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Anti-inflammatory effect of mud-bath applications on adjuvant arthritis in rats

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ABSTRACT

Objective. *The real effects of mud-bath applications on the inflammatory process are still not clarified. We studied these effects on rat adjuvant-induced arthritis.*

Methods. *Arthritis was induced in 30 rats by subplantar injection of Freund's complete adjuvant (FCA) into the right hind paw. Ten days after FCA injection, the rats were randomized in 3 groups of 10 each: the first one was submitted to a cycle of mud-bath applications, the second one was treated with indomethacin, the third one received only saline* per os *(control group). The paw volume, measured by plethysmometry, and the serum levels of TNFα and IL-1β were considered as evaluation parameters.*

Results. *FCA injection caused a progressive enhancement of paw volume and a rapid increase of TNFα and IL-1β serum levels. After the randomization, mud-bath applications reduced inflammation and at the end of the treatment the paw volume and the TNFα and IL-1β serum levels were significantly tapered in comparison to the controls ($p<0.01$).*

Conclusion. *The results of the study suggest an anti-inflammatory effect of mud-bath applications on adjuvant arthritis in rats. These results could explain the beneficial effects of thermal treatments observed in some inflammatory rheumatic diseases.*

Introduction

Spa therapy has been applied to patients with various rheumatic conditions since ancient times. Despite the difficulty to conduct randomized controlled trials, the efficacy of mud packs and thermal baths has been demonstrated in several open studies on musculoskeletal disorders (1).

A limit of spa therapy is the possibility that hot applications could induce a disease flare. Although some clinical trials have shown the beneficial effects of mud packs also in inflammatory rheumatic diseases such as rheumatoid arthritis (2) and ankylosing spondylitis (3), the real effects of these applications on the inflammatory processes are still not clarified.

Rat adjuvant-arthritis is widely used as experimental model, mimicking human arthritis. Freund's adjuvant induces an intense activation of macrophages, with production of high levels of pro-inflammatory cytokines and expansion of autoreactive T and B cells. T cells migrate to the joint and contribute to immune-mediated arthritis (4).

The aim of our work was to investigate the effects of a cycle of mud-bath applications on the paw volume and on the serum levels of pro-inflammatory cytokines TNFα and IL-1β in rat adjuvant-induced arthritis.

Materials and Methods

Animals

30 male Lewis rats (160-180 g) were obtained from Charles River (Calco, Italy) and maintained under standard conditions (light/dark cycle of 12 h; humidity: 55-60%; room temperature: 22±2°C) with food and water *ad libitum*. The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by U.S. National Institute of Health.

Induction of arthritis

Arthritis was induced in rats by a single subplantar injection of 0.1 ml Freund's complete adjuvant (FCA), a mixture containing 1 mg of heat killed *Mycobacterium tuberculosis* in 0.85 ml paraffin oil and 0.15 ml mannide monooleate, into the right hind paw. Paw volume was measured by plethysmometry (Basile, Comerio, Italy) just before and 2, 10, 15 and 20 days after adjuvant injection. The increase of paw volume with respect to the basal value (i.e. before FCA injection) was considered as a measure of joint inflammation.

Determination of IL-1β and TNFα in rat serum

Blood samples were collected from anesthetized rats through the tail vein on days 0.5 (12 h after inoculation of FCA), 2, 10, 15 and 20 after adjuvant injection. Serum IL-1β and TNFα levels were determined using ELISA kits specific for rats (Amersham Pharmacia Biotech, Cologno Monzese, Italy), in accordance with kit manufacture's indications.

Mud-bath

Thermal mud and water were obtained from Montegrotto Terme, a spa in the Euganean Thermal Area located near Padova in northern Italy.

The thermal water spurts from a well at 73C° and contains 6 g/l of mineral salts, in particular sodium chloride.

The mud is a blend of natural clay (94%) and organic substances (6%) produced from the maceration of algae, bacteria, protozoa and diatoms in special tanks where there is a continuous flow of thermal water.

Experimental design

Ten days after subplantar FCA injection, when arthritis was clearly developed, the animals were randomized in three groups of 10 rats each. The first group underwent thermal treatment, i.e. an application of 40-42°C mud for 15 minutes, followed by immersion in 37-38°C thermal water for 10 minutes. The second group was treated with indomethacin (Sigma, Milano, Italy) administered orally at a single daily dose of 3 mg/kg, suspended in 2% methylcellulose; this kind of drug administration, largely employed in rats to avoid gastrointestinal damage, allows to obtain a constant plasma concentration after 2 h and to mantain at least 40% of its value after 24 h (5). The third group, used as controls, received only saline per os. Each group received its respective treatment once a day from day 10 to day 20.

At the end of the treatments the rats were sacrificed and histological findings from paws were obtained. Serial sections were cut to a thickness of 5 µm, and stained with haematoxylin and eosin. Subsynovial inflammation, synovial hyperplasia, pannus formation, cartilage erosion and bone destruction were evaluated.

Statistical analysis

The time-course curves for paw volume, serum TNFα and IL-1B levels were subjected to two-way repeated measures analysis of variance (ANOVA) with post-hoc t-test.

Results

The volumes of the rat's paw are reported in Figure 1. The paw volume showed a rapid increase in the first 2 days after FCA injection (acute phase of inflammation) and a further progressive enhancement in the following days (systemic phase of inflammation).

After day 10, when the rats were randomized, mud-bath applications reduced inflammation and at day 20, at the end of the treatment, the paw volume was significantly reduced in comparison to the controls (1.95 ± 0.22 vs 2.34 ± 0.22 cm^3, $p<0.01$).

Indomethacin therapy caused a more rapid reduction of paw volume, significant both at day 15 (1.86 ± 0.11 vs 2.15 ± 0.33 cm^3, $p<0.05$) and at day 20 (2.03 ± 0.23 vs 2.34 ± 0.22 cm^3, $p<0.05$) in comparison to the controls.

Histhology, performed at the end of the treatments, showed the typical aspects of adjuvant arthritis in all rats. No significant differences were noted between the three groups.

The circulating levels of the pro-inflammatory cytokines are reported in Figures 2 and 3.

Basal serum levels of TNFα and IL-1β rapidly increased both 12 h after the FCAinjection, then significantly tapered after 2 days. In the following period, till randomization at day 10, TNFα levels increased again, while IL-1β levels continued to decrease.

At day 15, TNFα levels (Fig. 2) were significantly reduced both in rats treated with indomethacin ($p<0.01$) and in those submitted to mud-bath applications ($p<0.05$). At the end of the treatments, this reduction was similar in both groups of animals in comparison to the controls ($p<0.01$).

IL-1β levels (Fig. 3) showed a significant reduction at day 15 ($p<0.01$) and at day 20 ($p<0.01$) in rats submitted to thermal treatment and only at day 20 ($p<0.01$) in those treated with indomethacin, in comparison to the controls.

Discussion

Mud packs and thermal baths are among the oldest tools known to relieve symptoms of many rheumatic disorders. Spa therapy has been widely used in Europe, but, despite its popularity, the reported scientific evidence for its efficacy is poor and the mechanisms of action are still not identified (6, 7).

The principle mechanism of action of mud packs appears to be its analgesic effect, related to a neuroendocrine re-

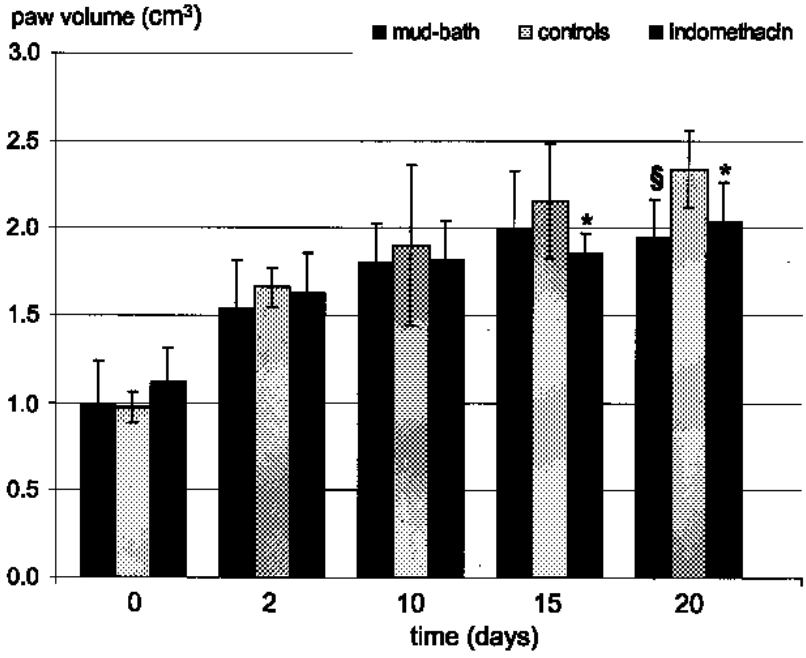

Fig. 1. Rats' paw volume before (time 0) and after Freund's adjuvant injection. Bars represent the mean ± SD for each group. Differences between groups were analyzed by two-way repeated measures analysis of variance (ANOVA) with the post-hoc t-test (*p < 0.05 vs controls, §p < 0.01 vs controls).

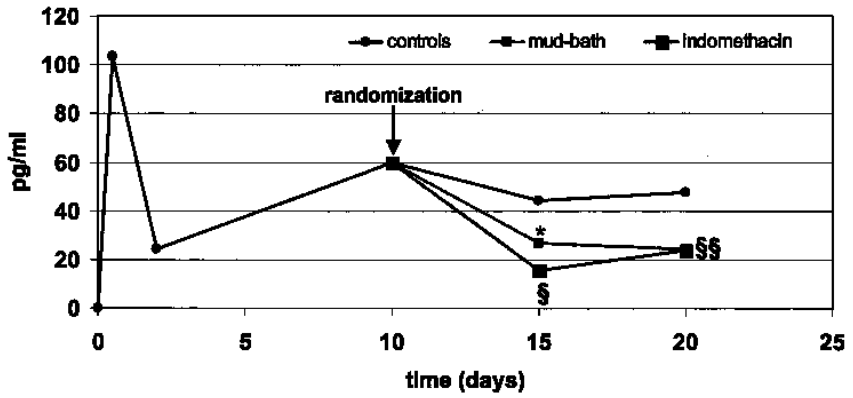

Fig. 2. Mean TNFα serum levels after Freund's adjuvant injection. Values are expressed as mean ± SD. Differences between groups were analyzed by two-way repeated measures analysis of variance (ANOVA) with the post-hoc t-test (*p < 0.05 vs controls, §p < 0.01 vs controls).

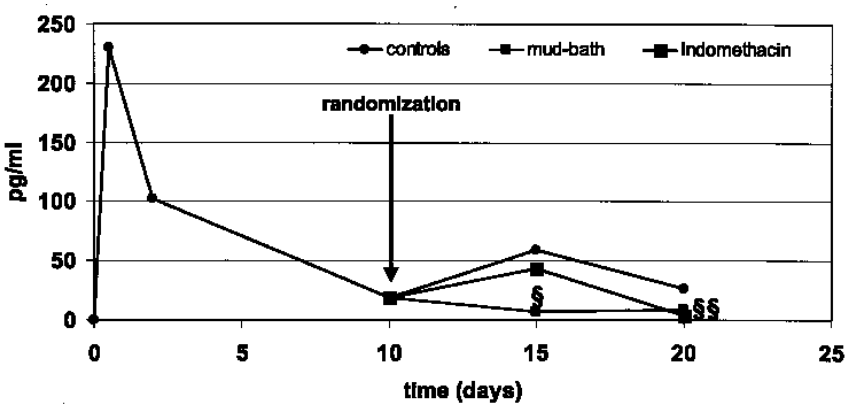

Fig. 3. Mean IL-1β serum levels after Freund's adjuvant injection. Values are expressed as mean ± SD. Differences between groups were analyzed by two-way repeated measures analysis of variance (ANOVA) with the post-hoc t-test (§p < 0.01 vs controls).

action causing an increase of serum levels of opioid peptides such as endorphins and enkephalins (8, 9). This beneficial effect could be nullified by inflammatory flares induced by heat applications. For this reason, the efficacy of mud packs and thermal baths in inflammatory joint diseases is still controversial and depends upon disease activity, while in degenerative diseases such as osteoarthritis (10) and fibromyalgia (11) is well documented.

Since experimental studies on the effects on inflammation of thermal applications are lacking, we decided to apply the mud and thermal water of Montegrotto Terme, a spa located near Padova in northern Italy, on rat adjuvant-induced arthritis. Rats develop severe local inflammation in the injected paw within 24 h after adjuvant injection, followed by systemic inflammation from day 10 up to day 28 (peaked typically at day 21) and by a slow remission after day 40. We performed the thermal applications on the rats at the same temperature and for the same length of time usually employed in patients submitted to thermal treatment in this spa.

It is well known that TNFα and IL-1β play a central role in the inflammatory synovitis both in experimental animal models and in human rheumatic diseases (12). In agreement with the literature (13), both the development of the adjuvant arthritis in the paw and the increase of TNFα serum levels have been observed in control animals.

Mud-bath applications led to a significant reduction of paw volume in comparison to the controls, higher than that obtained by indomethacin therapy at the end of the treatment. Moreover, TNFα and IL-1β serum levels were significantly lowered in rats submitted to thermal treatment and at the end of the experiment cytokines levels were similar to those observed in rats treated with indomethacin. The slow down of paw volume increase and the significant decrease of TNFα and IL-1β serum levels suggest an anti-inflammatory effect of mud-bath applications on rat adjuvant arthritis. It must be pointed out that the thermal treatment was applied from day 10 to day 20 after adjuvant injection, during the systemic phase of inflammation. We monitored the disease progression from the induction of arthritis until the disease peaked and the rats were sacrificed to perform hystologic evaluation.

We do not know why mud-bath applications have anti-inflammatory effects on the adjuvant-induced arthritis in rats. However it could be hypothized that pro-inflammatory cytokine TNFα and IL-1β levels might be down-regulated by means of the neuroendocrine reaction to the thermal treatment. Indeed further studies are needed to determine the mechanisms of action of mud packs and thermal baths on inflammatory processes. On the other hand, controlled clinical trials are mandatory to test the efficacy of thermal treatments in patients with inflammatory joint diseases.

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