whole body cold exposure. *Free Radical Biol Med* 16:299–305.
- Sollom AC, Kneebone N. 2007. Treatment of depression in people who have multiple sclerosis. *Mult Scler* 13:632–635.
- Stroud NM, Minahan CL. 2009. The impact of regular physical activity on fatigue, depression and quality of life in persons with multiple sclerosis. *Health Qual Life Outcomes* 7:68.
- Yamauchi T. 1989. Whole body cryotherapy is method of extreme cold -175°C treatment initially used for rheumatoid arthritis. *Z Phys Med Baln Med Klin* 15:311.