

Effect of whole body cryotherapy on uric acid concentration in plasma of multiple sclerosis patients

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SUMMARY

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Many clinical studies show lower concentration of uric acid (UA) in plasma multiple sclerosis (MS) patients than in healthy controls. UA has been suggested as a marker of disease activity. Increasing UA concentration has been proposed as a therapy for the treatment of neurodegenerative diseases including MS because of the neuroprotective properties of UA. Using whole body cryotherapy (WBCT) becoming popular in Poland because of improving functional activity of MS patients, decreased of spasticity and analgesic properties of cryogenic temperatures.

Material and methods. The study estimate UA concentration in plasma of MS patients (n=32) and healthy controls (n=35) before and after 10 (3 minutes) exposures of WBCT.

Results. UA concentration in plasma of MS patients 4.0 ± 0.57 mg/dl is lower than in healthy controls (5.1 ± 0.3 mg/dl). After using 10 exposures of WBCT we observed increase of UA concentration after WBCT treatment higher in MS patients (5.6 ± 0.74 mg/dl) than in controls subjects (5.5 ± 0.48 mg/dl).

Conclusions. Results of our study indicate significant increase of UA concentration in plasma of MS patients ($p < 0.001$) and healthy controls ($p < 0.01$). WBCT might to be the therapy which affected on the increase of the concentration of UA in MS patients.

Key words: multiple sclerosis, uric acid, whole body cryotherapy

STRESZCZENIE

Wpływ krioterapii ogólnoustrojowej na stężenie kwasu moczowego w osoczu chorych na stwardnienie rozsiane

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Wiele badań klinicznych wskazuje na obniżone stężenie kwasu moczowego w osoczu chorych na stwardnienie rozsiane (SR) w stosunku do grupy osób zdrowych. Podwyższenie stężenia kwasu moczowego zostało zaproponowane jako terapia leczenia chorób neurodegeneracyjnych w tym stwardnienia rozsianego ze względu na jego właściwości neuroprotektynne i antyoksydacyjne. Kwas moczowy jest rozpatrywany jako marker aktywności stwardnienia rozsianego. Zastosowanie krioterapii ogólnoustrojowej staje się w Polsce coraz bardziej popularne ze względu na poprawę funkcjonalną chorego zmniejszenie spastyczności oraz działanie analgetyczne temperatur kriogenicznych.

Materiał i metody. W badaniu oznaczono stężenie UA w osoczu chorych na SR (n=32) oraz w grupie osób zdrowych (n=35) przed i po zastosowaniu serii 10 (3 minutowych) zabiegów krioterapii ogólnoustrojowej.

Wyniki. Stężenie UA w osoczu chorych na SR (4.0 ± 0.57 mg/dl) jest niższe niż w grupie kontrolnej (5.1 ± 0.3 mg/dl). Po zastosowaniu serii 10 zabiegów krioterapii ogólnoustrojowej uzyskano podwyższenie wartości UA w osoczu wyższe w grupie chorych na SR (5.6 ± 0.74 mg/dl) niż grupie kontrolnej (5.5 ± 0.48 mg/dl).

Wnioski. Wyniki badań wskazują na podwyższenie poziomu UA w osoczu chorych na stwardnienie rozsiane $p < 0.001$ w porównaniu do osób zdrowych $p < 0.01$. Wydaje się, że krioterapia ogólnoustrojowa może być terapią wspomagającą leczenie SR i innych schorzeń neurodegeneracyjnych z w których stres oksydacyjny pełni ważną rolę.

Słowa kluczowe: stwardnienie rozsiane; kwas moczowy; krioterapia ogólnoustrojowa

Uric acid (UA) 7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione is a natural antioxidant, accounting for up to 60% of the free radical scavenging activity in human blood [1]. Many clinical studies show lower concentration of UA in plasma multiple sclerosis (MS) patients [1-4]. It is not clear whether low plasma UA concentrations are a cause or a consequence of MS. There is a possibility that persons with low plasma UA levels are unable to prevent against free radical toxicity, leading to the development of inflammation and the destruction of central neuron system (CNS) cells. From the other hand there is an opportunity that the inflammation and oxidative stress which occurs in MS leads to the consumption of UA to scavenge the excess free radicals produced, resulting in a lower UA level [1]. UA has been proposed as a marker of disease activity [4]. Increasing UA concentration has been suggested as a therapy for the treatment of neurodegenerative diseases including MS because of the neuroprotective properties of UA [5-7]. Moreover, a recent clinical study found that the administration of inosine (UA precursor) retarded the progression of MS in all 11 patients that received the drug and improved some of the symptoms of the disease in three of the patients [7]. During immunomodulatory treatment concentration of plasma UA significantly increased [1]. Hypothermia is a potent putative neuroprotectant and may inhibit generation of free radicals and oxidative stress. Using whole body cryotherapy (WBCT) becoming popular in Poland because of improving functional activity of MS patients, decreased of spasticity and analgesic properties of cryogenic temperatures [8].

Oxidative stress can damage lipids, proteins, and nucleic acids causing cell death in various cell types of the CNS [10-12]. Reactive oxygen species (ROS) leading to oxidative stress generated in excess primarily by macrophages which have been implicated as mediators of demyelination and axonal damage in MS. ROS cause damage to main cellular components resulting in cell death by necrosis or apoptosis. Activated microglial and astrocytes can produce NO at steady state concentrations as high as 1 μ M (high flux NO). During CNS pathology NO released react with O₂⁻ and formed reactive nitrogen species (RNS), such as peroxynitrite (ONOO⁻) which cause damage a variety of macromolecules, including proteins. ONOO⁻ is formed in a reaction which is limited only by the diffusion rates of the molecules [9]. Damage of neurons is tightly connected with peroxynitrite which is pathogenic factor of MS. NO produced by glial cells within MS lesions also has been shown to directly reduce axonal conduction. ONOO⁻ can mediate a variety of destructive interactions including oxidation, lipid peroxidation, DNA strand breakage, and nitration of

aminoacids, mainly tyrosine residues in proteins [13]. UA is a natural scavenger of ONOO⁻ [9].

In MS patients a disturbances in antioxidant defense system may be dependent not only on the lower activity of antioxidant enzymes but mainly on the lower concentration of an important non-enzymatic antioxidants such as uric acid [14]. Our previous studies indicated statistically significant increase of total antioxidative status (TAS) level in plasma after WBCT treatment in MS patients [14-16]. The authors emphasize the possible role of WBCT on UA concentration in plasma of MS patients. For that, in progressive phase of MS the antioxidative therapy such as WBCT should be taken into account.

Therefore, the aim of our study was to determine the effects of ten session of WBCT on UA concentration in plasma of MS patients and control groups.

MATERIAL AND METHODS

Subjects with clinically definite MS according to McDonald criteria were included to the study and patients were observed for one year before. MS patients (n=32) and healthy controls (n=35) participated in the study.

The MS sample consisted of 21 females and 11 males. Mean age was 47.6 \pm 13.5 years, mean weight BMI 20.1 \pm 9.7, mean enhanced disability status scale (EDSS) score was 4.3 \pm 1.8 and mean disease duration was 11.5 \pm 9.5 years (Tab. 1).

The patients were under Neurorehabilitation Ward control for 5 weeks and in that time they 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 internist examination. 35 healthy age, sex, diet matched volunteers as a control to MS were chosen.

Inclusion/exclusion criteria for this study were a diagnosis of SPMS 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 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 in Neurological Rehabilitation Division III General Hospital in Lodz, Poland and Department of Biochemistry Collegium Medicum in Bydgoszcz University of Toruń, Poland.

Experimental design. An experimental trial with WBCT took place in out-patient clinic "ATOS" in Lodz. One cycle of WBCT consisted from 10 exposures in a cryogenic chamber carried out daily from Monday to Friday with two day weekend break. The used cryogenic chamber had two rooms: the vestibule, with the temperature of -60°C , and the main chamber, with temperature between -110°C and -160°C . Liquid nitrogen was used as the coolant. Sessions in the chamber lasted 3 min. WBCT was applied every day between 10.30 and 11.30 am. The participants were exposed to ten sessions of extremely low temperature (-130°C) in a cryogenic chamber for the first time in their life. Each session lasted 3 min according to guidance of *Gregorowicz and Zagrobelny* [17] on the appropriated duration of exposure and temperature for adult patients and a list of medical conditions in which WBCT is unsuitable. Just before each session of WBCT systolic and diastolic blood pressure was measured. No illnesses occurred during the study period. The study was carried out at April 2010. Estimations of UA were made in 2 stages: before and after WBCT in examined group and healthy volunteers.

Blood samples were collected into cooled EDTA and centrifuged to isolate plasma and erythrocytes. In both groups the samples of blood were taken one hour before the first 10 days cycle of cryotherapy and one hour after the last immersion.

Estimation of uric acid. Blood samples were analyzed at the SYNEVO Laboratory at III General Hospital in Lodz. The concentration of uric acid was determined using a colorimetric enzyme assay on the Hitachi 917 analyzer. Results are expressed as mg/dl of uric acid. References range is 2.5 -7.0 mg/dl.

Statistical analysis. The data were analyzed using the STATISTICA 7.1 (StatSoft, Tulsa, OK) statistical package. Results were statistically elaborated. Due to nonparametric distribution the Wilcoxon test was used to analyze changes. Results were compare with healthy subjects. Results of UA measured in mg/dl were considered statistically significant ($p<0,01$). The results in both groups were uniform.

RESULTS

In our study we evaluated the effect of WBCT on UA concentration in plasma of MS patients and healthy subjects. WBCT has an effect on the main antioxidant in human blood – uric acid which was distinctly higher after WBCT in MS patients and healthy subjects ($p<0.001$; $p<0.01$ respectively, Tab. 2). Increasing UA level has been proposed as a therapy for the treatment of neurodegenerative diseases including MS because of the neuroprotective properties of UA

[9,7]. UA level in plasma of MS patients was distinctly lower than in healthy volunteers (4.0 ± 0.57 mg/dl, $5,1\pm 0.3$ mg/dl, respectively $p<0.01$, Tab. 2).

DISCUSSION

CNS is particularly susceptible to ROS-induced damage due to the high oxygen demands of the brain and low concentration of antioxidants [9,11-14]. It contains antioxidants enzymes, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and nonenzymatic such as antioxidants glutathione, vitamins A,C,D, coenzyme Q and uric acid etc. Enzymatic and non enzymatic antioxidants like vitamins, micro and macro elements can regulate progress and function different immunological cells [9]. Extremely fast production in CNS of several ROS including: $\text{O}_2^{\cdot-}$, HO_2^{\cdot} , H_2O_2 , $\cdot\text{OH}$ and NO^{\cdot} (mainly produced by macrophages structures responsible for demyelination and axons disruption) takes place [11]. In our previous studies we observed reduced total antioxidative status (TAS) in plasma and SOD activity in erythrocytes of MS patients [14-16]. In MS deregulation of free radical metabolism and antioxidant capacity are altered [13] and low concentration of main antioxidant such as uric acid have been studied. *Guerrero et al.* [18] observed that lower uric acid levels in MS patients are connected with clinical relapse of MS and correlated with disability of MS patients assessed by EDSS (expanded disability status scale) score. Lower plasma UA level in MS may represent constitutive loss of protection against nitric oxide and peroxynitrite [19]. UA may assist in the removal of superoxide by preventing against the degradation of superoxide dismutase (SOD), the enzyme that is responsible for clearing superoxide from the cell [5].

Alternatively, increasing UA levels has been proposed as a therapy for the treatment of neurodegenerative diseases, such as MS [1, 5, 6]. UA has been found to both prevent and alleviate the symptoms of experimental allergic encephalomyelitis (EAE), the animal model of MS, in mice [6, 7].

There is a need for developing new therapies such as WBCT especially in progressive phase of MS that are more process-specific and can be used in specific patient subpopulations [19]. Generation of ROS might be partly inhibited by hypothermia that is known as a potent putative neuroprotectant and may decreased as well the generation of free radicals as oxidative stress [20]. Treatment with WBCT may improve both survival and neurological outcome in MS patients [8].

WBCT is becoming popular in treatment neurological disorders [8, 14, 15]. However, changes that

occur in the human body under cryogenic temperatures conditions are still not entirely understood. Hypothermia affects thermoregulatory system which attempts to maintain a constant core temperature (around 37°C) by means of skin vasoconstriction and by increasing the metabolic rate through shivering [21, 22]. During a cold exposure skin temperature decreases rapidly due to vasoconstriction and direct skin cooling, most profoundly in the unprotected extremities. Directly after WBCT, all skin temperatures increased rapidly within 15 minutes. Exposure to low temperatures leads to an intensified heat production in order to maintain the balance between heat and its loss. Cold-induced thermoregulation is associated with an increase in lipid metabolism [21]. The human body uses energy derived mainly from the conversions of carbohydrates and lipids [22].

There are not data concerning the treatment of MS patients with WBCT (-110°C – -160°C), however cooling (7°C – 26°C) especially heat sensitive MS patients as a factor improved functional activity of MS patients was applied [23, 24, 25].

WBCT distinctly elevates the level of uric acid more in plasma of MS patients ($p < 0,001$) than in healthy subjects ($p < 0,01$). Increasing UA level has been proposed as a therapy in MS [5, 6]. Our previous studies indicated statistically significant increase of TAS level in plasma after WBCT treatment in MS patients [14-16].

CONCLUSIONS

It seems that WBCT may be used as adjuvant therapy in MS and other diseases with oxidative stress background. WBCT might to be the therapy which effected increased concentration of UA in MS patients.

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