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THE BIOCHEMICAL PAIN MEDIATORS AND THE ROLE OF THE THERMAL STAY IN THE GLOBAL APPROACH TO THE PATIENT

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Abstract

INTRODUCTION : patients suffering from skeletal pathology frequently undergo thermal treatments, thank to their efficacy in controlling pain symptoms accompanied with skeletal conditions. Aim of the present investigation has been obtain a better understanding about the possible influences of mud bath therapy, the most frequently used thermal method for the treatment of skeletal pathologies, on several neuropeptides involved in pain transmission. MATERIALS AND METHODS : 48 patients with osteoarthritis (OA) were enrolled in the study and randomized in Group A (12 days mud pack treatment and 500 mg acetaminophen twice a day) and Group B (only drug treatment with 500 mg acetaminophen twice a day). Blood samples were collected for the assay of P substance, CGRP, β-NGF, γ-NP. RESULTS : the statistical analysis has shown a significant difference between baseline and final values in both groups, for all the investigated parameters. The results suggested an efficacy synergy between thermal treatment and pharmacological therapy in group A. DISCUSSION : often in rheumatic patients pain treatment is unsatisfactory and this has led patients and physicians to seek other therapeutic interventions. Mud bath therapy could be taken in account in order to develop a "combined therapy" with the main anti-inflammatory drugs, to decrease dosage and increase the patients' safety. CONCLUSION : in agreement with the data of other authors, it seems that the regular use of mud pack therapy, together with the change of living environment and the spa scene seem to improve the quality of life and to control the main substances involved in neurogenic inflammation.

Key words : pain, osteoarthritis, mud bath therapy, QoL

Introduction

Several patients attending Spa suffer from skeletal pathologies, in particular osteoarthritis (OA). OA is a multifactorial disease with several risk factors influencing the normal repair mechanisms of cartilage [1,2]. Patients often seek medical help because of pain and this symptom, together with the limitation of movements,

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largely accounts for physical disability with high costs for health care systems and population [3,4].
Often, in these patients, pain treatment is unsatisfactory ; in fact many variables, influencing symptoms' quality and intensity, have to be taking into account arranging the therapy : age, general health conditions, disease evolution, etc [5-8].
Inflammatory changes in synovial membranes and production of inflammatory cytokines [9-11] play a dominant role in the development of pain, accompanying OA.
Actually the goals of management of patients with rheumatic diseases include the control of pain, the improvement in joint function and the avoidance of toxic effects of therapy [12,13]. The current therapy of osteoarthrosis is largely symptomatic and based on analgesic and anti-inflammatory drugs, but a dissatisfaction with these interventions has led many patients and therapists to seek other interventions, like as spa therapies, which represents a widely used method in traditional medicine as treatment for several rheumatic diseases [14-19]. Mud bath therapy, affecting chondrocytes activities, modulating cytokines production and decreasing pain, might exert beneficial effects on cartilage homeostasis and inflammatory reactions [20,21].
Beneficial effects of the treatment on the pain might depend at least in part, on the induced increases of opioïd peptides, and other part on the ability to modulate some inflammatory peptides. Recently studies related to the pathogenesis of osteoarthritic pain have focused attention on small sensitive fibers (C fibers and A delta fibers) containing neuropeptides, which would be involved in synoviocytes proliferation and consequently release of prostaglandin E2 (PGE2) and collagenases [22,23]. It has been shown that nociceptive fibers release P Substance (PS), calcitonin gene related peptide (CGRP), β-nerve growth factor (beta-NGF) and γ-neuropeptide [26] contributing to the neurogenic inflammation and to the decrease of proteoglycan synthesis [24,25].
In order to delve the knowledge about the mechanisms of action of mud bath therapy on the pain symptomatology, the present study investigated if the thermal treatment exerts an influence on these biochemical markers released from the nociceptive fibers.

Materials and Methods

After the study design has been approved by the local Ethical Committee, 48 patients were recruited in family physicians ambulatories, on the basis of the following inclusion criteria :
• lumbar osteoarthritis diagnosed according the American College of Rheumatology guidelines,
• first diagnosis of lumbar osteoarthritis at least 8 years before,
• no drugs assumption other than acetaminophen,
• no acute joint inflammation.
The enrolled patients signed an informed consent, in accordance with the second declaration of Helsinki and were divided into two groups of treatment.
Sample size calculation was performed using the difference in means formula for simple two-group designs with the following criteria : an equal number of cases and controls

(ratio of controls to cases = 1), a desired power of test (1-beta) of 0.85, an alpha error of 0.05 (level of statistical significance Z = 1.96). A list for allocating patients by simple randomisation was constructed using a sequence of natural random numbers from a computer-generated sequence.
Treatments may then be allocated to patients in sequence using numbered opaque envelops containing treatment allocations :
• Group A : for 12 days the patients (mean age 67.38 ± 6.48) underwent a cycle of hot mud pack and took 500 mg acetaminophen twice a day (combined treatment) ;
• Group B : patients (mean age 67.46 ± 5.87) received only pharmacological treatment for 12 days (500 mg acetaminophen twice a day).
Group A patients underwent the 12 hot mud packs in a thermal bath location of Region Veneto (Italy) according to the standard protocol employed in this thermal basin : "mature" thermal mud was applied to the whole body for 15 to 20 minutes at a temperature of 39°-40° C, followed by a shower and a thermal bath at 37°-38° C for 10 to 12 minutes.
The "maturation" process is caused by the development of a typical microflora, mainly represented by blue-green algae (*cyanophyceae*) and diatoms [14] producing sulpholipide compounds, supplied with an anti-inflammatory activity, which are released in the thermal mud [26,27].
This analysis has been carried out in order to exclude a possible negative effect of heat application on osteoarthritis joints and to investigate a possible interaction of mud pack with some neuropeptides.
Blood samples were collected for each patient before and after the end of the treatments, for the assay of P substance (PS), calcitonin gene related peptide (CGRP), β-nerve growth factor (β-NGF), γ-neuropeptide (γ-NP), neuropeptides implicated in neurogenic inflammation.
PS is a tachykinin that causes vasodilatation strictly linked to neurogenic inflammation in a dose-dependent manner. CGRP is a potent vasodilatator and is frequently co-localized with PS. γ-NP is present throughout the central and peripheral nervous systems and is a potent vasoconstrictor. Beta-NGF is a neurotrophin involved in the development and maintenance of peripheral nociceptive and sympathetic neurons.
Blood samples were collected in vacutainer tubes containing EDTA, and centrifuged at 1600xg for 15 minutes at 4ºC, transferred to other tubes and kept at -70ºC until further analysis. Each measurement was performed in duplicate.
The statistical analysis of the results was performed with the paired Student's *t*-test on differences between values before and after each treatment. Homogeneity of variance between the two groups for each neuropeptide was checked with the Levene's test. When variances resulted equal, the ANOVA test was performed to compare the effects of the two different treatments.

Results

Table 1 shows the serum levels of PS, CGRP, Beta-NGF and Gamma-NP, as mean ± SD, before and after the treatments in the group A.

	Group A (mean ± S.E.M)		
	Before	After	***p*-values***
γ-Neuropeptide (ng/mL)	0.02 ± 0.004	0.08 ± 0.01	$p<0.01$
CGRP (ng/mL)	0.38 ± 0.04	0.17 ± 0.01	$p<0.01$
P Substance (pg/mL)	571.3 ± 0,01	513.8 ± 0,01	$p<0.01$
β-NGF (ng/mL)	36.6 ± 2,93	34.3 ± 2,96	$p<0.01$

* degrees of freedom=23.

Table 1. Mean and S.E.M of plasma concentrations of γ-NP, CGRP, PS and β--NGF before and after treatment in Group A patients. Group A (N=24) received a mud pack treatment and 500 mg acetaminophen twice a day for 12 days.

A significant difference ($p<0.01$) between baseline and after treatment values was present for each measured markers in this group. Particularly, PS, CGRP, beta-NGF samples showed decreased concentrations, while increased serum levels were observed for γ-NP.

Table 2 reports the results of treatment of group B patients (500 mg of acetaminophen twice a day) on the same parameters as group A.

	Group B (mean ± S.E.M)		
	Before	After	***p*-values***
γ-Neuropeptide (ng/mL)	0.06 ± 0.01	0.08 ± 0.01	$p<0.01$
CGRP (ng/mL)	0.21 ± 0.02	0.15 ± 0.02	$p<0.01$
P Substance (pg/mL)	559.7 ± 0,01	529.7 ± 0,01	$p<0.01$
β-NGF (ng/mL)	37.0 ± 2,09	34.8 ± 1,96	$p<0.01$

* degrees of freedom=23.

Table 2. Mean and S.E.M of plasma concentrations of γ-NP, CGRP, PS and β--NGF in Group B patients. Group B patients (N=24) received 1 g/die acetaminophen for 12 days.

There is a significant difference ($p<0.01$) between neuropeptide levels before and after treatment, but not all the samples in the group showed the same behavior. Concordance of variation with respect to the mean was observed in 87.5 %, 79.2 %, 95.8 % and 79.2 % for PS, CGRP, beta-NGF and γ-NP respectively.

The Levene's test for the homogeneity of variance showed a difference between baseline concentrations of γ-NP and CGRP of the two groups, as the p values of this test were respectively 0.045 and 0.013 for baseline values of γ-NP and CGRP. No significant diffe-

rence of variance was evident for the other data and an ANOVA test was performed to check any difference after the two therapies on the plasma concentrations of the studied neuropeptides.
After the treatments significant statistical differences were not evident between the two groups evaluated parameters.
In order to evaluate the impact of thermalism in subjects suffering from osteoarthrosis, the 48 enrolled patients have been subdivided into two subgroups, Aa (more than 3 cycles of MPT) and Bb (less than 3 cycles of MPT), on the basis of the undergone number of cycles of mud bath therapy in the last 6 years, and replayed a questionnaire in order to collect : anamnestic data, intensity and duration of articular pain and disability, number of undergone cycle of thermal treatment.
The degree of disability was evaluated asking the subjects to attribute a score on the base of their difficulties in performing several daily living activities : standing with arm up, climbing 10 steps, walking 2 blocks, walking 3 blocks, standing up and sitting down (chair), standing up and sitting down (bed), showering, doing light works, doing heavy works.
Table 3 shows the results of the submitted questionnaire, expressed as disability scores in performing several activities of daily living.

	Group Aa (M±SD) 24 patients	**Group Bb** (M±SD) 24 patients	***p***
Standing with arms up	3.79 ± 2.81	3.25 ± 2.56	>0.01
Climbing 10 steps	3.08 ± 2.21	4.31 ± 2.07	**<0.01**
Walking two blocks	1.10 ± 0.95	1.25 ± 1.12	>0.01
Walking three blocks	1.21 ± 0.85	1.69 ± 0.75	**<0.01**
Standing up and sitting down (chair)	3.40 ± 1.66	5.06 ± 2.05	**<0.01**
Standing up and sitting down (bed)	3.17 ± 1.24	4.31 ± 1.49	**<0.01**
Showering	1.33 ± 1.02	1.94 ± 0.98	**<0.01**
Doing light works	1.02 ± 0.81	1.50 ± 0.85	**<0.01**
Doing heavy works	3.56 ± 1.41	4.85 ± 0.92	**<0.01**

Table 3. Activities of daily living disability score (M±SD) in Groups Aa and Bb patients and relative statistical differences.

Group A patients' declared a higher number of undergone cycles of thermal treatments, a low score of disability, even if they had a longer history of OA.
High significant statistical differences were evident between group A and B in relation to seven activities of daily living, as Student's t test has evidenced.

Discussion

PS is a tachykinin causing vasodilatation linked to neurogenic inflammation in a dose-dependent manner. CGRP is a potent vasodilatator and is frequently co-localized with PS [28]. γ-NP is present throughout the central and peripheral nervous systems and is a potent vasoconstrictor. Beta-NGF is a neurotrophin involved in peripheral nociception [29].
So, in order to have a better understanding about the thermal treatments mechanisms of action, we have hypothized a possible interaction of mud pack with some neuropeptides involved in neurogenic inflammation and we have investigated the serum levels of P substance (PS), calcitonin gene related peptide (CGRP), beta-nerve growth factor (β-NGF) and gamma-neuropeptide (γ-NP) in osteoarthritis patients treated with hot mud pack therapy with respect to a control group not treated with hot applications but with acetaminophen [30].
PS, CGRP, β-NGF serum values have shown a statistically significant decrease while γ-NP increases in both the studied groups of treatment.
In this study all the assayed neuropeptides are implicated in neurogenic inflammation and are released in the periphery, even more in presence of cytokines like as Il-1 and TNF-α [31,32]. These results could explain, at least in part, the already shown influence on the damaging substances such as sensitizing agents : nitric oxide (NO), tumor necrosis factor α (TNF-α), prostaglandin E2 (PGE2) [33,34]. It could be possible that lower concentrations of these substances, restoring a correct sensibility of small sensory fibers to the noxious stimuli, hence reduce neuropeptides release [35] and again γ-NP increased level could exert an inhibitory effect on β-NGF release, as reported in scientific literature [36].
Treatment with acetaminophen produces the same effects than hot mud packs, but Group A (combined therapy) shows an absolute greater effect in modifying the levels of the investigated neuropeptides [37].
Our data seem suggest the hot mud packs influencing the serum levels of the main neuropeptides involved in neurogenic pain mechanisms, could amplify the analgesic and anti-inflammatory properties of acetaminophen [38].
These data suggest that Group A suffered from OA for more years than group B, used less drugs than group B, used less physiotherapy and had a lower degree of disability performing activities of daily living. Consequently the quality of life seems to be better in group A.
This result is important because able to induce a positive reaction in the patients who perceive that their symptoms betterment is rationally justified.
It would be a limit to consider the pain experience only from a neurologic and biochimic point of view, underestimating emotional components such as anxiety, trouble, fear and apprehension [39].
Thermal location represents the place where the patients meet the global approach to the health way, implement with the disease therapy and with a great attention to the personal emotional condition too this is the holistic point of view, considering the patient such as a unity : body-mind-spirit [40,41].

Considering a disease such as a help request from the suffering people, the medical staff has to taking into account that every therapy needs to be applied in a context of positive communication. The people should perceive solidarity-faith-hope [42-44].
Several scientific researches have shown that positive emotions develop an important influence on the immune system inducing increase in beta-endorphin serum levels, improvement of lung oxygenation and blood circulation and muscles relaxation [45-48].
Thermal environment favors the relationships between the patients and the sharing of common deficit and fears.
The friendly alliance between people and the environment able to offer human and therapeutic resources to reach the well-being, could induce the patients towards new styles in living and managing their disorders and symptoms.

Conclusion

Concluding our results, in agreement with those of other authors, show that the regular use of mud pack therapy, which is one of the oldest form of therapy for osteoarthritis, seem to improve the quality of life and to control the main substances involved in neurogenic inflammation. Many factors are positively involved, such as change of living environment, spa scenary, physical and mental relaxations and the absence of work duties.
The combined therapy in the thermal environment could be taken in account in order to reduce the total amount of taken drugs, consequently decreasing the social costs for rheumatic disease therapies and improving patients' safety and quality of life [49].

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