

Influence of Cryogenic Temperatures on Inflammatory Markers in Patients with Ankylosing Spondylitis

Agata Stanek^{1*}, Grzegorz Cieślak¹, Joanna Strzelczyk², Sławomir Kasperczyk³, Karolina Sieroń-Stoltny¹, Andrzej Wiczkowski², Ewa Birkner³, Aleksander Sieroń¹

¹Department and Clinic of Internal Diseases, Angiology and Physical Medicine, Medical University of Silesia, Batorego 15, 41-902 Bytom, Poland

²Department of Biology, Medical University of Silesia, Zabrze, Poland

³Department of Biochemistry, Medical University of Silesia, Zabrze, Poland

Received: 28 April 2009

Accepted: 11 August 2009

Abstract

The aim of this study was to estimate the influence of cryogenic temperatures used for whole-body cryotherapy on inflammatory markers in patients with ankylosing spondylitis (AS) and healthy volunteers. The study involved 32 male persons: 16 patients with AS and 16 healthy volunteers. All subjects were exposed to a cycle of 10 daily procedures of whole-body cryotherapy at a temperature of -120°C lasting 2 minutes with subsequent kinesitherapy. In both groups, before and after a cycle of whole-body cryotherapy with subsequent kinesitherapy, serum C-reactive protein, fibrinogen, mucoprotein, soluble intercellular adhesion molecule-1 levels and erythrocyte sedimentation rate were estimated. The results of this study indicate that cryogenic temperatures used for whole-body cryotherapy decrease the levels of inflammatory markers both in patients with ankylosing spondylitis and healthy volunteers.

Keywords: cryogenic temperatures, whole-body cryotherapy, inflammatory markers, C-reactive protein, mucoprotein, soluble intercellular adhesion molecule-1

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease of the sacroiliac joints and the spine. The etiology of AS is unknown. It is still not clear which precise mechanisms determine the interactions between host factors (HLA-B27 and other genes, cytokines, T lymphocytes) and microbial factors leading to the manifestation and chronicity of AS. Antigen HLA-B27 is found in only 6% of the general population, but it occurs in approximately 93% of individuals suffering from AS. AS is almost three times

more common in men than in women. It typically affects young people, with onset usually between 15 and 30 year of life. Chronic spinal inflammation can develop a complete fusion of the vertebrae, a process called ankylosis. Ankylosis causes total loss of mobility of the spine. AS is also a systemic rheumatic disease. It can also produce an inflammatory process in peripheral joints of limbs, as well as in several organs such as eyes, heart, lungs and kidneys. The main symptoms of the disease are pain and stiffness in the low back, upper buttock area, neck, and the remaining regions of the spine. The onset of pain and stiffness is usually gradual and these symptoms progressively worsen over months [1-7].

*e-mail: astanek@tlen.pl

As pharmacotherapy is not sufficient to improve the clinical state of patients with AS, physiotherapy seems to be one of the cornerstones of successful long-term management of AS. It is applied for three major reasons [8-14]:

- to maintain or restore spinal mobility,
- to maintain or improve posture,
- to improve chest expansion.

One of the most efficient methods of physical medicine used in the treatment of many diseases of the motional system is cryotherapy using extremely low temperatures (below -100°C) applied for a short time (2-3 minutes) to stimulate physiological reactions of the human organism, in order to make more effective pharmacological treatment and kinesitherapy (Fig. 1) [15-20].

The action of cryogenic temperatures causes several favorable, physiological reactions in human organisms, such as: analgesic effect, neuro-muscular effect, anti-inflammatory and antiedematous effects, and circulatory effect. The actual indications for cryotherapy include: autoimmune diseases of joints and periarticular tissues (ankylosing spondylitis, rheumatoid arthritis, psoriatic arthritis, myositis and fibromyositis), degenerative, post-traumatic and overloading lesions of the motional system, fibromyalgia, osteoporosis, gout, diseases of central nervous system with muscular hypertension, disseminated sclerosis, radicular syndromes, diseases of peripheral nervous system, depressive syndromes and vegetative neurosis, vital restitution, assistance of endurance and force training, acceleration of post-exertion restitution in active sportsmen [15-25] and more.

As the mechanism of positive therapeutic effects of cryotherapy are still not completely known, the aim of the study was to examine the influence of cryogenic temperatures used for whole body cryotherapy on markers of inflammatory status in patients suffering from AS and healthy volunteers.

Experimental Procedures

Patients

The research protocol has been reviewed and approved by the Bioethical Committee of the Medical University of Silesia in Katowice (permission No. NN-013-144/1/02) and all analyzed subjects gave their informed, written consent for inclusion in the trial.

The study involved 32 male persons: 16 patients with ankylosing spondylitis (experimental group, mean age 47 ± 4.7 years) and 16 healthy volunteers (control group, mean age 43 ± 3.9 years) with no significant difference in mean age between them.

Criteria of Inclusion

All patients included in the trial fulfilled the modified New York Criteria for definite diagnosis of AS, which serves as the basis for the ASAS/EULAR recommendations [26].

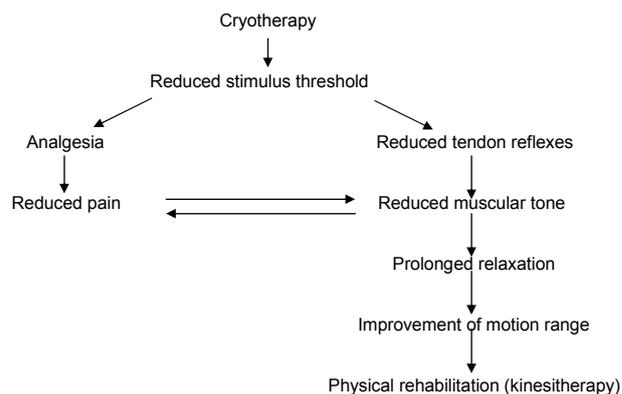


Fig. 1. The therapeutic mechanisms of cryotherapy according to Knight [17].

According to modified New York Criteria (1984), the radiographic presence of sacroiliitis is a necessary condition for diagnosis of AS.

Using routine plain x-ray examination, the severity of sacroiliac joint disease is divided into 4 grades [27]:

- grade I – non-specific blurring of joint margin;
- grade II – loss of definition of the sacroiliac joint margins, partial bone bridging or pseudowidening, initial joint space widening, followed by joint space narrowing and the indistinctness of the sacroiliac joint;
- grade III – numerous severe erosions, widening of joint space, partial ankylosing and definite sclerosis on both sides of the joint;
- grade IV – ultimate fusion of the sacroiliac joint (complete ankylosing).

The presence of sacroiliitis of a grade \geq II bilaterally or grade III or IV unilaterally fulfills radiologic criterion for the diagnosis of AS.

Aside from sacroiliitis, the patient must exhibit 1 of the following 3 clinical criteria for a diagnosis of AS to be established:

- low-back pain and stiffness present for more than 3 months with palliation by exercise and no relief with rest,
- limitation of lumbar spinal motion in sagittal and frontal planes,
- limitation of chest expansion relative to age- and sex-matched individuals.

Definite ankylosing spondylitis is present if the radiologic criterion is present in addition to at least 1 clinical criterion. Probable ankylosing spondylitis is present if three clinical criteria are present alone or if the radiologic criterion is present but no clinical criteria are present [26].

All patients with AS were HLAB27 positive and they exhibited III and IV radiographic grade of sacroiliac joint disease. They did not suffer from any other diseases and had no associated pathologies.

They were exposed to whole-body cryotherapy procedures during sanatorium treatment at a health resort in Goczałkowice Zdrój.

They had not taken non-steroidal and anti-inflammatory drugs in the month before the cryotherapy treatment.

Healthy volunteers were qualified to a complex treatment (called cryorehabilitation) including cryotherapy (treated as an assisting component) and subsequent kinesitherapy in order to obtain biological renovation, resulting in: leveling of the physical and psychological fatigues, quickening of post-exertion regeneration, intensification of muscle contraction force, plus improvement of physical condition, proper motor function and general feeling.

They were exposed to whole-body cryotherapy procedures at the same health resort in ambulatory conditions.

Criteria of Exclusion

All patients included in the trial had no commonly accepted contraindications for cryotherapy such as: intolerance of cold, cryoglobulinemia, Raynaud's disease, hypothyroidism (increasing risk of hypothermia), acute diseases of respiratory tract, neoplastic disease (due to adaptive intensification of local blood supply), instable angina pectoris, severe valvular heart defects (in the insufficiency of circulation stage), cardiac failure, severe arrhythmias, purulent-gangrenous skin lesions, vegetative neuropathies (due to predisposition to hyperhidrosis), local blood flow disturbances, cachexia and hypothermia, as well as claustrophobia and mental diseases (due to inability to comply with safety rules in a cryogenic chamber) [15-20, 25, 28, 29].

Cryotherapy Procedure

All patients of both groups were exposed for 10 consecutive days to a cycle of whole-body cryotherapy procedures lasting 2 minutes a day, with subsequent 60-minute routine kinesitherapy.

Whole-body cryotherapy procedures were performed in the Cryoflex chamber, which consists of two compartments: the antechamber and the proper chamber. The temperature in the antechamber was -60°C , whereas in the proper chamber it reached -120°C . After the adaptation process in the antechamber lasting 30s, subjects were exposed to cryogenic temperatures for 2 minutes in the proper chamber. During the procedure of whole-body cryotherapy all subjects were dressed in swim suit, cotton socks and gloves, wooden shoes and face and ear guards. The subjects were walking around the chamber during the whole cryotherapy procedure without touching each other. The program of kinesitherapy was arranged individually for each subject. No complications or side effects of cryotherapy exposure were observed.

Blood Sample Acquisition

On the first and last day of a cycle of whole-body cryotherapy with subsequent kinesitherapy in subjects from both groups, the following markers of inflammatory process were estimated: serum C-reactive protein (CRP), mucoprotein (the complex of α_1 acute phase proteins, consisting mainly of α_1 -acid glycoprotein, $_{3-5}\text{S}\alpha_1$ -glycoprotein, haptoglobin, and several others, which is soluble in per-

chloric acid solution and precipitable by phosphotungstic acid) and soluble intercellular adhesion molecule-1 (sICAM-1) levels, plasma fibrinogen concentration as well as erythrocyte sedimentation rate (ESR).

Samples of whole blood (volume 5 ml) were drawn from a basilic vein of each subject. A part of collected blood samples was placed directly into a tube with citrate in order to determine erythrocyte sedimentation rate. The remaining blood samples were decanted and centrifuged, and then separated heparinized plasma and serum, respectively, were immediately stored at -70°C until biochemical analyses were made. Analyses were performed at 25°C .

Laboratory Analyses

Serum CRP concentration was determined by means of the turbidimetric method using the photometric-turbidimetric test for the quantitative determination of human C-reactive protein (CRP) in serum and plasma (Human Gesellschaft für Biochemica and Diagnostica mbH, Germany) ($\lambda = 340 \text{ nm}$) [30]. For this method the linearity is kept up to 25 mg/dl and the lower detection limit is 0.1 mg/dl. No prozone phenomenon was observed up to 40 mg/dl. Reference values for adults are up to 0.5 mg/dl.

Serum mucoprotein concentration was determined by means of colorimetric method described by Winzler in modification of De La Huerga et al. [31] using a kit produced by Aqua-Medica (Poland) and spectrophotometer Specol ($\lambda = 680 \text{ nm}$). For this method the reference values for males range between 0.45 and 1.17 g/l.

Plasma fibrinogen concentration was determined by means of the modified Clauss method [32] using a Multifibren U Kit (Dade Behring Inc., USA) and multiparametric analyzer Kone Lap. For this method the measurement range lies between 0.8 and 12 g/l. The precision of the method was calculated with Control Plasma N and Control Plasma P for 5 days in 8-fold determination. The coefficient of variation in the series was 2.9% and 7.2% for Control Plasma N and control Plasma P, respectively. From day to day it was 1.6% and 3.4%, respectively. Normal reference values range between 1.8 and 4.5 g/l.

Serum sICAM-1 concentration was determined by means of an enzyme-linked immunosorbent assay using a human sICAM-1 ELISA BMS201CE kit (Bender MedSystems GmbH, Austria) and a PowerVave XS absorption analyzer (Biotek, USA) ($\lambda = 450 \text{ nm}$). For this method the limit of detection for sICAM-1, defined as the analyte concentration resulting in an absorption significantly higher than the absorption of the dilution medium (mean plus three standard deviations) was determined to be 3.3 ng/ml. The overall intra-assay coefficient of variation has been calculated to be 4.1%, whereas overall inter-assay coefficient of variation has been calculated to be 7.66%. According to manufacturer data the reference levels for healthy blood donors ranged between 130 and 300 ng/ml. Normal levels may vary, depending on the collective serum used, ranging up to 400 ng/ml.

Erythrocyte sedimentation rate (ESR) was measured by means of standard method.

Table 1. Comparison of particular inflammatory marker values before and after the end of a cycle of whole-body cryotherapy procedures with subsequent kinesitherapy in groups of patients with ankylosing spondylitis and healthy volunteers, with statistical evaluation.

Parameter	Group	Before cryotherapy procedures with subsequent kinesitherapy (mean value \pm SD)	After cryotherapy procedures with subsequent kinesitherapy (mean value \pm SD)	Statistical significance
Serum CRP concentration [mg/dl]	Ankylosing spondylitis	6.37 \pm 8.09	2.17 \pm 4.31	p=0.002
	Healthy volunteers	0.00 \pm 0.00	0.00 \pm 0.00	p=0.00
Serum mucoprotein concentration [g/l]	Ankylosing spondylitis	1.43 \pm 0.24	1.02 \pm 0.25	p<0.001
	Healthy volunteers	1.06 \pm 0.07	0.92 \pm 0.14	p=0.002
Plasma fibrinogen concentration [g/l]	Ankylosing spondylitis	3.55 \pm 0.66	2.98 \pm 0.72	p<0.001
	Healthy volunteers	1.97 \pm 0.58	1.71 \pm 0.45	p=0.02
Serum sICAM-1 concentration [ng/ml]	Ankylosing spondylitis	331.0 \pm 103.0	262.0 \pm 76.9	p=0.001
	Healthy volunteers	290.0 \pm 86.0	286.0 \pm 81.2	p=0.836
Erythrocyte sedimentation rate [mm/h]	Ankylosing spondylitis	19.19 \pm 14.72	12.06 \pm 11.06	p=0.001
	Healthy volunteers	1.50 \pm 0.63	1.13 \pm 0.50	p=0.028

Statistical Data Analysis

The database was established in the software MS Excel 2000. Statistical analysis was undertaken using the Statistica 6.0 PL statistical package. For each parameter the indicators of descriptive statistics were determined (mean value and standard deviation SD).

The normality of the data distribution was checked using the Shapiro-Wilk test, while the homogeneity of the variance applying Levene test. In order to compare the differences between control group and the AS group, an independent sample Student *t* test was used or, alternatively, the Mann-Whitney U test. Correlations between particular parameters were statistically verified by means of Spearman's non-parametric correlation test.

Differences at the significance level of $p < 0.05$ were considered statistically significant.

Results

The obtained results are shown in Table 1 and Figs. 2-6.

In healthy volunteers initial values of C-reactive protein, mucoprotein, fibrinogen and s-ICAM-1 concentration, as well as erythrocyte sedimentation rate value, were contained within a range of reference values, though in the case of s-ICAM-1 they were close to the upper limit of the range.

In patients suffering from ankylosing spondylitis, initial values of C-reactive protein, mucoprotein, fibrinogen and s-ICAM-1 concentrations, as well as erythrocyte sedimentation rate value, were significantly higher ($p < 0.001$) as compared to the control group of healthy volunteers.

After a cycle of cryotherapy procedures in patients with ankylosing spondylitis, a statistically significant decrease in

C-reactive protein concentration (6.37 \pm 8.09 and 2.17 \pm 4.31 mg/dl – before and after therapy, respectively, $p = 0.002$) (Table 1, Fig. 2), mucoprotein concentration (1.43 \pm 0.24 g/l and 1.02 \pm 0.25 g/l – before and after therapy, respectively, $p < 0.001$) (Table 1, Fig. 3), fibrinogen concentration (3.55 \pm 0.66 g/l and 2.98 \pm 0.72 – before and after therapy respectively, $p < 0.001$) (Table 1, Fig. 4), sICAM concentration (331 \pm 103 g/l and 262 \pm 76.9 – before and after therapy, respectively, $p < 0.001$) (Table 1, Fig. 5), as well as in erythrocyte sedimentation rate (19.19 \pm 14.72 and 12.06 \pm 11.06 mm/h – before and after therapy, respectively, $p = 0.001$) (Table 1, Fig. 6) were obtained. Cryotherapy significantly decreased mucoprotein concentrations (1.06 \pm 0.007 and 0.92 \pm 0.14 g/l – before and after therapy, respectively, $p = 0.002$) (Table 1, Fig. 3), fibrinogen concentration

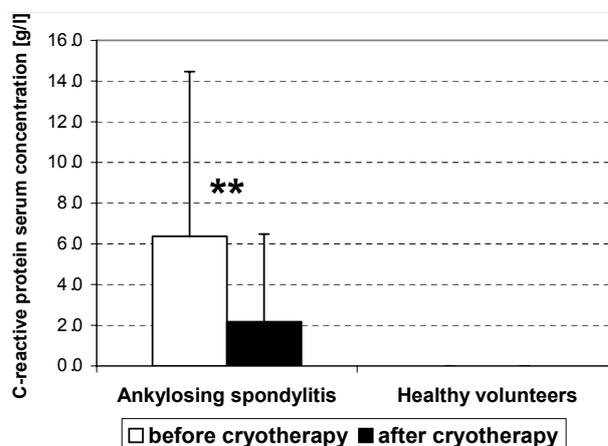


Fig. 2. Comparison of C-reactive protein (CRP) serum concentration before and after a cycle of whole-body cryotherapy procedures with subsequent kinesitherapy in ankylosing spondylitis group and control group of healthy volunteers.

* $p < 0.05$, ** $p < 0.01$

(1.97 ± 0.58 and 1.71 ± 0.45 g/l – before and after therapy, respectively, $p=0.02$) (Table 1, Fig. 4) and erythrocyte sedimentation rate (1.5 ± 0.63 and 1.13 ± 0.5 mm/h – before and after therapy, respectively, $p=0.028$) (Table 1, Fig. 6) also in the control group of healthy volunteers.

Discussion of Results

In the present study, before beginning the whole-body cryotherapy cycle an increased CRP, mucoproteins, fibrinogen and sICAM-1 concentrations, as well as elevated ESR, were observed in patients with ankylosing spondylitis as compared to the control group. As a result of a cycle of cryotherapy procedures, a statistically significant decrease in C-reactive protein, mucoproteins, fibrinogen and sICAM-1 concentrations, as well as ESR, were found, which is indicative for anti-inflammatory effect.

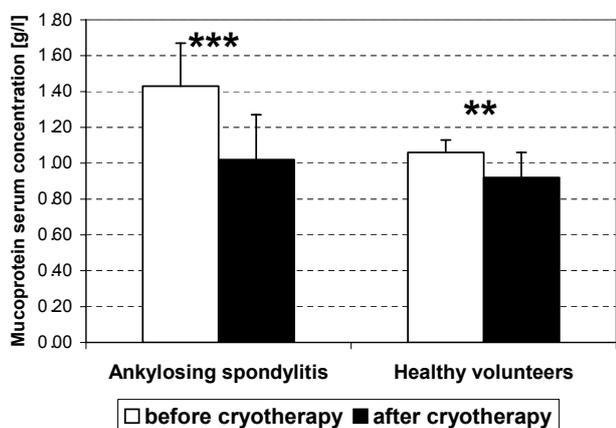


Fig. 3. Comparison of mucoprotein serum concentration before and after a cycle of whole-body cryotherapy procedures with subsequent kinesitherapy in ankylosing spondylitis group and control group of healthy volunteers.
* $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$

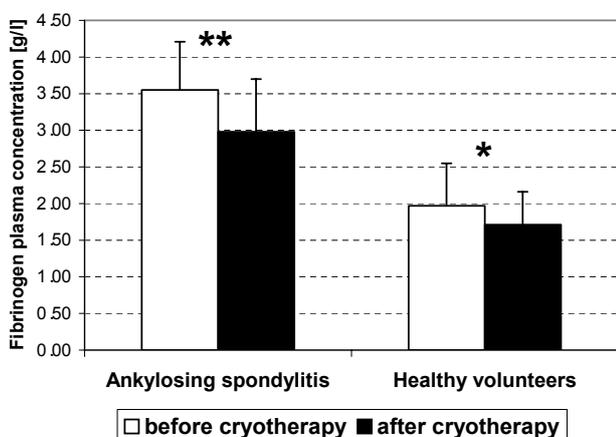


Fig. 4. Comparison of fibrinogen plasma concentration before and after a cycle of whole-body cryotherapy procedures with subsequent kinesitherapy in ankylosing spondylitis group and control group of healthy volunteers.
* $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$

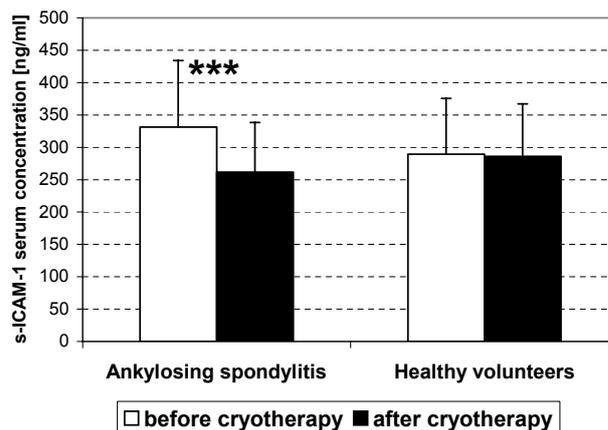


Fig. 5. Comparison of soluble intercellular adhesion molecule-1 (sICAM-1) serum concentration before and after a cycle of whole-body cryotherapy procedures with subsequent kinesitherapy in ankylosing spondylitis group and control group of healthy volunteers.

* $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$

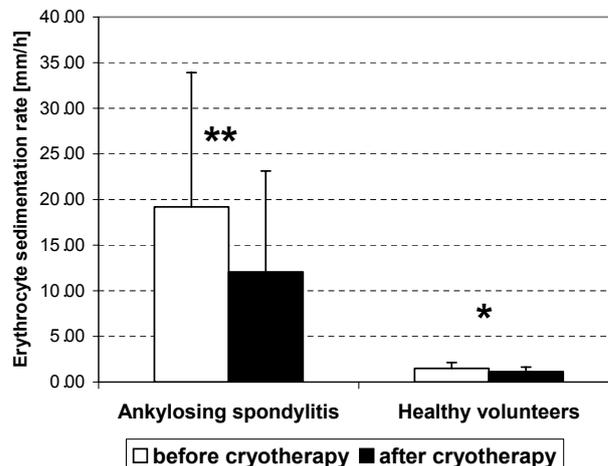


Fig. 6. Comparison of erythrocytes sedimentation rate in blood before and after a cycle of whole-body cryotherapy procedures with subsequent kinesitherapy in ankylosing spondylitis group and control group of healthy volunteers.

* $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$.

Cryotherapy significantly decreased mucoproteins and fibrinogen concentrations, as well as ESR, also in the control group of healthy volunteers.

The inflammatory response of tissues is associated with vasodilatation, increased vascular permeability, recruitment of immunologic cells, and the release of inflammatory mediators and cytokines from these cells. The macrophage-derived cytokines IL-1 and IL-6 are primarily responsible for the acute phase response, related to a protective change in plasma protein production by hepatocytes. The main important acute phase proteins are C-reactive protein (CRP), fibrinogen and mucoproteins. The erythrocyte sedimentation rate (ESR) is an index of the acute phase response, mainly reflecting the concentrations of fibrinogen and the α -globulins, but also other immunoglobulins that are not acute

phase reactants. The acute phase markers most frequently used as indicators of inflammatory activity in patients with ankylosing spondylitis are ESR and CRP [33-35].

Soluble intercellular adhesion molecule-1 (sICAM-1) is a member of the immunoglobulin supergene family, expressed on the surface of several cell types, including leukocytes and endothelial cells [36, 37]. ICAM-1 is induced on fibroblasts and endothelial cells by inflammatory mediators such as IL-1, TNF-alpha and IFN-gamma. It plays an important role in the migration of leukocytes to the inflammation sites [38]. Elevated levels of sICAM-1 are found, i.e. both in serum [39, 40] and synovial tissue [41] in patients with rheumatoid arthritis, and they correlate with the disease activity [39]. In patients with rheumatoid arthritis, a weak positive correlation between serum sICAM-1 levels and serum CRP levels was confirmed [42]. The increased serum sICAM-1 levels were also observed in patients with spondyloarthropathies and in these patients positive correlations were found between sICAM-1 and erythrocyte sedimentation rate, as well as serum C-reactive protein and interleukin 6 levels, but not with serum TNF-alpha level. These results suggest that sICAM-1 levels may reflect the acute phase of inflammation [43].

The results obtained in the present study show that whole-body cryotherapy evokes an anti-inflammatory effect in objects exposed to its action.

Whole-body cryotherapy is a relatively new therapeutic method of physical medicine with a history of about 20 years. Thus, research works on mechanisms of therapeutic action of cryogenic temperatures are still carried on.

In available literature there are very few reports on the influence of whole-body cryotherapy on inflammatory markers in patients with inflammatory diseases of the motional system. So far the beneficial influence of whole-body cryotherapy on these markers was confirmed only in our preliminary study [23], in which a 2-week cycle of whole-body cryotherapy procedures caused a significant decrease in serum C-reactive protein and seromucoid concentration.

On the other hand, Banfi et al. [44] confirmed a slight, but not significant, decrease in serum C-reactive protein concentration, a significant decrease in serum sICAM-1 and pro-inflammatory cytokines IL-2 and IL-8 levels, as well as a significant increase in anti-inflammatory cytokine IL-10 levels in rugby players, who underwent a cycle of 5 daily whole-body cryotherapy procedures during their regular training.

It was also found that cold and exhausting exercise modulate cytokine production, upregulating expression of IL-6 and IL-1 receptor antagonist but downregulating IL-1beta and TNF-alpha in monocytes of healthy men. These changes in cytokine expression appear to be linked to enhanced catecholamine secretion and subsequent c-AMP production [45].

Taking into account the above data and the fact that in the present study the values of inflammatory parameters were decreased by whole-body cryotherapy also in healthy volunteers, it seems that cryogenic temperatures could influence directly the immune response, probably through

the modification of IL-1beta, IL-2, IL6, IL8 and IL-10 release from macrophages. The effect is beneficial in patients with inflammatory diseases, but one cannot exclude its potentially unfavourable influence on the immune system in healthy subjects.

One of the mechanisms of anti-inflammatory action of whole-body cryotherapy may be linked to stabilization of lysosome membranes and subsequent inhibition of the release of active enzymes from lysosomes [46]. It seems that this effect could be related to increased ACTH and cortisone blood concentrations, due to both whole-body cryostimulation and physical training. It was observed in experimental study that ACTH and cortisone modifies the activity of lysosomal enzymes in rats [47, 48]. It was also found that long-lasting physical training causes significant increase in corticosterone level, as well as a slight lowering of lysosomal enzyme activity in rats [48].

In healthy individuals (men and women) after a single, 2-minute cryostimulation treatment, a statistically significant increase in the serum ACTH concentration without any significant changes in cortisol level was observed [49]. Moreover, in patients with rheumatoid arthritis exposed to a cycle of whole-body cryotherapy procedures a statistically significant increase in serum cortisol concentration was reported after a single treatment as well as after 7 and 14 procedures accompanied by kinesitherapy [50].

However, Woźniak et al. [51] did not observe any statistically significant changes in serum cortisol concentration, neither after a single session of cryostimulation of untrained men nor after 6 and 10 days of cryostimulation procedures accompanied by training. The concentration of cortisol increased significantly only after the first 6 days of training without cryostimulation and remained at that level after the tenth day of training. In another study [52] in healthy women exposed to a cycle of whole-body cryotherapy applied 3 times a week for 12 weeks, the first session caused only temporary, insignificant increases in plasma ACTH and cortisol concentrations. In weeks 4-12, plasma levels of these hormones were significantly lower than in week 1, probably due to habituation, suggesting that cryostimulation does not stimulate the pituitary-adrenal cortex axis.

These contradictory results suggest that probably an increase in ACTH and cortisol levels is not the only mechanism of lysosomal membrane stabilization caused by whole-body cryostimulation.

The other mechanism of anti-inflammatory action of whole-body cryotherapy resulting in stabilization of lysosome membranes could be related to its beneficial influence on antioxidant enzyme activity [53]. In our own clinical study we observed increased activities of antioxidant enzymes, an increase in the level of plasma total antioxidant status and lack of changes in the level of malondialdehyde MDA (lipid peroxidation marker) in patients with ankylosing spondylitis after a cycle of repeated whole-body cryotherapy procedures [54]. However, it should be emphasized that a single session of whole-body cryostimulation could induce disturbances of prooxidant-antioxidant balance in the form of lowering total oxidative status (TOS)

and temporarily decreasing total antioxidative status (TAS) with subsequent elevation on the next day, resulting in increasing oxidative stress [55]. It is suggested that repeated exposure to cryogenic temperatures may cause adaptive changes in the form of increases in antioxidative capacity and antioxidant enzyme activity, resulting in the formation of prooxidant-antioxidant balance at higher levels, assisting the anti-inflammatory effect and protecting tissues against the increased generation of reactive oxygen species and oxidative stress caused by training [56-58].

Clinical evaluation of patients involved in the present study confirmed a more favorable subjective estimation of clinical effects of whole-body cryotherapy with subsequent kinesitherapy compared with separate kinesitherapy in all persons exposed to this method of physiotherapy. In patients with AS, a clinical improvement was related mostly to subsidence or considerable reduction of pain intensity and to a decrease in recurrence rate of pain syndrome, as well as to the reduction of neurotonia, relaxation and subsidence of sleep disorders. In healthy volunteers exposed to whole-body cryotherapy during the biological revival process, a clinical improvement was related mostly to augmentation of physical efficiency, reduction of neurotonia, relaxation and subsidence of sleep disorders [59].

Moreover, in patients with AS a significant improvement of spine mobility in the form of an increase in the values of thoracic spine mobility range measured in Otto's test, lumbar spine mobility range measured in Shober's test, respiratory chest expansion range, lumbar left-lateral and right-lateral flexure range and cervical left-lateral and right-lateral rotation range, as well as a decrease in the values of: "finger-floor", "occiput-wall" and "chin-thorax" dimensions was observed. In patients exposed to whole-body cryotherapy with subsequent kinesitherapy, percentage changes in the values of particular parameters were more distinct as compared to patients in whom solely kinesitherapy was used, mainly in cases of lumbar and thoracic spine mobility parameters [60].

Beneficial clinical effects of whole-body cryotherapy in patients with AS, as well as promising results of presented laboratory reports indicate the necessity of further studies in order to find a final explanation of anti-inflammatory effects of whole-body cryotherapy in patients with ankylosing spondylitis.

Conclusion

Cryogenic temperatures used for whole-body cryotherapy as a component of complex treatment decreases levels of inflammatory markers both in patients with ankylosing spondylitis and healthy volunteers.

Acknowledgements

The project was supported by grant No. 3P05B156 25 from the State Committee for Scientific Research, Republic of Poland.

References

1. ALBU A., PARASCA I., TALU S., POANTA L. Ankylosing spondylitis - a systemic disease. *Rom. J. Intern. Med.* **42**, 685, **2004**.
2. GAUSE A., SCHNABEL A. Rheumatology 2003 - part I: research news concerning pathogenesis, epidemiology, diagnosis, and therapy of chronic inflammatory joint diseases. *Med. Klin.* **98**, 523, **2003**.
3. KATARIA R.K., BRENT L.H. Spondyloarthropathies. *Am. Fam. Physician* **69**, 2853, **2004**.
4. KIM T.H., UHM W.S., INMAN R.D. Pathogenesis of ankylosing spondylitis and reactive arthritis. *Curr. Opin. Rheumatol.* **17**, 400, **2005**.
5. MARKER-HERMANN E., FRAUENDORF E., ZEIDLER H., SIEPER J. Pathogenesis of ankylosing spondylitis - mechanisms of disease manifestation and chronicity. *Z. Rheumatol.* **63**, 187, **2004**.
6. REVEILLE J.D., ARNETT F.C. Spondyloarthritis: update on pathogenesis and management. *Am. J. Med.* **118**, 592, **2005**.
7. SIEPER J. Spondyloarthritis. *Z. Rheumatol.* **6**, 185, **2004**.
8. ANALAY Y., OZCAN E., KARAN A., DIRACOGU D., AYDIN R. The effectiveness of intensive group exercise on patients with ankylosing spondylitis. *Clin. Rehabil.* **17**, 631, **2003**.
9. DAGFINRUD H., KVIEN T.K., HAGEN K.B. Physiotherapy interventions for ankylosing spondylitis. *Cochrane Database Syst. Rev.* **4**, CD002822, **2004**.
10. FRANSEN M. When is physiotherapy appropriate? *Best Pract. Res. Clin. Rheumatol.* **18**, 477, **2004**.
11. METZGER D., ZWINGMANN C., PROTZ W., JACKEL W.H. Whole-body cryotherapy in rehabilitation of patients with rheumatoid diseases - pilot study. *Rehabilitation* **39**, 93, **2000**.
12. SMIDT N., DE VET H.C., BOUTER L.M., DEKKER J., ARENDZEN J.H., DE BIE R.A., BIERMA-ZEINSTRAS.M., HELDERS P.J., KEUS S.H., KWAKKEL G., LENSSEN T., OOSTENDORP R.A., OSTELO R.W., REIJMAN M., TERWEE C.B., THEUNISSEN C., THOMAS S., VAN BAAR M.E., VAN 'T HUL A., VAN PEPPEN R.P., VERHAGEN A., VAN DER WINDT D.A. Effectiveness of exercise therapy: a best-evidence summary of systematic reviews. *Aust. J. Physiother.* **51**, 71, **2005**.
13. VLAK T. Spondyloarthropathies - clinical evaluation and physical therapy. *Reumatism* **51**, 29, **2004**.
14. YURTKURAN M., AY A., KARAKOC Y. Improvement of clinical outcome in ankylosing spondylitis by balneotherapy. *Joint Bone Spine* **72**, 303, **2005**.
15. OLSON J.E., STAVRINO V.D. A review of cryotherapy. *Phys. Ther.* **52**, 840, **1972**.
16. FRICKE R. Ganzkörperkältetherapie. *Z. Phys. Med. Baln. Med. Klin.* **15**, 311, **1989**.
17. ZAGROBELNY Z. (Ed.). Whole-body and local cryotherapy; Wydawnictwo Medyczne Urban & Partner, Wrocław **2003** [In Polish].
18. PODBIELSKA H., STREK W., BIALY D. (eds.). Acta of Biomedical Engineering: Whole Body Cryotherapy; Indygo Zahir Media, Wrocław **2007**.
19. SIEROŃ A., CIEŚLAR G. (Eds.). Cryotherapy - therapeutic application of cold; α -medica press: Bielsko-Biała, **2007** [In Polish].
20. KNIGHT K.L. Cryotherapy in sport injury management; *Human Kinetics: Champaign*, **1995**.

21. JONDERKO G. Current state of knowledge about the application of cryotherapy for treatment of musculoskeletal diseases. *Chir. Narz. Ruchu Ortop. Pol.* **55**, 31, **1990** [In Polish].
22. ZGRADIC I., DINIC S., POPOVIC R. Personal experience in cryotherapy treatment. *Med. Pregl.* **40**, 29, **1987**.
23. SKRZEP-POLOCZEK B., WIŚNIEWSKA B., ROMUK E., BIRKNER E., JAGODZIŃSKI L., STANEK A., SIEROŃ A. The effects of whole-body cryotherapy on ankylotic spondylitis patients. In: Ring H. (Ed.). *Proceedings of 2nd World Congress of the International Society of Physical and Rehabilitation Medicine – ISPRM “Reflection on Advances in Rehabilitation – Future Challenges”*; 2003 May 18-22; Prague; Monduzzi Editore: Bologne, pp. 391-395, **2003**.
24. YAMAUCHI T., NOGAMI S., MIURA K. Various applications of extreme cryotherapy and strenuous exercise program - focusing on chronic rheumatoid arthritis. *Physiother. Rehab.* **5**, 35, **1981**.
25. GREGOROW H. Whole body cryotherapy - indications and contraindications, the procedure and its clinical and physiological effects. *Acta Bio-Opt. Inform. Med.* **4**, 119, **1998**.
26. VAN DER LINDEN S., VALKENBURG H.A., CATS A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum.* **27**, 381, **1984**.
27. BRAUN J., GOLDER W., BOLLOW M., SIEPER J., VAN DER HEIJDE D. Imaging and scoring in ankylosing spondylitis. *Clin. Exp. Rheumatol.*, **20**, (28), 178, **2002**.
28. ROBERTSON V.J., CHIPCHASE L.S., LAAKSO E.L., WHELAN K.M., MCKENNA L.J. Guidelines for the clinical use of electrophysical agents 2001, Australian Physiotherapy Association: Victoria, **2001**.
29. JOCH W., ÜCKERT S. Ausdauerleistung nach Kälteapplikation. *Leistungssport* **33**, 17, **2003**.
30. DOWNTON S.B., COLTON H.R. Acute phase reactants in inflammation and infection. *Seminars Hematol.* **25**, 84, **1988**.
31. DE LA HUERGA J., DUBIN A., KUSHNER D.S., DYNIEWICZ H.A., POPPER H. Studies of serum mucoprotein (seromucoïd). I. A turbidimetric method. *J. Lab. Clin. Med.* **47**, 403, **1956**.
32. THOMAS L. *Clinical and laboratory diagnostics*, 5th ed.; TH Books Verlags GmbH: Frankfurt/Main, **1998**.
33. BAL A., UNLU E., BAHAR G., AYDOG E., EKSIÖGLU E., YORGANCIOGLU R. Comparison of serum IL-1beta, sIL-2R, IL-6, and TNF-alpha levels with disease activity parameters in ankylosing spondylitis. *Clin. Rheumatol.* **26**, 211, **2006**.
34. LIU B., GUO C.Y., LIU W.Q., WU N., XING Q. The value of erythrocyte sedimentation rate and C-reactive protein in evaluating disease activity in ankylosing spondylitis. *Zhonghua Nei Ke Za Zhi* **44**, 566, **2005**.
35. YILDIRIM K., ERDAL A., KARATAY S., MELIKOGLU M.A., UGUR M., SENEL K. Relationship between some acute phase reactants and the Bath Ankylosing Spondylitis Disease Activity Index in patients with ankylosing spondylitis. *South Med. J.* **97**, 350, **2004**.
36. ROTHELIN R., DUSTIN M.L., MARLIN S.D., SPRINGER T. A human intracellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol.* **37**, 1270, **1986**.
37. STAUNTON D.E., MARLIN S.D., STRATOWA C., DUSTIN M.L., SPRINGER T.A. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell* **52**, 925, **1988**.
38. DUSTIN M.L., ROTHLEIN R., BHAN A.K., DINARELLO C.A., SPRINGER T.A. Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry and function of a natural adherence molecule (ICAM-1). *J. Immunol.* **137**, 245, **1986**.
39. POTOČNIK A.J., KINNE R., MENNINGER H., ZACHER J., EMMRICH F., KROCZEK R.A. Expression of activation antigens on T cells in rheumatoid arthritis patients. *Scand. J. Immunol.* **31**, 231, **1990**.
40. KLIMIUK P.A., FIEDORCZYK M., SIERAKOWSKI S., CHWIECKO J. Soluble cell adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) in patients with early rheumatoid arthritis. *Scand. J. Rheumatol.* **36**, 345, **2007**.
41. KOCH A.E., SHAH M.R., HARLOW L.A., LOVIS M.R., POPE R.M. Soluble intercellular adhesion molecule-1 in arthritis. *Clin. Immunol. Immunopathol.* **71**, 208, **1994**.
42. COGALGIL S., TAYSI S. Levels of antioxidant proteins and soluble intercellular adhesion molecule-1 in serum of patients with rheumatoid arthritis. *Ann. Clin. Lab. Sci.* **32**, 264, **2002**.
43. WENDLING D., RACADOT E., AUGE B., TOUSSIROT E. Soluble intercellular adhesion molecule 1 in spondylarthropathies. *Clin. Rheumatol.* **17**, 202, **1998**.
44. BANFI G., MELEGATI G., BARASSI A., DOGLIOTTI G., D'ERIL G., DUGUÉ B., CORSI M. Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. *J. Thermal Biol.*, **34**, 55, **2009**.
45. RHIND S.G., CASTELLANI J.W., BRENNER I.K., SHEPARD R.J., ZAMECNIK J., MONTAIN S.J., YOUNG A.J., SHEK P.N. Intracellular monocyte and serum cytokine expression is modulated by exhausting exercise and cold exposure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**, R66, **2001**.
46. WOŹNIAK A., MILA-KIERZENKOWSKA C., DREWA T., DREWA G., WOŹNIAK B., OLSZEWSKA D., MALINOWSKI D., RAKOWSKI A., BRZUCHALSKI M. Activity of chosen lysosome's enzymes in plasma of canoeists after whole-body cryotherapy. *Med. Sport.* **17**, 322, **2001** [In Polish].
47. KOSTULAK A. The effect of ACTH and cortisone on the behavior of arylsulphatases in the salivary glands of hite rat. *Acta Histochem.* **58**, 63, **1977**.
48. TONCSEY H., FRENKL R. Studies on the lysosomal enzyme system of the liver in rats undergoing swimming training. *Int. J. Sports Med.* **5**, 152, **1984**.
49. ZAGROBELNY Z., HALAWA B., JEZERSKI C., WAWROWSKA A. The influence of a single whole body cryostimulation on selected hemodynamic indices and hormone concentrations in the serum of healthy individuals. *Pol. Tyg. Lek.* **48**, 303, **1993** [In Polish].
50. ZAGROBELNY Z., HALAWA B., NEGRUSZ-KAWECKA M., SPRING A., GREGOROWICZ H., WAWROWSKA A., ROZWADOWSKI G. Hormonal and hemodynamic changes caused by whole body cooling in patients with rheumatoid arthritis. *Pol. Arch. Med. Wewn.* **87**, 34, **1992** [In Polish].
51. WOŹNIAK A., WOŹNIAK B., DREWA G., MILA-KIERZENKOWSKA C., RAKOWSKI A. The effect of whole-body cryostimulation on lysosomal enzyme activity in kayakers during training. *Eur. J. Appl. Physiol.* **100**, 137, **2007**.
52. LEPPÄLUOTO J., WESTERLUND T., HUTTUNEN P., OKSA J., SMOLANDER J., DUGUÉ B., MIKKELSSON M. Effects of long-term whole-body cold exposures on plas-

- ma concentrations of ACTH, beta-endorphin, cortisol, catecholamines and cytokines in healthy females. *Scand. J. Clin. Lab. Invest.* **68**, 145, **2008**.
53. OLLINGER K., BRUNK U.T. Cellular injury induced by oxidative stress is mediated through lysosomal damage. *Free Radic. Biol. Med.* **19**, 565, **1995**.
54. STANEK A., CIEŚLAR G., BIRKNER E., JAGODZIŃSKI L., SKRZEP-POLOCZEK B., ROMUK E., SIEROŃ A. Changes in antioxidant status in patients with ankylosing spondylitis during cryotherapy. *Eur. J. Clin. Invest.* **35**, (2), 26, **2005**.
55. LUBKOWSKA A., CHUDECKA M., KLIMEK A., SZYGUŁA Z., FRĄCZEK B. Acute effect of a signal whole-body cryostimulation on prooxidant-antioxidant balance in blood of healthy, young men. *J. Thermal. Biol.* **33**, 464, **2008**.
56. WOŹNIAK A., WOŹNIAK B., DREWA G., MILA-KIERZENKOWSKA C. The effect of cryostimulation on the prooxidant-antioxidant balance in blood of elite kayakers after training. *Eur. J. Appl. Physiol.* **101**, 533, **2007**.
57. JANISZEWSKI M. Cryotherapy as a factor supporting rehabilitation. *Med. Sport.* **2**, 57, **1998** [In Polish].
58. URSO M.L., CLARKSON P.M. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, **189**, 41, **2003**.
59. STANEK A., CIEŚLAR G., MATYSZKIEWICZ B., ROSMUS-KUCZIA I., SIEROŃ-STOŁTNY K., KOCZY B., SIEROŃ A. Subjective estimation of therapeutic efficacy of whole-body cryotherapy in patients with ankylosing spondylitis. *Balneol. Pol.* **47**, 24, **2005** [In Polish].
60. STANEK A., SIEROŃ A., CIEŚLAR G., MATYSZKIEWICZ B., ROSMUS-KUCZIA I. Influence of whole-body cryotherapy on parameters of spine mobility in patients with ankylosing spondylitis. *Ortop. Traumat. Rehab.* **7**, 549, **2005** [In Polish].

