SERUM HEAT SHOCK PROTEINS IN KNEE JOINT OSTEOARTHRITIS PATIENTS TREATED WITH GRASS THERMAL THERAPY

Elena Verzelloni[1*], Francesco Russo[2], Giovanni Agostini[2], Patrizia Manica[3], Angela Conte[4]

[1]Department of Human and Environmental Sciences, University of Pisa, Italy. [2]Department of Surgery, University of Pisa, Italy. [3]Garniga Terme, Trento, Italy. [4]Department of Agricultural Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy. verzelloni.elena@unimore.it

Summary

In thermal resort of Garniga in Bondone Alp, Trento (Italy), spa therapy of osteoarthritis is carried out with applications on body of fermenting grass from Bondone prairies, 1600 m above sea-level. During application heat is developed and grass compounds are released. The mechanism of therapy is not well known. Heat shock proteins 70 (Hsp 70) and 60 (Hsp 60) were determined in serum of fifteen patients with knee joint osteoarthritis treated with standard therapy (11 applications in 2 weeks). Clinical assessments were carried out and serum was collected before and after the third and the ninth application and after one, three and six months. Hsps 70 and 60, were determined by immunoassay.
Funtional impairment (Lequesne's index), pain rating, quality of life indexes, analgesic and/or NSAID consumption significantly improve after treatment up to the end of the follow-up period. Improvement of physical activity and of psychic and physical wellbeing is also obtained. Significant increasing of Hsp 70 level is observed after the third application with a maximum after the 9th application then decreases progressively. No variations of Hsp 60 serum level are found. No adverse reactions were reported.
Conclusion. Heat produced by grass applications determines a controlled repeated heat shock which increases extra-cellular and probably intracellular Hsp 70 at joint level increasing cellular defences and modulating local and general immunity responses. Possible roles of grass-released compounds remain to be investigated.

Keywords: Hsp 70, Hsp 60, serum Hsps, knee joint osteoarthritis, grass thermal therapy, thermal treatment, osteoarthritis.

Heat shock proteins (Hsps) are normally expressed in cells (constitutive forms) and are involved in protein folding, protein interaction and transport. Hsps are up-regulated in response to stresses (inducible forms) such as thermal, mechanical, osmotic, and chemical stresses. Growth factors, hormones, and physical activity also up-express Hsps. Viral, bacterial, and parasitic infections, fever, inflammation, autoimmunity as well of other pathological condition increase the Hsp level. Hsps are considered to be intracellular however several Hsps have been demonstrated in biological fluids (i.e. serum, liquor, sinovia, saliva…). The role of extracellular Hsps is not well understood however several conditions that increase intracellular Hsp levels increase also the extracellular concentration of Hsps (1-4).

Heat applications have a therapeutic tradition for osteoarthritis in several country including Italy, Austria, Germany, Belgium, France, Spain…). In thermal resort of Garniga in Bondone Alp, Trento (Italy), spa therapy of osteoarthritis is carried out with applications on body of grass collected from Bondone prairies, 1600 m above sea-level. The mechanism of therapy is not well known (5).

We have determined the concentration of Hsp 70 and Hsp 60 in serum before, during and after the standard treatment in 15 male patients with knee joint osteoarthritis to evaluate if thermal application induces a measurable variation of these molecules.

Methods

15 patients with knee joint osteoarthritis (radiologically demonstrated osteoarthritis in the knee joints) were enrolled for this study. 8 patients had idiopathic generalized osteoarthritis (OA), 5 patients have idiopathic OA localized to knee joints, 2 patients have secondary OA. Associated pathologies are: hypertension (5 patients), hyperlipidemia (5 patients), type-2 diabetes mellitus (2 patients), atopic allergy (1 patient). Patients have a medical prescription for thermal therapy in a thermal resort and all patients before and after treatments are clinically evaluated for systemic diseases and for articular and skeletal muscle disorders. The following investigation are carried out: complete medical examination, anthropometrical measurements, anamnesis, blood pressure measurement, ECG…as well, anatomic localization of complaint (articular vs non articular) determination of the nature (inflammatory vs non inflammatory) of the pathological process, determination of the extent of involvement (monoarticular, polyarticular, focal, widespread) determination of chronology (onset, acute, chronic), formulation of a differential diagnosis, preceding treatments, drug therapy. Daily activities, job activity, and physical activities, depression, anxiety… are also evaluated. Specific knee joint examination are also carried out in this study: knee examination and radiography, spontaneous and induced pain evaluation, knee mobility and deambulation, Lequesne's index determination…Physician and patient evaluated the effectiveness, the compliance, the tolerance, and the possible adverse effects.

The following blood analyses are routinely carried out: blood cell count, Hb, glucose, urea, creatinine, SAST, SALT, Erythrocyte sedimentation rate (ESR) Westergren, PCR, TSH, FT3, FT4. Complete urine analysis are also performed.

In this study we have determined blood Hsp 70 and Hsp 60 with the methods described by Pokeley et al. (1, 2). Blood collection and clinical evaluation have been performed before treatments and after the 3rd and the 9th application and one, three and six months after treatment. All patients (adult and cognitively intact) were informed on the aim of this study and on the parameters investigated and give their informed consensus for clinical evaluation and blood collection during treatment and follow-up. This study was planned and carried out through in compliance with the Helsinki Declaration.

Means ± Standard Deviation are reported. Paired and unpaired Student's t-test were used as appropriate. A p value (2-sided) < 0.05 was considered as statistically significant.

Results

Patients have an age which ranges between 49 and 65 years (mean ± SD; 59.3 ± 4.9 years). Three subjects have a body mass index (BMI) of 30 Kg/m^2 (obese), ten with a BMI between 25-30 are overweight with a mean of 26.6 ± 1.9 Kg/m^2. The means of ESR and of PCR are 9.1 ± 7.9 mm/1st h and 9.1 ± 2.3 mg/100ml respectively. The other haematological parameters are within normal ranges.

Grass is collected on Bondone Alp (Viotte prairies) at 1500-1600 m on the sea level. After mowing, grass is allowed to ferment and is used for therapy. In this study fresh collected fermenting grass has been used. However grass may be maintained for months at –20 °C without loss of its fermenting capacity and its therapeutic properties. The more important species present in Viotte grass are *Anthyllis vulneraria* L. (*alpestris* and *purpurescens*), *Arnica Montana* L., *Hypericum perforatum* L., *Thymus vulgaris* L., *Carlina acaulis* L., *Pulsatilla vulgaris* Mill..

After mowing, grass spontaneously ferments and during fermentation its temperature reaches in 1-2 days about 60°C. Grass at this temperature is used for the treatment.

During treatments the body of patient is lying on a bed of fermenting grass and the body is covered by 10-20 cm of fermenting grass for 20 min. Only neck and head remain uncovered. At the end of bath in the fermenting

grass patients remain for 30-45 min. in a reaction bed wrapped in a wool blanket. The reaction to heat application decreases progressively in about 3-4 h.

The patient takes a grass bath every day for 5 days. After one day of rest a second cycle of 6 grass baths is performed for a total number of 11 grass baths in two weeks.

The serum Hsp 70 concentration is significantly increased (only with paired Student's t-test) of 29.0 % and 63.6 % after the 3rd and 9th grass bath respectively (Table 1). After the 9th bath Lequesne's index is significantly (with unpaired student's t-test) decreased of 50.8 %.

Table 1. Serum Hsp 70 concentration and Lequesne's index before, during, and after grass bath therapy.

Time of evaluations	Hsp 70 * (νg/ml)	Lequesne's index *
Before treatment	278.0 ± 138.4 (100-470)	6.1 ± 2.0 (2-12)
After 3rd grass bath	358.7 ± 138.6 (140-520)	-
After 9th grass bath	454.7 ± 164.2 (200-660)	***3.1 ± 2.4 (0-8)
After 1 month	425.3 ± 142.1 (180-630)	***3.3 ± 1.6 (1-8)
After 3 months	**282.7 ± 101.1 (130-480)	**4.6 ± 2.0 (2-10)
After 6 months	226.7 ± 75.4 (160-390)	5.0 ± 2.0 (1-10)

*mean ± SD (range).
All the Hsp 70 and Lequesne's index values have $p < 0.05$ respect to before treatment with paired Student's t-test except for the values ** that are not significant.
*** values with $p < 0.05$ respect to before treatment also with unpaired Student's t-test.

After 1 month Hsp concentration and Lequesne's index remain almost the same respect to the value observed at the 9th application. During follow-up period a progressive increase of Lequesne's index is observed. However after 6 months the index remains lower respect the initial value. The difference is significant evaluated by paired t-test. After three months the Hsp 70 level is similar to the before treatment value while after 6 months its is significantly lower (paired t-test).

The mean ± SD of the Hsp 60 are: before treatment 285.3 ± 127.0 ng/ml; after 9th grass bath 291 ± 134.7 ng/ml; after 6 months 240.7 ± 106.3 ng/ml. The variations observed are not significant also with paired Student's t-test even if after six months a small decrease of serum Hsp 60 level is observed.

A scale with 4 values have been used to evaluate the tolerability and the efficacy of the treatment. The values of the scale are: 3 – very good; 2 – good; 1 – fair good; 0 – insufficient. For tolerability the mean ± SD of the values from physician was 2.47 ± 0.72 (range 1-3) from patients 2.47 ± 0.61 (range 1-3) and for efficacy the mean of the values from physician was 1.87 ± 0.34 (range 1-2), from patients 2.33 ± 0.47 (range 2-3) with significant difference ($p < 0.0025$ with impaired t-test) between the two evaluation.

The mean value ± SD of visual analogical scale of pain before treatment was 2.5 ± 1.03 (range 1.0-5.9) and after the 9th treatment 1.5 ± 0.86 (range 0.4-3.5). The difference is significant with unpaired Student's t-test ($p < 0.01$). All the other clinical evaluations (NSAID consumption, mobility…) improve after the thermal treatment (data not shown) and the improvement is maintained during the 6 months of follow-up.

Discussion

The bath in fermenting grass or phytobalneotherapy is a very ancient use in a restricted area of Alps (Trento province and Sud-Tyrol in Italy, and in Austria) for the treatment of several types of osteoarticular pathologies in which inflammatory phase is not present or it is very low such as the osteoarthritis of knee of the patients of

our study. In an investigation of about 2000 patients (5) it has been shown that the treatment is beneficial for more then 80% of patients. Pain is decreased, NSAID consumption is decreased, articular movements, evaluated by various tests, are more broad and flexible, velocity of body movements is increased as well physical activity of patients. The treatment with grass baths greatly improves also skin appearance, mood and general wellbeing. Possible adverse effects are: increased articular pain during the bath (10% of patients) and asthenia (8% of patients) for 3 or 4 days after the 3rd or 4th bath. These adverse effects do not require interruption of the treatment. Almost all patients ask to can back to Garniga Resort the following year.

In all patients with knee joint arthritis of this study we have observed all the above reported beneficial effects. The statistically significant difference between physician and patient evaluation of the efficacy of the treatment may be partially due to the enjoyment of Garniga Resort, to the beautifulness and climate of Alps, to the increased physical activity, and to the improving of general wellbeing.

The mechanism of action of phytobalneotherapy for osteoarthritis is not known. It is possible however that the main action is due to heat application. Our observation that Hsp 70 increase in blood during grass bath therapy demonstrates that heat application is able to up-regulate the expression of Hsp 70 in treated patients.

It has been shown in cell cultures and in experimental animals that the over-expression of Hsp 70 protects from cellular injuries and from apoptosis. The protective and anti-apoptotic function of Hsp 70 has been also demonstrated in chondrocytes (6-8). It has been recently reported that up-regulation of Hsp 70 may contribute to the robustness and active matrix production of chondron which is a basic unit of articular cartilage that includes the chondrocyte and its pericellular matrix (9).

Some functions of intracellular Hsps have been clearly demonstrated while the physiological function(s) of extra-cellular Hsps are currently not well defined. It has been suggested that extra-cellular Hsps have a fundamental role in physiological and immunological mechanisms possibly involving the maintenance of tolerance to these highly conserved molecules, the regulation of immune response, and the modulation of innate immuno system (1-4)

Release of Hsps have been demonstrated from many cell types such as blood mononuclear cells, glial cells, vascular cells…the release is not simply due to cell damage even if a part of serum Hsps may derive from damaged cells and/or cellular turnover. Since Hsp 70 does not have a peptide leader sequence for secretion, the secretion may not be via the ER/Golgi route. A possible route is the transport of Hsp 70 via the Golgi into lysosomal lipid rafts prior to exocytosis (10). During grass bath treatment the tissues more exposed to heat are skin, subcutaneous tissue, tissues of vessels and blood cells near to body surface. In these tissues and cells an up-regulation of Hsps is expected and may give the grater contribution to the Hsp 70 increase in serum. In joint tissues and muscles more exposed to heat an up-regulation of Hsps is also expected. It is noteworthy that experimental animal maintained at 42° C an increase of Hsps is observed also in the heart (11). Other factors, together with heat, may contribute to the over expression of Hsps. For example hyaluronan oligosaccharides up-regulate Hsp 72 expression by enhancing the activation of Heat Shock Factor 1 under stress condition and suppress cell death (12).

The increased physical activity which is observed after the treatment as a consequence of the improvement of knee osteoarthritis may also contribute to the increase of Hsp 70 in serum during treatment and in the months which follow. The physical exercise in fact increases serum Hsp 70 in man and experimental animals (4, 13-15). It has been demonstrated that moderate physical activity increases the Hsp 70 expression also in joint tissues and protects cartilage from degeneration (7). Up-regulation of Hsp 70 is involved in the mechanisms which determine muscle hypertrophy after exercise (16).

An increase of serum Hsps and in particular of Hsp 60 and its antibody has been observed in some chronic diseases like hypertension and cardiovascular diseases (17, 18). In our study the serum concentration of Hsp 60 is not increased during and after treatment while Hsp 70 after 6 months after treatment is significantly

decreased, with paired Student t-test, respect to the value before treatment. In a recent study the level of serum Hsp 70 does not appear related to vascular risk factors (including hypertension), echocardiographic left ventricular mass indexes, prevalent cardiovascular diseases or traditional serum biomarkers (19). In any case the clinical significance and the possible functions of the serum increase of Hsps in these pathological conditions is yet to be elucidated. Osteoarthritic patients with hypertension, stabilized cardiovascular diseases, preceding stroke, atherosclerosis, varicose veins may be treated with phytobalneotherapy (5). It has been shown that thermal treatments improve impaired vascular endothelial function in patients with coronary risk, attenuates neointimal thickening with enhanced expression of Hsp 72 and suppression of oxidative stress, attenuates leukocyte-endothelial interactions and decrease serum TNFα and Il-1beta (20-23).

The released compounds (essential oils, terpenes, volatile compounds…) from fermenting grass which is rich of officinal aromatic species deserve consideration. It has been shown that some of these compounds are biologically active substances which may enter in the body by breathing or through the skin (5). Although their possible therapeutic effects are not known and are actually under investigation no adverse effects has been reported like allergic reactions, rhinitis, dyspnea, wheezing, asthma attack, urticaria, atopic dermatitis…

To conclude phytobalneotherapy of knee joint osteoarthritis patients with fermenting Bondone grass in Garniga spa causes a controlled repeated heat shock which ameliorates their pathological conditions and determines an increase of serum Hsp 70 during treatment and in following months. The increase is probably due not only to the applications of heat on the body surface, but also to the increase of physical activity and to the remodelling of muscular and joint tissues that follows the treatment. The increase of extra-cellular and intracellular Hsp 70 enhances cellular defences and modulates general and local immunity responses.

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