

Modulation of Neurogenic Inflammation in Osteoarthritis Patients Undergoing a Combined Treatment of Mud Packs, Thermal Baths, and Acetaminophen: A Preliminary Study

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Abstract

Introduction: Osteoarthritis (OA) is the most common joint disease. Certain neuropeptides contribute to both the continuance of symptoms and cartilage damage resulting from the disease. Symptomatic drugs are frequently used for pain control, but dissatisfaction with such an intervention has led many patients to seek other treatments, 1 of which is thermal heat from mud packs.

Our intentions in the present study were to investigate a possible interaction between thermal treatment and the main neuropeptides involved in OA pathogenesis and to identify any possible detrimental effect of heat application on OA joints.

Materials and Methods: Forty-eight patients with lumbar OA were enrolled in the study and randomized to Group A (12 days of mud-pack treatment at 39-40° C plus thermal bath at 37°-38° C and 500 mg acetaminophen twice per day) or Group B (drug treatment only, 500 mg acetaminophen twice per day).

Blood samples were collected for assays of gamma neu-

ropeptide (γ NP), calcitonin gene-related peptide (CGRP), substance P (SP), and beta nerve-growth factor (β NGF).

Results: Statistical analysis has shown a significant difference between baseline and final values of the measured parameters in both groups. In group A, all the samples showed a variation of the same sign as the mean. Evidence for a greater modification of baseline levels of CGRP and SP in Group A was obtained by statistical analysis.

Discussion: Our data suggest that hot mud packs/thermal baths do not have a negative effect on OA patients. Heat treatment seems to increase the effect of pharmacological therapy on some neuropeptides involved in the pathogenesis of OA. Thus, it is possible that a combined therapy such as that used in Group A could be tried with the main antiinflammatory drugs used in OA in an effort to decrease dosages and increase the patients' safety.

Key words: osteoarthritis, osteoarthritis, pain, neuropeptides, heat, mud packs, acetaminophen

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Osteoarthritis (OA) is the most common type of joint disease worldwide.¹ Patients often seek medical help because of pain; this symptom, together with limitation of movements, largely accounts for the physical disability associated with the disease.²

Associated Factors

Many factors seem to be important to the pathogenesis of OA, all of them leading to an imbalance between cytokines and growth factors involved in the maintenance of cartilage integrity.^{3,4} This process is often accompanied by synovitis, affecting disease progression.

In fact, neuropeptides exert a proinflammatory effect on synovia and can contribute to maintenance of symptoms and progression of damage.⁵ With this in mind, for this study we examined 4 neuropeptides:

1. **gamma neuropeptide (γ NP)**, a potent vasoconstrictor present throughout the central and peripheral nervous systems;
2. **calcitonin gene-related peptide (CGRP)**, a potent vasodilator that is frequently co-localized with SP⁶;
3. **substance P (SP)**, a tachykinin that causes vasodilation strictly linked to neurogenic inflammation in a dose-dependent manner⁷; and
4. **beta nerve-growth factor (β NGF)**, a neurotrophic factor involved in the development and maintenance of peripheral nociceptive and sympathetic neurons.⁸

Treatment Goals and Options

The management goals of patients with rheumatic diseases include 1) control of pain, 2) improvement in joint function, and 3) avoidance of toxic effects from therapy.⁹

Symptomatic drugs, such as analgesics and antiinflammatories, are generally used,¹⁰ but a dissatisfaction with these interventions has led many patients to seek other interventions.¹¹ Among the physical therapies used in the care of chronic musculoskeletal disorders, mud therapy and thermal baths with hot mineral water have a traditional role, especially in several European countries.¹² Their efficacy and tolerability have been demonstrated in some rheumatic diseases even if the exact mechanism of action is not completely understood.¹²⁻¹⁴

Beneficial effects of thermal baths might depend, at least in part, on the induced increased levels of opioid peptides and on their ability to modulate some inflammatory mediators.¹⁵⁻¹⁸

To have a better understanding of a possible therapeutic role of hot treatments, and to detect any possible detrimental effect of heat application on joints of patients with rheumatic disease, we investigated the serum levels of neuropeptides mentioned earlier— γ NP, CGRP, SP, and β NGF—in lumbar OA patients treated with hot mud-pack therapy followed by a thermal bath with respect to a control group not treated with hot applications. Note that both groups were treated identically twice daily with 500 mg acetaminophen.

Material and Methods

After the study design was approved by the local ethical committee, 48 patients were recruited from family physicians' offices on the basis of the following inclusion criteria:

1. lumbar osteoarthritis diagnosed according the American College of Rheumatology guidelines,
2. first diagnosis of lumbar osteoarthritis at least 8 years before, and
3. no drug consumption other than acetaminophen.

The presence of any acute pathology was considered an exclusion criterion.

The enrolled patients signed an informed consent in accordance with the second declaration of Helsinki and were randomly divided into 2 equal groups for treatment.

Sample-size calculation was performed using the difference-in-means formula for simple 2-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, and an alpha error of 0.05 (level of statistical significance $Z=1.96$).

A list for allocating patients by simple randomization was constructed using a sequence of natural random numbers from a computer-generated sequence.

Treatments were then allocated to patients in sequence using numbered opaque envelopes containing treatment allocations.

Group A (combined treatment): For 12 days patients ($n=24$, mean age 67.38 ± 6.48) underwent a cycle of hot mud-pack treatments combined with a thermal bath and took 500 mg acetaminophen twice per day.

Group B: For 12 days patients ($n=24$, mean age 67.46 ± 5.87) received only pharmacological treatment (500 mg acetaminophen twice per day).

Group A patients had hot mud packs applied daily for 12 days in a thermal bath location at Abano Terme, Italy, according to the standard protocol employed at this spa. "Mature" thermal mud at a temperature of 39° to 40° C was applied to the whole body for 15 to 20 minutes, followed by a shower and a thermal bath for 10 to 12 minutes at 37° to 38° C.

"Mature" thermal mud consists of bromine-iodine natural mineral thermal water mixed with natural clay that has undergone a process of maturation at 70° C for at least 2 months.¹⁹

The "maturation" process is caused by development of a

typical microflora, mainly represented by blue-green algae (cyanophyceae) and diatoms, which produce sulfolipide compounds.²⁰ These compounds, when applied to the body, have antiinflammatory effects.^{21,22}

Our study was designed to examine possible positive or negative effects of heat application on osteoarthritic joints and to investigate a possible interaction of mud packs with the aforementioned neuropeptides.²³

Blood samples were collected for each patient before and after the end of the treatments for the assay of γ NP, CGRP, SP, and β NGF, the neuropeptides implicated in neurogenic inflammation.

Blood samples were collected in vacutainer tubes containing ethylene diamine tetra-acetic acid, and centrifuged at 1600 relative centrifugal force for 15 minutes at 4° C, transferred to other tubes, and kept at -70° C until further analysis. Each measurement was performed in duplicate.

Statistical analysis of the results was performed with the paired Student's *t*-test on differences between values before and after each treatment. The 2-sample *t*-test was used to compare after-treatment neuropeptide levels and the differences from baseline for each investigated neuropeptide.

Results

All 48 patients completed the study.

Table 1 shows the results of treatment of group A patients (mud-pack treatments and thermal bath plus 1000 mg of acetaminophen in 2 divided doses) on γ NP, CGRP, SP, and β NGF levels, as mean \pm standard error of the mean (SEM). A significant difference ($P<.01$) between baseline and after-treatment values was present for each measured neuropeptide in this group. Particularly, each sample varied in the same sign as the mean: ie, a decrease in CGRP, SP, and β NGF, and an increase of γ NP plasma concentrations were consistently observed.

Table 1. Mean and SEM of plasma concentrations of γ NY, CGRP, SP, and β NGF before and after treatment in Group A patients. Group A ($n=24$) received 12 days of mud-pack treatment at 39 to 40° C plus thermal bath at 37° to 38° C and 500 mg acetaminophen twice per day.

	Group A (Hot Applications, mean \pm SEM)		
	Before	After	<i>P</i> values*
γ NP (ng/ml)	0.02 \pm 0.004	0.08 \pm 0.01	$P<.01$
CGRP (ng/ml)	0.38 \pm 0.04	0.17 \pm 0.01	$P<.01$
SP (pg/ml)	571.3 \pm 11	513.8 \pm 10	$P<.01$
β NGF (ng/ml)	36.6 \pm 2.93	34.3 \pm 2.96	$P<.01$

*Degrees of freedom = 23.

Table 2 reports the results of treatment of group B patients (1000 mg of acetaminophen in 2 divided doses) on the same parameters as for group A. There is a significant difference ($P<.01$) between neuropeptide levels before and after treatment.

No evidence of differences in mean neuropeptide levels after treatment could be observed with the 2-sample *t*-test.

Table 2. Mean and SEM of plasma concentrations of γ NY, CGRP, SP, and β NGF in Group B patients. Group B patients (n=24) received 1 g acetaminophen for 12 days.

	Group B (mean \pm SEM)		
	Before	After	P values*
γ NP (ng/mL)	0.06 \pm 0.01	0.08 \pm 0.01	$P < .01$
CGRP (ng/mL)	0.21 \pm 0.02	0.15 \pm 0.02	$P < .01$
SP (pg/mL)	559.7 \pm 9.5	529.7 \pm 10	$P < .01$
β NGF (ng/mL)	37.0 \pm 2.09	34.8 \pm 1.96	$P < .01$

*Degrees of freedom = 23.

However, as reported in Table 3, the comparison of mean differences from baseline between Group A and Group B were significant for CGRP and SP.

Table 3. Mean and SEM of differences from baseline in plasma concentrations of γ NY, CGRP, SP, and β NGF in Group A and Group B patients.

	Group A	Group B	P values*
γ NP (ng/mL)	0.06 \pm 0.01	0.02 \pm 0.03	Not significant
CGRP (ng/mL)	-0.21 \pm 0.04	-0.06 \pm 0.01	$P < .01$
SP (pg/mL)	-57.50 \pm 7.29	-29.96 \pm 8.82	$P < .05$
β NGF (ng/mL)	-2.28 \pm 0.40	-2.23 \pm 0.52	Not significant

*Degrees of freedom = 23.

Discussion

Osteoarthritis is an arthropathy characterized by inflammatory changes in synovial membranes with production of inflammatory cytokines playing a dominant role in development of pain, even if its etiology is not completely understood.²⁴⁻²⁷

Recent studies have focused attention on small sensitive fibers (C fibers and A δ fibers) containing neuropeptides, which would be involved in synoviocytes proliferation and consequent release of prostaglandin E₂ (PGE₂) and collagenases.²⁸

It has also been shown that primary nociceptive fibers releasing SP and CGRP contribute to a decrease in proteoglycan synthesis, with consequent early cartilage damage.²⁹

In this study, all the assayed neuropeptides are implicated in neurogenic inflammation and are released in the periphery, and even more are released in the presence of cytokines such as interleukin 1 and tumor-necrosis factor α (TNF α).³⁰

Some proposed mechanisms for the apparent efficacy of thermal baths in OA are related to increased levels of opioid peptides,^{31,32} partially explaining both the reported analgesic effect of thermal-therapy application and the decreased serum levels of several inflammatory mediators.^{33,34}

In the present research, we have described the effects of mud packs on the main neuropeptides involved in neurogenic inflammation.

In our study, CGRP, SP, and β NGF serum values showed a

statistically significant decrease while γ NP increased significantly in both studied treatment groups.

These results could be explained, at least in part, by the demonstrated influence of mud packs on the substances functioning as sensitizing agents: nitric oxide, TNF α , and PGE₂.³¹⁻³⁵ It could be possible that lower concentrations of these substances restore a correct sensibility of small, sensory fibers to the noxious stimuli and hence reduce neuropeptide release.^{18,36}

The increased level of γ NP, exerting an inhibitory effect on β NGF release could account for its decreased level.^{37,38}

On another aspect of the procedure, a concern could be expressed about potential pathogens developing on thermal mud. Some years ago the Department of Clinical Microbiology of the medicine faculty at the University of Padua conducted several analyses to establish if some microorganisms would be able to survive in the environment of thermal water (1996, unpublished data). *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were tested in thermal water at 56° C. The results showed that:

- 1 000 000 colony-forming units/ml of *E. coli* and *C. albicans* were totally destroyed after 5 days of contact with thermal water at 56° C, and
- *S aureus* was destroyed after 5 days of contact with thermal water at 56° C.*

As thermal mud's maturation process occurs at the constant temperature of 70° C for 2 months, it is quite possible that this treatment is effective to eliminate the main species of human pathogens present in mud.

More research is needed to produce a more complete analysis of thermal mud's microbiologic characteristics.

Conclusion

Sole treatment with acetaminophen produced the same effects as hot mud packs, but evidence for a greater modification of baseline levels of CGRP and SP in Group A was obtained by statistical analysis.

Our data seem to suggest that hot mud packs do not have a negative effect on patients suffering from osteoarthritis—and, influencing serum levels of several of the main neuropeptides involved in neurogenic pain mechanisms could amplify the analgesic and antiinflammatory properties of acetaminophen.³⁹⁻⁴¹ This combined therapy could be taken into account to reduce the total amount of drug therapy, consequently both decreasing the social costs for rheumatic-disease therapies and improving patients' safety.^{42,43}

We believe that further investigations are needed to establish the best synergism between pharmacological therapy and mud-pack treatment, which is generally considered an integrative and not a substitutive support, and to develop rational and effective guidelines for such treatments in the management of musculoskeletal disorders. Considering the high use of non-steroidal antiinflammatory drugs and their frequently associated, sometimes-important side effects in the osteoarthritis population, even a small reduction in drug-associated toxicity

*It was impossible to obtain a colony number for *S aureus*.

would have a major impact on the costs in the healthcare system and on patients' quality of life.

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