

THE EFFECT OF MUD-BATH THERAPY ON BONE STATUS IN RATS DURING ADJUVANT SUBCHRONIC ARTHRITIS

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Summary: Introduction: We studied influence of mud-bath on bone status in male Wistar rats with subchronic arthritis. Methods: Arthritis was induced by 2 subplantar injections of Freund's adjuvans with heat-killed *Streptococcus pyogenes* into paw. Groups: intact (int) on chippings; (con) arthritis on chippings; (san38) arthritis on hot sand; (mu38) arthritis on hot mud; (mu21) arthritis on mild mud. Bone mineral density (BMD, g/cm²) was measured by dual energy X-ray absorptiometry and femurs were tested biomechanically. Bone markers osteocalcin (OC), PINP and CTX were analysed in bone. Results: BMD of right femur decreased vs. left in san38 (p = 0.030) and mu38 (p = 0.047). Fracture load of right/left femur (N) decreased in experimental groups, significantly in san38 (p = 0.05). Fracture threshold of neck decreased in right vs. left in experimental groups, but significantly in san38 (p = 0.05). OC decreased in mu38 vs. con (1.84 ± 0.14/2.62 ± 0.23). PINP decreased in int vs. san38 (p = 0.005) and mu21 (p < 0.001). CTX decreased in int vs. mu38 (p = 0.006) and mu21 (p = 0.005). Conclusion: The hot bath appears indifferent in relation to osteoporosis, while cold mud-bath shows good effect on bone metabolism. The cold mud-baths help to reduce arthritic inflammation and pain and thereby lead to higher mobility with positive consequence on bone.

Key words: Mud-bath; Rat; Arthritis, Bone

Introduction

Mud has been a therapeutic tool in use for 25 centuries. Mud packs have a place as a non-pharmacological tool in certain clinical settings, such as degenerative articular processes, selected skin disorders, and others. The mud down-regulates beta-endorphin and stress hormones in patients with osteoarthritis by reducing inflammation and pain, thus diminishing sources of stress (1, 2).

Mennuni (3) has demonstrated the positive effect of mud-bath treatment on patients with spine osteoarthritis (4). In advanced spondyloarthritis an increase in spinal BMD is observed; proximal femur measurements appear to be less affected by disease-related new bone formation (5, 6).

Many studies have demonstrated the influence of mud-bath therapy on inflammation, but not on bone metabolism. Some authors have emphasized the content of calcium in several mineral clays and potential transdermal transport of calcium ion into the body (7). The mud-bath induces a decrease in cytokines with bone-resorbing effect. On the contrary the warm leads to hyperemia and loss of bone mineral (8), and the arthritic patients have also increased levels of pro-inflammatory cytokines (9), which may have an ultimate impact on bone tissue, resulting in changes in BMD (2, 10, 11).

Active osteoblasts exprime aminoterminal propeptide of procollagen type 1 (PINP) and osteocalcin (12). Osteocalcin

(OC) and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) are valuable markers for detection of bone loss in routine clinical practice (6). We analyse markers from bone tissue: osteocalcin, PINP and CTX.

This study aimed to determine the effect of repeated mud-baths on bone status in male albino Wistar rats with subchronic adjuvant arthritis following unloading of painful pads. To determine the effect of bath temperature on inflammatory processes, we added a group with a hot sand bath, and we also added another group with a cold mud-bath. Patients usually use a hot mud-bath, and therefore our experiment contained a group with mud-bath at 38 °C. Bone status was diagnosed using DXA, markers of local bone production, and the load necessary for fracture of femurs.

Material and methods

Freund's adjuvans preparation. A mixture of 5 g paraffin oil, 5 g lanoline and 5 ml of heat-killed *Streptococcus pyogenes* B stock (Department of Microbiology, University Hospital, Hradec Kralove, CZ) was maintained in an ultrasound bath for 60 minutes to obtain a suspension.

Arthritis induction. The arthritis was induced by an injection of 100 µl of Freund's adjuvans suspension into the plantar surface of the right hind paw on the first and eighth day of experiment. "Intact" sham animals were injected

similarly with saline solution. The acute arthritic phase lasted 1 week, followed by chronic, mild joint swelling.

Animals. All animal procedures were performed in accordance with the guidelines issued by the Local Ethical Committee (No. 27139/2006-30/300). Adult albino Wistar rats (Biotest Ltd., Konarovice, CZ) were placed in plastic cages according to standard conditions. The rats were divided into 5 groups (six rats in each group); their initial body weight was 265 ± 7 g. All painful manipulations were performed under total ether anesthesia. All the rats received 34 bath-mud applications for 20 minutes 4–5 times per week. **Group 1: int** – intact control (without arthritis), on dry chippings at 21 °C. **Group 2: con** – rats with arthritis, with bath on dry chippings at 21 °C. **Group 3: san38** – rats with arthritis, with bath on hot dry sand at 38 °C. **Group 4: mu38** – rats with arthritis, with bath on hot wet mud at 38 °C. **Group 5: mu21** – rats with arthritis, with bath on wet mud at 21 °C. The rats were sacrificed under ether anesthesia by exsanguination from the abdominal aorta after 50 days of experiment.

Methods. Bone mineral density (BMD) was performed in Osteocentre, University Hospital and Medical Faculty in Hradec Kralove by dual-energy X-ray absorptiometry DXA, Hologic (Waltham, MA USA). BMD was measured whole body (Total), all bones of body (Net) and in three parts: lumbar and caudal vertebrae and diaphysis of the femurs. After animal sacrifice, both femurs and proximal tail vertebrae were carefully excised and stored at -80 °C until required. Tail vertebrae were selected: frozen samples (150 mg) were minced in a phosphate buffer (1.5 ml) and then disrupted and homogenized in a MagNA Lysor instrument (Roche Applied Science, Germany). The supernatant was separated and the concentrations of the bone markers OC, CTX-I and PINP in the bone homogenate were assayed using a commercial rat ELISA kit manufactured by the company Usnlife Sciences & Technology Co., Ltd., China (OC RatLaps™ EIA, $\mu\text{g/l}$; CTX-I, RatLaps™ EIA, $\mu\text{g/l}$; PINP, Rat/Mouse PINP EIA, $\mu\text{g/l}$).

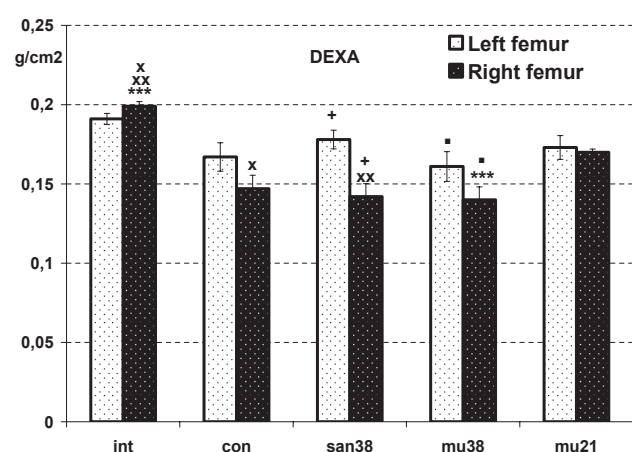


Fig. 1: DXA of both femurs (R1 and R2; g/cm²)

Bone biomechanical testing. Both femurs were de-frosted in vet mull (saline solution). The mechanical properties of the femurs were measured using a special custom-made testing machine (Martin Kosek & Pavel Trnecka, Hradec Kralove, CZ). This method has been described in detail previously (13, 14).

Statistical analyses (Holm-Sidak and Dunn's methods, One way Anova, *paired t-test*) were performed using software "SigmaStat 3.1" Jandel Scientific, San Rafael, CA, USA. The data were expressed as mean \pm SE or median (percentile 25–75%). One character represents statistical significance $p < 0.05$, two characters represent $p < 0.01$ and three characters represent $p < 0.001$.

Results

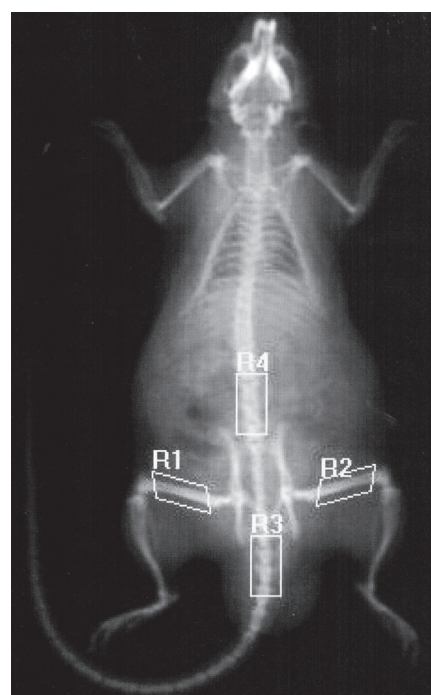


Fig. 2: BMD evaluation in areas of the rat skeleton

Bone mineral density of the right femur (g/cm²) was significantly lower in groups con ($p < 0.05$), san38 ($p < 0.01$) and mu38 ($p < 0.001$) compared with group int. BMD of the right femur was significantly decreased versus BMD of the left femur in groups san38 ($p = 0.030$) and mu38 ($p = 0.047$).

The load for three-point fracture of right and left femurs was lower in all experimental groups compared with group int, and was significantly so in groups con (left femur $p = 0.009$; right femur $p = 0.015$) and san38 (left $p = 0.063$; right $p = 0.012$). When we compare the force for fracture for group con, then there was statistically significant increase only in group mu38 on the left femur ($p = 0.049$).

The bone mineral density of lumbar vertebrae was increased in group mu38 compared with group int or con ($p = 0.05$). There was an increase in tail BMD in group mu21 compared with group int ($p = 0.05$).

The load for fracture of femur neck was lower in all experimental groups compared with group int, and was significantly in groups con (right neck $p < 0.005$), san38

(right neck $p < 0.05$). On the other hand the load for fracture of femur neck was higher in mu38 vs con (left neck $p < 0.01$).

In contrast to intact rats, the load for neck fracture of right femurs in comparison with left femurs was lower in all experimental groups, but significantly only in group san38 ($p = 0.05$) and mu38 ($p = 0.05$).

Tab. 1: DXA lumbal spine (R4), tail (caudal vertebra; R3), total and net BMD (g/cm^2)

	Lumbal vertebra	Caudal vertebra	Total	Net
int	0.217 ± 0.004	0.210 ± 0.008	0.158 ± 0.004	0.201 ± 0.006
con	0.216 ± 0.006	0.214 ± 0.006	0.160 ± 0.004	0.203 ± 0.005
san38	0.217 ± 0.006	0.218 ± 0.005	0.159 ± 0.003	0.205 ± 0.007
mu38	0.227 ± 0.008 $p = 0.05$ vs. int, con	0.220 ± 0.004	0.159 ± 0.004	0.216 ± 0.006
mu21	0.220 ± 0.005	0.223 ± 0.002 $p = 0.05$ vs int	0.160 ± 0.001	0.205 ± 0.004

Tab. 2: Three point fracture of left and right femurs (N) and the force for fracture of left and right neck (N)

	Force for fracture of left femur (N)	Force for fracture of right femur (N)	Force for fracture of left neck (N)	Force for fracture of right neck (N)
int	229 ± 12	224 ± 11	142 (134–157)	150 (135–168)
con	176 ± 11 $p = 0.009$ vs. int	186 ± 7 $p = 0.015$ vs. int	130 (113–136)	117 (106–120) $p < 0.005$ vs. int
san38	195 ± 11 $p = 0.063$ vs. int $p = 0.250$ vs. con	174 ± 12 $p = 0.012$ vs. int $p = 0.408$ vs. con	139 (128 – 177)	115 (107–146) $p < 0.05$ vs. int
mu38	204 ± 6 $p = 0.049$ vs. con	182 ± 8 NS vs. con	169 (160–196) $p < 0.01$ vs. con	130 (129–154)
mu21	195 ± 12 $p = 0.250$ vs. con	177 ± 7 $p = 0.385$ vs. con	140 (131–182)	139 (124–161)

Tab. 3: Concentration of osteocalcin, PINP, and CTX from homogenate of tail bone ($\mu\text{g}/\text{l}$)

	OC	PINP	CTX
int	2.3 ± 0.2	11.4 ± 1.2	0.275 ± 0.032
con	2.6 ± 0.2	23.3 ± 1.9 $p < 0.001$ vs. int	0.598 ± 0.043 $p = 0.006$ vs. int
san38	2.4 ± 0.2	24.4 ± 4.3 $p = 0.005$ vs. int	0.586 ± 0.083 $p = 0.006$ vs. int
mu38	1.8 ± 0.1 $p = 0.005$ vs. con $p = 0.049$ vs. int	23.6 ± 3.4 $p = 0.005$ vs. int	0.660 ± 0.085 $p = 0.006$ vs. int
mu21	2.6 ± 0.2 $p = 0.005$ vs. mu38	34.2 ± 3.1 $p < 0.001$ vs. int $p = 0.014$ vs. con NS vs san38 $p = 0.044$ vs mu38	0.783 ± 0.132 $p = 0.005$ vs. int NS vs con, san38, mu38

A lower concentration of osteocalcin was observed in group mu38 compared not only with groups con ($p = 0.005$) and int ($p = 0.049$), but also with group mu21 ($p = 0.005$). There were higher concentrations of PINP in all experimental groups compared with intact rats, and the increase was statistically-significant in all groups: group con ($p < 0.001$), group san38 ($p = 0.005$), group mu38 ($p = 0.005$) and group mu21 ($p < 0.001$). Compared with group con, the concentration of PINP in bone homogenate was increased significantly only in group mu21 ($p = 0.014$). There were higher concentrations of CTX in all experimental groups in comparison with group int.

Discussion

The increased bone turnover and activity of inflammation are important in the pathophysiology of arthritis-related osteoporosis (6). The results of the study demonstrate the efficacy of the mud-bath treatment on patients affected by lumbar spine osteoarthritis with decrease of serum levels of TNF α and IL-1beta (15) and with the improvement of symptoms specifically those of pain, articular functionality and quality of life (3). Usually the patients have many procedures during 21 days but only 4–8 mud-baths. Such a time is short for development of bone changes in non osteoporotic individuals. That is why we decided for 34 baths during 50 days in rats regarding to their rapid metabolism.

Some authors have shown significant increase in spinal BMD in parallel with increased spondyloarthritis (new bone formation, sclerosis and syndesmophytes) and this may influence spinal BMD measurements using DXA methods (5). Subchronic aseptic inflammation induction in hind paw possibly influences bone metabolism, leading to increase of regional BMD in spine and caudal vertebrae in rats treated with hot mud. Grassi proposed an inducing effect of mud on the activity of plasma hormones, cytokines and endorphins (2). Pizzoferrato demonstrated that plasma cortisol decreased after first session of mud therapy yet (10). There is release of ACTH and analgesic beta-endorphin in response to thermal stressing (11).

The proximal femur (DXA, BMD) measurements seem to be less affected by disease-related new bone formation (5). Our results showed that a sub-chronic lesion of the right lower pads affected the right femur BMD relative to the left, this effect was most pronounced by hot bath (san38 and mu38).

Many authors have shown the positive effect of mud-bath treatment in osteoarthritis (3), by reducing inflammation and pain (1, 2). On the other hand, thermal stress leads to hyperemia associated with loss of bone mineral (16). The inflammatory damage to pads and associated pain and distress contributed to unloading of the right pads for alleviation. This holding-up of the right leg for relief leads over time to lower BMD in the right femur, seen in all experimental groups except group mu21. The bone loading is

necessary to prevent bone loss (17) and the bone structure can be achieved through impact exercise (18). In contrast, prolonged inactivity leads to atrophy, loss of muscle and bone mass (19). We assume with regard to the mentioned factors a positive effect of cold mud on inflammatory injury and consequently on BMD.

The markers of bone metabolism were analysed in bone homogenate from caudal-tail vertebrae, which do not serve for locomotion and are not influenced directly by inflammation at the pads, in contrast to the femurs. Higher concentrations of PINP were found in all experimental groups, indicating increased bone formation activity and bone collagen formation. Concurrently all experimental groups had higher concentrations of CTX, a marker of bone resorption. As a marker of osteoformation, osteocalcin concentration was lower in the hot mud group. Thus, decreased femur growth (results are not presented) was induced not only by subchronic inflammatory processes in the pads, but also by hot mud-bath treatment. Arends suggested that increased bone turnover plays an important role in the pathophysiology of osteoarthritis-related osteoporosis (6).

The subchronic inflammation in right lower pads led to decrease in the force needed for three point fracture of left and right femurs particular in all experimental groups. We found similar decline in the BMD of femurs but without statistical significance.

The subchronic inflammatory injury in male rats leads to enhancement of bone metabolism in both groups treated with hot bath. We concluded an indifferent effect of warmth on the fracture threshold, as well as on BMD and on bone metabolic turnover. While the cold mud-bath shows positive effect on bone turnover metabolism and resistance to fracture. We presumed, that the cold mud-baths help to reduce arthritic inflammation and pain and thereby lead to higher mobility with positive consequence on bone.

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References

1. Giacomino MI, de Michele DF. Is mud an anti-inflammatory? *An Med Interna*. 2007; 24: 352–3.
2. Grassi M, Lucchetta MC, Rini GB, Raffa S. Fangotherapy in chronic degenerative rheumatic diseases. *Clin Ter*. 2003; 154: 45–8.
3. Mennuni G, Di Franco M, Petracchia L, et al. Spa therapy in osteoarthritis: experience in Cervia *Clin Ter* 2009; 160: 115–9.
4. van der Weijden MA, van Denderen JC, Lems WF, Heymans MW, Dijkmans BA, van der Horst-Bruinsma IE. Low bone mineral density is related to male gender and decreased functional capacity in early spondylarthropathies. *Clin Rheumatol*. 2011; 30: 497–503.
5. Kaya A, Ozgocmen S, Kamanli A, Ardicoglu O. Bone loss in ankylosing spondylitis: does syndesmophyte formation have an influence on bone density changes? *Med Princ Pract*. 2009; 18: 470–6.

6. Arends S, Spoorenberg A, Bruyn GA, et al. The relation between bone mineral density, bone turnover markers, and vitamin D status in ankylosing spondylitis patients with active disease: a cross-sectional analysis. *Osteoporos Int.* 2011; 22: 1431–9.
7. Szántó Z, Papp L. Effect of the different factors on the iontophoretic delivery of calcium ions from bentonite. *J Control Release.* 1998; 56: 239–47.
8. Bellometti S, Galzigna L, Richelmi P, Gregotti C, Bertè F. Both serum receptors of tumor necrosis factor are influenced by mud pack treatment in osteoarthrotic patients. *Int J Tissue React.* 2002; 24: 57–64.
9. Mamani-Matsuda M, Kauss T, Al-Kharrat A, et al. Therapeutic and preventive properties of quercetin in experimental arthritis correlate with decreased macrophage inflammatory mediators. *Biochem Pharmacol.* 2006; 72: 1304–10.
10. Pizzoferrato A, Garzia I, Cenni E, Pratelli L, Tarabusi C. Beta-endorphin and stress hormones in patients affected by osteoarthritis undergoing thermal mud therapy. *Minerva Med.* 2000; 91: 239–45.
11. Giusti P, Cima L, Tinello A, et al. Stress hormones liberated by fangothrapy. ACTH and beta-endorphin levels under heat stress. *Fortschr Med.* 1990; 108: 601–3.
12. Duncan RL, Akanbi KA, Farach-Carson M C. Calcium signals and calcium channels in osteoblastic cells. *Semin. Nephrol.* 1998; 18: 178–190.
13. Gradosova I, Zivna H, Svejkovska K, Palicka V, Tichy A, Zivny P. The role of atorvastatin in bone metabolism in male albino Wistar rats. *Pharmazie.* 2011 Aug; 66: 606–10.
14. Turner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. *Bone.* 1993; 14: 595–608.
15. Cozzi F, Carrara M, Sfriso P, Todesco S, Cima L. Anti-inflammatory effect of mud-bath applications on adjuvant arthritis in rats. *Clin Exp Rheumatol.* 2004; 22: 763–6.
16. Bellometti S, Bertè F, Richelmi P, Tassoni T, Galzigna L. Bone remodelling in osteoarthrotic subjects undergoing a physical exercise program. *Clin Chim Acta.* 2002; 325: 97–104.
17. Qin W, Bauman WA, Cardozo C. Bone and muscle loss after spinal cord injury: organ interactions. *Ann N Y Acad Sci.* 2010; 1211: 66–84.
18. Hind K, Gannon L, Whatley E, Cooke C, Truscott J. Bone cross-sectional geometry in male runners, gymnasts, swimmers and non-athletic controls: a hip-structural analysis study. *Eur J Appl Physiol.* 2011; DOI 10.1007/s00421-011-2008-y.
19. Utz JC, Nelson S, O'Toole BJ, van Breukelen F. Bone strength is maintained after 8 months of inactivity in hibernating golden-mantled ground squirrels, *Spermophilus lateralis*. *J Exp Biol.* 2009; 212: 2746–52.

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