Rheumatol Int (2007) 28:39–45
DOI 10.1007/s00296-007-0371-0

ORIGINAL ARTICLE

The efficacy of Brazilian black mud treatment in chronic experimental arthritis

Zélia Maria Nogueira Britschka · Walcy Rosolia Teodoro · Ana Paula Pereira Velosa · Suzana Beatriz Veríssimo de Mello

Received: 17 April 2007 / Accepted: 18 May 2007 / Published online: 12 June 2007

Abstract Studies have demonstrated the beneficial effects of fangotherapy on relieve of pain improving function of rheumatic patients. Herein, we investigated the effect of Brazilian black mud in protect articular damage in chronic arthritis induced in rats. Mud was daily applied (40°C/30 min) during the course of arthritis and was compared with warm water and no treated groups. At 21th day after arthritis induction synovial fluid and membrane were analyzed regarding cellular influx, hyperplasia and vascular proliferation. Cartilage structure, cell count, proteoglycan and collagen amount were also analyzed by three pathologists blinded to the treatment. Mud treatment diminished leukocyte migration into the synovial membrane and articular cavity when compared with both control groups. Regarding cartilage, an increase in collagen, number of chondrocytes and more conserved tissue structure was observed in mud-treated animals. These results demonstrate a protective effect of Brazilian mud on this model of arthritis, suggesting that this therapy may be useful as a complementary approach to treat articular diseases.

Keywords Mud therapy · Zymosan-induced arthritis · Synovial membrane · Cartilage

Introduction

Rheumatoid arthritis (RA) is an auto-immune disease in which inflammation of the synovial lining cells produces pain, swelling and progressive erosion of joints. It is believed that synovial inflammation is the primary event, which leads to cartilage destruction. Like other chronic diseases, the comprehensive management of RA involves more than classical therapy. Non-pharmacological therapies include exercises, rehabilitation procedures and complementary alternative medicine [1].

Fangotherapy consists of local application or whole body immersion in the mud; it has been used in Europe and Asia, and has been recommended as a complementary therapy for a great variety of rheumatic diseases [2–4], muscular pain disorders [5], and skin treatment [6]. Balneotherapy, combined with conventional treatment, has been associated with the improvement of the inflammatory signals of arthritis [7–9].

Some hypothesis has been suggested to explain their anti-inflammatory effects; however, none of them are conclusive. One of the discussion points is related to the mud maturation process, which can cause some alterations to the mud's physical characteristics [10–14]. Another point of discussion is the high heat retention capacity attributed to thermal mud associated with its analgesic effect, through opioid peptides as encephalin [11] and endorphins [15]. It has also been suggested that some of the positive effects of thermal mud could be attributed to the mineral composition which could be absorbed through the skin during application [4].

Studies on RA and OA patients treated with mud from Abano and Montegrotto Termes (Italy) have shown a decrease in the serum levels of tumour necrosis factor α (TNF-α), prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) [3, 16], of radical-mediated peroxidations, nitric oxide and myeloperoxidase [17, 18]. There are also evidences showing reduction of inflammatory parameters in clinical trials using fangotherapy from the Dead Sea [7, 8, 19].

Z. M. N. Britschka · W. R. Teodoro · A. P. P. Velosa · S. B. V. de Mello (✉)
Department of Internal Medicine, Rheumatology Division, Faculdade de Medicina da Universidade de São Paulo, Av. Dr. Arnaldo, 455, São Paulo 0124-6903, SP, Brazil
e-mail: svmello@usp.br

However, these clinical studies did not consider the possibility that spa therapy led to significant improvements in physical and mental activities resulting in amelioration of quality of life [20]. Recently, in the first experimental study with mud therapy Cozzi et al. [21] described the efficacy of mud from Abano and Montegrotto on adjuvant-induced arthritis in rats. They related the reduction of paw volume and TNF-α and IL-1β seric levels after mud or indomethacin treatment in comparison with the control group. It is important to emphasize that all these studies with mud therapy did not exclude temperature influence on amelioration of the inflammatory process.

Peruíbe, a city located in southern São Paulo State in Brazil, has a muddy coast know as Black Mud. This clay is formed by a mixture of sulphurous water and 30 minerals of volcanic origin, such as sulfur, natrium, zinc and calcium (personal communication). Its application has been regionally accepted by patients and physicians as an alternative treatment for RA and OA.

The present study was undertaken to first test, the ability of Brazilian black mud to protect the articular damage in zymosan induced arthritis in rats as well as to verify the temperature influence on this effect.

Materials and methods

Animals

Thirty male Wistar rats weighing 200–250 g at the beginning of the experiments were employed for zymosan-induced arthritis (Zy-IA). The animals were allowed a standard pellet diet and water ad libitum. During experimental procedures, the animals were anaesthetized to avoid any stress condition. The Animal Ethics Committee of COBEA (Brazilian College of Experimental Animals) has approved all experimental procedures performed on animals in accordance with procedures set by UFAW (The Universities Federation for Animals Welfar).

Zymosan-induced arthritis (Zy-IA)

Male rats were anaesthetized with 0.4 ml of 1.0 mg/kg of xylazine and 0.75 mg/kg of ketamine, and submitted to an intra-articular injection of 1.0 mg de zymosan (Sigma, Chemical Company, St. Louis, MO, USA) diluted in 50 µl of saline on the left knee, the right knee received same volume of saline [22, 23]. Animals were euthanased in a CO_2 chamber 21 days after the intra-articular challenge. The joints were washed twice with 0.5 ml saline, containing 10 mM EDTA, and the synovial wash was collected for the determination of total and differential cell counts using a Neubauer chamber and stained smears, respectively. Synovial membranes were surgically excised, paraffin-embedded and routinely processed.

Mud treatment

Black mud was obtained in Peruíbe, SP, Brazil. The mud was stored for 6 months in closed buckets with 10–15 cm fresh sea water collected from the same location as the mud. This maturation time is sufficient to provide mud free of contaminations.

Thirty rats were randomly distributed in three groups of 10 animals: untreated (control), water treated (temperature control) and mud treated (test). The protocol of treatment consisted of monitoring Zy-IA rats during 30 min walking in cages containing 300 ml of mud or warm water (40°C). This amount of mud or water was sufficient to cover all the knee joints of the animals, allowing them to walk freely in the cages. Concerning behavior response, no difference was observed in consequence of both treatments. The same procedure was performed daily during 3 weeks after arthritis induction.

Morphological analysis

All pieces used for histological analysis was fixed in 10% buffered formalin, embedded in paraffin and sectioned at 4 µm. Rats whole joints were decalcified in 6.0% EDTA. After inclusion sections (4 µm) were cut in the sagittal plane containing the long axis of the femur and analyzed histologically using hematoxylin-eosin (H&E), alcian blue 1.0% pH 2.5 for proteoglycan and Masson trichrome for collagen. The matrix stained with picro-Sirius red (collagen constituent) was also evaluated in polarized light microscopy.

The synovial membranes H&E stained were semi-quantitatively evaluated for vascular proliferation and lining hyperplasia giving a 0–3 score (0 = absence of alterations, 3 = high severity). These observations were made by three pathologists blinded to the treatment.

At × 400 of magnification, infiltrating leukocyte cells were counted by an eye-piece systematic point sampling grid with 100 points and 50 lines to count the fraction of points over-laying infiltrating cells. Ten microscopic fields were counted to obtain a final result expressed as a percentage of cells [24].

In cartilage tissue, the number of chondrocytes was also evaluated by an eye-piece systematic point sampling grid. A morphometric study of the cartilage was done by image analysis system (Leica Q500iW, Leica Imaging System. Cambridge, UK) in slices stained with picro-Sirius red. In brief, 25 fields of each slide were analyzed; and collagen amount expressed as a percentage of relative birefringent tissue/cartilage area.

Mankin's grade [25], a tissue-damage score (0–6), was used to verify the structure or erosion of cartilage:

0 = normal tissue, 1 = tissue with surface irregularities, 2 = pannus and surface irregularities, 3 = clefts to transitional zone, 4 = clefts to radial zone, 5 = clefts to calcified zone and 6 = complete disorganization. Proteoglycan content (0–4) was also evaluated by Mankin's grade; this score was attributed by three pathologists blinded to the treatment.

Statistical analysis

Results are expressed as mean ± s.e.m. To compare the differences between means, we used one-way ANOVA followed by student Newman Keuls test. The chosen level of significance was 0.05.

Results

Efficacy of mud treatment on inflammatory parameters

Figure 1 shows total and differential leukocyte counts in the synovial fluid knee joint of rats challenged intraarticularly with 1.0 mg of zymosan. This experimental model is characterized by a large infiltration of MN cells into the joint, indicating a chronic inflammatory response. The treatment consisted of daily paws immersion on warmed water or mud during 21 days. Our data clearly show that mud treatment significantly impaired the inflammatory process, reducing leukocyte migration into articular cavity when compared with an untreated group (PMN, $P = 0.008$; MN, $P = 0.03$). However, warm water treatment did not promote any alteration in cellular influx when compared with untreated control rats; this observation almost excluded the effect of heating in the anti-inflammatory efficacy of the mud.

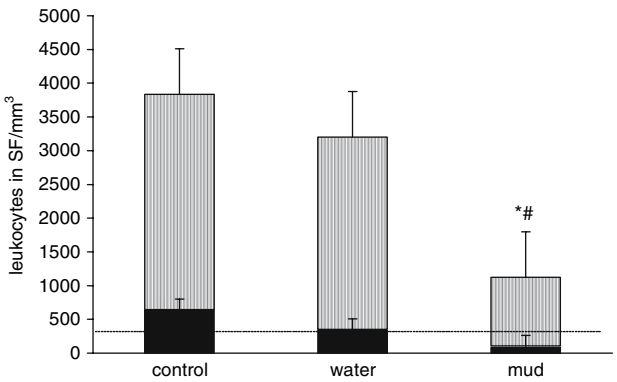


Fig. 1 Total and differential leukocyte counts in the joint wash of rats with Zy-IA. Untreated group ($n = 10$), water-treated group ($n = 10$) and mud treated group ($n = 10$). *PMN* polymorphonuclear (*dark bar*), *MN* mononuclear (*light bar*). Data are expressed as mean ± s.e.m. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. warm water control. *Hatched line* represents the cellularity in the synovial wash of the right knees injected with saline

Microscopic analysis of the synovial membrane of Zy-IA rats is summarized in Fig. 2a and depicted in Fig. 2b. Intense vascular proliferation, hyperplasia of the membrane and high cellular infiltration were observed in untreated animals. We did not verify significant difference between synovium extracted from untreated rats and those submitted to warm water treatment (Fig. 2a). It is important to note that mud treatment promotes a significant reduction in all analyzed parameters: cellularity ($P = 0.0001$ vs. control; $P = 0.02$ vs. water), hyperplasia ($P = 0.006$ vs. control and $P = 0.003$ vs. water) and vascular proliferation ($P = 0.003$ vs. untreated and $P = 0.06$ vs. water). An example of the arthritic lesion evoked by Zy intraarticular injection is showed in Fig. 2b1. The effect of mud treatment on reducing the inflammatory process in the synovial membrane is illustrated in Fig. 2b2. The surface of the membrane exhibited a single cell layer of synoviocytes, less number of vessels and reduction in cellular infiltration.

Efficacy of mud treatment on protection of cartilage damage

Morphological changes including focal and diffuse erosion of cartilage were observed in blades stained with H&E (not shown) and Masson trichrome in joints from untreated Zy-IA animals (Fig. 3a) and animals treated with warm water (Fig. 3b). Frequently, pannus were seen on the surface of the affected cartilages. In some cases, the inflammatory process had extended through the epiphyseal bone into the adjacent bone marrow. Erosion or complete disarrangement of the articular cartilage was seen in most joints (arrows). It is important to emphasize that mud treatment, here employed also preserved the articular space and cartilage integrity when compared with both control arthritic groups, as shown by arrow in Fig. 3c.

Cartilage structure was evaluated by Mankin's score (0–6), which summarize the alteration of the tissue. The attributed score (4.9 for untreated and 5.27 for water treated rats) reflects serious damage in cartilage that exhibited surface irregularities followed by clefts until the calcified zone (Fig. 4). The score of 3.5 attributed for cartilages from mud-treated animals denote more conserved structure with less erosion ($P = 0.03$ vs. control and $P = 0.04$ vs. water).

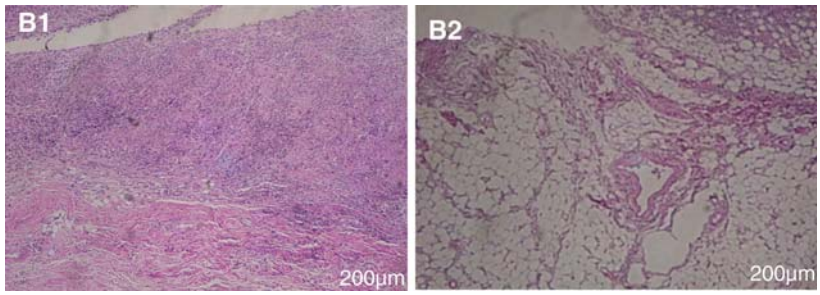
The effectiveness of mud treatment to prevent cartilage damage can also be observed in Figs. 5 and 6, cartilage from Zy-IA control groups presented reduction in the number of chondrocytes and loss of collagen net, respectively. The treatment with mud promotes an remarkable increase (50%) in the number of chondrocytes as shown in Fig. 5 as well as the preservation of collagen amount evidenced in blade stained with picro-Sirius red ($P = 0.04$ vs. water and $P = 0.03$ vs. control) (Fig. 6). Proteoglycan staining, although reduced in Zy-IA was not altered by mud treatment (not shown).

A

Histological Parameters	Animals		
	Control (untreated) (n=10)	Water Treated (n=10)	Mud Treated (n=10)
Hyperplasia	2.8 0.08	2.6 0.18	1.73 0.23*#
N° of vessels	2.4 0.23	2.3 0.24	1.73 0.22*#
Cell counts	25.21 x 10^{-2} 1.52	21.06 x 10^{-2} 2.67	13.8 x 10^{-2} 2.13*#

Semiquantitative evaluation for synovial vascular proliferation and lining hyperplasia (0-3). Cell number were quantified by sampling grid and expressed as percentages (number of points overlying the cells divided by total number of points overlying tissue). Results are expressed as means s.e.m. *p <0.05 vs. control; # p<0.05 vs. warm water

Fig. 2 **a** Summarize the parameters evaluated in the synovial tissue from rat knee with Zy-IA in the 21st day. The synovium from control animals stained with H&E is depicted in **b1**. Sub-synovial infiltration and maintenance of the synovial lining could be observed in samples obtained from mud-treated rats (**b2**). Original magnification ×100



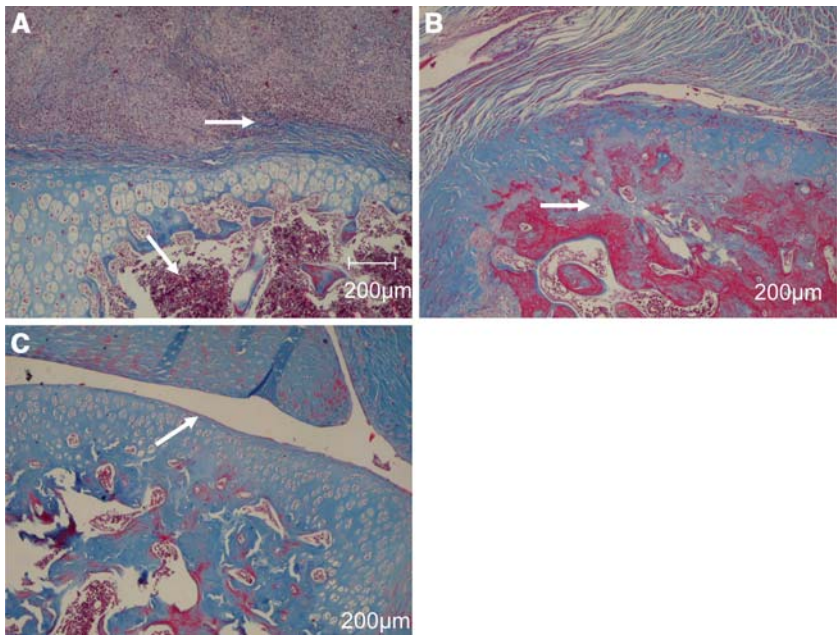


Fig. 3 Photomicrographs of knee joint from rats with Zy-IA in the 21st day stained with Masson trichrome. The pattern observed in untreated animals is shown in **a**, in the water treated group in **b**. *Arrows* indicate cartilage erosion with the development of fibrous cartilage, overgrown pannus and subchondral bone damage. **c** The pattern of the joint from mud-treated group, *arrow* shows the maintenance of the articular space and cartilage tissue. Original magnification × 100

Discussion

Topical application of Brazilian black mud can produce the reduction of articular damage as suggested by following observations. First, the schedule of treatment here employed suppresses cellular infiltration into the articular cavity and synovial membrane, reducing inflammation of Zy-IA. Second, cartilage tissue was also preserved since the number of chondrocytes, the structure of the tissue and lost of collagen amount were impaired by mud treatment, and third, the protective effect of mud was more efficacious than water applied at the same temperature.

It is important to emphasize the importance of the use of experimental model to access mud efficacy excluding the influence of medication or psychological factors. For this purpose, we choose a chronic experimental model (Zy-IA), which partially resembles RA in human, with progressive synovitis and articular deformity. Acute phase of Zy-IA is characterized by increase in vascular permeability and polymorphonuclear infiltration, 7 days after the intraarticu-

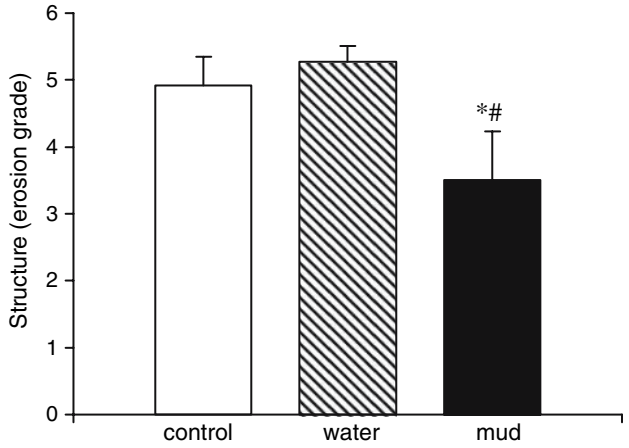


Fig. 4 Histological evaluation of cartilage structure of Zy-IA in rats in 21st day. The score was attributed by Mankin's graduation (0–6). Untreated group (*open bar*) ($n = 4$), warm water-treated group (*crossed light bar*) ($n = 5$) and mud-treated group (*dark bar*) ($n = 4$). Data are expressed as mean ± s.e.m. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. warm water

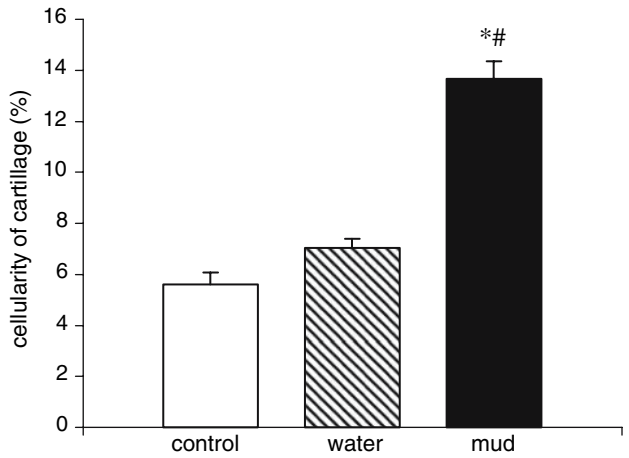


Fig. 5 Morphometric analysis of cellularity cartilage of Zy-IA animals. Untreated (*open bar*) group ($n = 4$), warm water-treated group (*crossed light bar*) ($n = 5$) and mud-treated group (*dark bar*) ($n = 4$). Chondrocyte number quantified by an eyepiece systematic point sampling grid is expressed as a percentage (number of points overlying the cells divided by total number of points overlying tissue). Data are expressed as mean ± s.e.m. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. warm water

lar challenge, a chronic arthritis can be observed, characterized by mononuclear cell infiltration, synovial hypertrophy and pannus formation [26, 27]. Corroborating this finding, our data in the 21st day after arthritis induction show the predominance of mononuclear cells in the synovial infiltrate. At this time mud, but not water treatment reduced significantly both polymorphonuclear and mononuclear cells influx into the articular cavity. In a previous study on antigen-induced arthritis in rabbits, we did not detect any difference on these parameters using a short-time therapy (personal communication). Experimental studies using

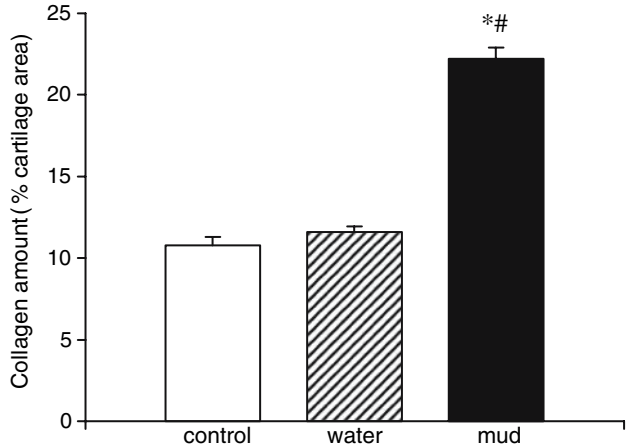


Fig. 6 Collagen amount in the cartilage from Zy-IA animals. Untreated group (*open bar*) ($n = 4$), water-treated group (*crossed light bar*) ($n = 5$) and mud-treated group (*dark bar*) ($n = 4$). Collagen amount is expressed as a percentage of relative birefringent tissue/cartilage area. Data are expressed as mean ± s.e.m. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. warm water

hyperthermia or local application of heat showed reduction of the development of chronic and proliferative inflammation, whereas exacerbate the acute process [28–30].

Destructive alterations present on RA patients were also observed on Zy-IA rats. The synovium of untreated control and treated water groups showed evident hyperplasia with predominance of monocytes as well as neovascularization. The synovial membrane from Zy-IA joints treated with mud maintains a single cell-layer of synoviocytes, a slight vascular proliferation and less cellular infiltration. The persistent synovitis during the acute phase of arthritis, as well as the neutrophil infiltration with consequent release of lytic enzymes is one of the steps that will reflect on cartilage degradation, on inhibition of proteoglycan and collagen synthesis [31]. In control rats, the cartilage damage was well characterized by matrix clefts, loss of chondrocytes, collagen and proteoglycan amount. Excluding proteoglycan content, warm mud application stimulated both hypercellularity and collagen synthesis. It is known that collagenases act mainly on collagens I, II and III while gelatinases degrade collagens and proteoglycans in response to IL-1 and TNF-α secreted by synoviocytes and chondrocytes [32], suggesting that mud therapy can have a possible inhibitory effect on different metalloproteinases. Recently, Bellometti et al [33] investigated the effectiveness of fangotherapy in the reduction of the damage of cartilage by influence of the decrease of seric concentrations of MMP-3 while seric levels of MMP-8 and MMP-9 increased. Thus, it is possible that the results found in our study, concerning the content of proteoglycans could be due to the action of different metalloproteinases released as a result of mud application.

The increased staining of collagen observed in cartilages of mud-treated animals may possibly represent a tentative of tissue repair. These histological observations are in agreement with Bellometti et al. [16], which have reported decrease of TNF-α and a significant increase of IGF-1 after mud treatment that can act as an extra-cellular inhibitor of metalloproteinases and stimulator of proteoglycan synthesis. In RA patients with fever lower than 41°C, these cartilage-degrading enzymes were found more active, whereas above this temperature their activities decrease [34, 35]. Bellometti et al. [36] have also demonstrated that mud therapy combined with physical exercise was effective in increase anabolic parameters of bone metabolism through inducing proinflammatory cytokines. These results were corroborated by Schimidt [37] who described heat treatment and exercises associated with reducing of pain, and impairment of muscle tone, mobility and blood flow.

The concerning mechanism of anti-inflammatory action of mud, the experimental study with adjuvant arthritis has shown a significant reduction of paw volume simultaneously to the impairment of TNF-α and IL-1β serum levels [21]. Similar results were verified in humans submitted at whole body Abano-Montegrotto Terme mud immersion at 40°C/15 min followed by a sulphur bath at 37–38°C [38]. It is know that TNF-α is the cytokine firstly activate with the consequent induction of other proinflammatory mediators [31]. The decrease of the level of TNF-α can be associated with a reduction of LTB_4 and PGE_2 serum levels that occur simultaneously with pain amelioration [3]. IL-1, IL-8 and LTB_4 are potent chemoatractants for leukocytes; consequently, a reduction of their levels on the inflammatory site could be responsible for the observed impairment of cell influx here detected. Basili [39] speculated that mud-pack treatment might counteract the heat-stress-related effects on platelet and endothelial cell function. In their studies, plasma samples from healthy volunteers subjected to a cycle of 12 daily mud-pack applications showed no changes in proinflammatory cytokines (TNF-α, Il-1β) or adhesion molecules (sP-selectin, sE-selectin and sVCAM). It has been suggested that IL-6 could be the responsible for mud efficacy since a decrease of IL-6 levels was correlated with the impairment of neutrophil infiltration. Fairchild et al. [40] showed the reduction in TNF-α, IL-6 and IL-1β concentration in monocyte culture submitted to 40°C during 24 h. However, Samborski et al. [41] showed an increase of IL-6 levels and activation of the inflammatory process 1 h after therapy with hot mud. Therefore, the exact mechanism of action of fangotherapy is until now unknown.

It has also been proposed that the mechanism of action of the mud appears to be related to a neuroendocrine action. Bellometti and Galzigna [15] claim that the action of fangotherapy on remission of the pain is based on the fact of the heat slowly transferred to the body can modulate the inflammatory response through a neuroendocrine activation, promoting an increase of seric levels of opioids peptides, such as endorphins and encephalins. Sauna-induced hyperthermia also resulted in an increase of plasma β-endorphin and ACTH [42], indicating that heat treatment can trigger off a well-defined neuroendocrine reaction.

The contribution of temperature to the efficacy of mud therapy could not be excluded. Temperature per se could influence the inflammatory reaction; heat and cold may have anti- or proinflammatory effect depending on the etiologic agent, length of the therapy as well as the phase of the disease. Experimental studies evaluating inflammatory parameters such as oedema, pain and articular tissues damage corroborate this affirmation [28, 29]. Additionally, data of balneotherapy and physical therapy in osteoarthritic patients showed the beneficial effect of hot temperature in the amelioration of pain, metabolism of cartilage and secondary inflammation [37]. The contribution of temperature to the absorption of ions or substances that could be responsible by the anti-inflammatory efficacy of the mud is suggested by the assertion that heating is evoked during mud pack therapy promoting microcirculatory changes [43]. Studies with a microthermometer are in process in our laboratory to determine intraarticular temperature changes during mud therapy in rats.

Conclusion

Together our results demonstrate the beneficial effect of the Brazilian black mud in the amelioration of arthritis, reducing inflammation and inducing tissue repair of cartilage. There is a lot still to be determined about the mud's anti-inflammatory mechanism of action; however it may constitute in future in new substance potently useful to treat pain and inflammation as a co-adjuvant of traditional therapy.

These data corroborate traditional human treatment employing mud, which consists of a cycle of twelve applications for resulting in relief of pain, indicating that the timing, length, and frequency of procedure may be important factors in reduction of joint inflammation by heat treatment.

Acknowledgments The authors are grateful to Department of Pathology of Medical School of São Paulo University especially to Ana Lúcia Garippo and to Dr. Edwin Rocher Parra for his help in the morphometric analyses. We thank Cassia Arruda, Maria Aurora Gomes da Silva, Maria de Fátima de Almeida and Maria Luisa Guzzo for skillful technical assistance. We are indebted to Peruibe city concil who provided us with Black Mud. This work was partially supported by CAPES.

References

1. Taibi DM, Bourguignon C (2003) The role of complementary and alternative therapies in managing rheumatoid arthritis. Fam Community Health 26(1):41–52

2. Sukenik S, Buskila D, Neumann L, Keluiner-Baumgarten A (1992) Mud pack therapy in rheumatoid arthritis. Clin Rheumatol 11(2):243–247
3. Bellometti S, Galzigna L (1990) Serum levels of a prostaglandin and a leukotriene after thermal mud pack therapy. J Invest Med 46(4):140–145
4. Flussner D, Abu-Shakra M, Friger M, Codish S, Sukeni S (2002) Therapy with mud compress for knee osteoarthritis: comparison of natural mud preparations with mineral-depleted mud. J Clin Rheumatol 8(4):197–203
5. Grossi F, Mastroiani S, Conigliaro R, Quadrani V, Gatti R (1993) Effetti di tratamenti termali balneo-fangoterapici sulla patologia reumática extra-articolare in rapporto ad attivitá sportiva. Clin Ter 143:417–420
6. Carabelli G (1998) Effetti dell"uso di un fango su cute normale, secca e seborroica. Clin Ter 149:271–275
7. Sukenik S, Buskila D, Neumann L, Kleiner-Baumgarten A, Zimlichman RS, Horowitz J (1990) Sulphur bath and mud pack treatment for rheumatoid arthritis at the Dead area. Ann Rheum Dis 49:99–102
8. Sukeni S (1998) Balneotherapy for rheumatic diseases at the Dead Sea. Int Dermatol 37(9):717–718
9. Elkayam D (1991) Effect of spa therapy in Tiberias on patients with rheumatoid arthritis and osteoarthritis. J Rheumatol 18:1799–1803
10. Cara S, Carcangia G, Padalino G, Palomba M, Tamanini M (2000) The bentonites in pelotherapy: chemical mineralogical and technological properties of materials form Sardinia deposits (Italy). Appl Clay Sci 16:117–124
11. Carretero MI (2002) Clay minerals and their beneficial effects upon human health: a review. Appl Clay Sci 21:155–163
12. Veniale F (2004) Formulation of muds for pelotherapy: effects of "maturation" by different mineral waters. Appl Clay Sci 25(3, 4):135–148
13. Galzigna L, Cheschi-Berrini E, Moschin E, Tolomio C (1998) Thermal mud-pack as an anti-inflammatory treatment. Biomed Pharmacother 52:408–409
14. Galzigna L, Moretto C, Lalli A (1996) Physical and biochemical changes of thermal mud after maturation. Biomed Pharmacother 50:306–308
15. Bellometti S, Galzigna L (1999) Function of the hypothalamic adrenal axis in patients with fibromyalgia syndrome undergoing mud-pack treatment. Int J Clin Pharmacol Res 19(1):27–33
16. Bellometti S, Cecchettin M, Galzigna L (1997) Mud pack therapy in osteoarthrosis. Changes in serum levels of chondrocyte markers. Clin Chim Acta 268:101–106
17. Bellometti S, Cecchettin M, Lalli A, Galzigna L (1996) Mud pack treatment increase serum antioxidant defenses in osteoarthritic patients. Biomed Pharmacother 50:37–39
18. Bellometti S, Poletto M, Gregotti C, Richelmi P, Berte F (2000) Mud bath therapy influences nitric oxide, myeloperoxidase and glutathione peroxides serum levels in arthritic patients. Int J Clin Pharmacol Res 20(3–4):69–80
19. Neumann L (2001) The effect of balneotherapy at the Dead Sea on the quality of life of patients with fibromyalgia syndrome. Clin Rheumatol 20:15–19
20. Cherkin D (1998) Spa therapy: panacea or placebo? Med Care 36(9):1303–1305
21. Cozzi F, Carrara M, Sfriso P, Todesco S, Cima L (2004) Anti-inflammatory effect of mud-bath applications on adjuvant arthritis in rats. Clin Exp Rheumatol 22:763–766
22. Ramos AMO, Soares MLM, Rocha LRM, Jerônimo SMB, Rocha FAC, Patrício FRS (2001) Zymosan-induced arthritis in rats: histopathological, histochemical, and biochemical study of the articular cartilage. Rev Bras Reumatol 41(4):205–212
23. Rocha FAC, Rocha JCS, Peixoto MEB, Jancar S, Cunha FQ, Ribeiro RA (2003) Effect of nitric oxide syntase in articular inflammatory pain and cellular influx of zymosan-induced arthritis in rats. Rev Bras Reumatol 45(4):206–217
24. Pinto CA (2003) Morphometric evaluation of tumor matrix metalloproteinase 9 predicts survival after surgical resection of adenocarcinoma of the lung. Clin Cancer Res 9:3098–3104
25. Mankin HJ, Dorfman MD, Lippiello L, Zarins A (1971) Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips II Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg 53A(3):523–537
26. Keystone EC, Schorlemmer HU, Pope C, Allison AC (1977) Zymosan-induced arthritis: a model of chronic proliferative arthritis following activation of the alternative pathway of complement. Arthritis Rheum 20:1396–1401
27. Gegout P, Gillet P, Chevrier D, Guingamp C, Terlain B, Nette RP (1994) Characterization of zymosan-induced arthritis in the rat: effects on joint inflammation and cartilage metabolism. Life Sci 55:321–326
28. Schmidt KL, Ott VR, Röcker G, Schalter H (1979) Heat, cold and inflammation. Z Rheumatol 38:391–404
29. Ostberg JR, Taylor SL, Baumann H, Repasky EA (2000) Regulatory effects of fever-range whole-body hyperthermia on the LPS-induced acute inflammatory response. J Leukoc Biol 68(6):815–820
30. Oosterveld FG, Rasker JJ (1994) Treating arthritis with locally applied heat or cold. Semin Arthritis Rheum 24(2):82–90
31. Vassalli P (1992) The patophysiology of tumor necrosis factors. Ann Rev Immunol 10:411–452
32. Nagase H (1997) Activation mechanisms of matrix metalloproteinases. Biol Chem 378:151–160
33. Bellometti S, Richelni P, Tassoni T, Berte F (2005) Production of matrix metalloproteinases and their inhibitors in osteoarthritic patients undergoing mud bath therapy. Int J Clin Pharmacol Res 25:77–94
34. Castor CW, Yaron M (1976) Connective tissue activation: VIII. The effect of temperature studied in vitro. Arch Phys Med Rehabil 57:5–9
35. Harris ED, McCroskery PA (1974) The influence of temperature and fibril stability on degradation of cartilage collagen by rheumatoid synovial collagenases. N Engl J Med 290:1–6
36. Bellometti S, Bertè F, Richelmi P, Tassoni T, Galzigna L (2002) Bone remodeling in osteoarthrosic subjects undergoing a physical exercise program. Clin Chim Acta 325:97–104
37. Schimidt KL (1991) Physical therapy and balneotherapy of arthritis. Ther Umsch 48:46–51
38. Cecchettin M, Bellometti S, Lalli A, Galzigna L (1995) Serum interleukin 1 changes in arthrosic patients after mud-pack treatment. Phys Rheab Kur Med 5:92–93
39. Basili S (2001) Effects of mud-pack treatment on plasma cytokine and soluble adhesion molecules levels in healthy volunteers. Clin Chim Acta 314:209–214
40. Fairchild KD, Viscardi RM, Hester L, Singh IS, Hasday JD (2000) Effects of hyperthermia on cytokine production by cultures human mononuclear phagocytes from adults and newborns. J Interferon Cytokine Res 20:1049–1055
41. Samborski W, Sobieska M, Mackiewicz T, Stratz T, Mennet M, Muller W (1992) Can thermal therapy of ankylosing spondylitis induce an activation of the disease? Z Rheumatol 51:127–131
42. Vescosvi PP, Gerrra G, Pioli G, Pedrazzoni M, Maninerri L, Passeri M (1990) Circulating opioid peptides during thermal stress. Horm Metab Res 22:44–46
43. Poensin D, Carpentier PH, Féchoz C, Gasparini S (2003) Effects of mud pack treatment on skin microcirculation. Joint Bone Spine 70:367–370