Prophylactic effect of plaster and cataplasm contained ketoprofen in rats with adjuvant arthritis

Seong-Soo Kang and Seok-Hwa Choi*

Department of Surgery, College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Korea

This study demonstrates that prophylactic effect of plaster and cataplasm contained ketoprofen in adjuvant arthritis therapy by X-ray. Adjuvant arthritis was induced by a single injection of Freund’s complete adjuvant. Mature female Sprague-Dawley rats were designated to 3 groups such as nontreated control, plaster-treated (PT) and cataplasm-treated (CT), each of which was composed of ten animals. The PT and the CT groups showed reduced primary paw swelling, but secondary paw swelling was not affected. Bony changes were observed in all regions of the femur and tibia of the non-adjuvant-injected leg and the adjuvant-injected leg. The mean radiographic scores of the PT and the CT groups were significantly lower than those of the control group from day 0 to 7 of the experimental period (P<0.05, P<0.01). The CT rats showed reduced poly-arthritis development than the PT rats. Our results suggest that radiographic assessment of bony changes is more suitable for measuring changes in long bones such as femur or tibia than in vertebrae. The prophylactic effect of CT prominently suppressed edematous swelling and bony changes in arthritic limb compared with PT.

Key words: Adjuvant arthritis, plaster, cataplasm, ketoprofen.

Introduction

Arthritis in rat induced by intradermal injection of mycobacterial adjuvant is widely used as a model for the evaluation of compounds with anti-inflammatory or anti-rheumatic activity [22]. While the clinical appearance of the arthritis has been described, less is known about the time course of the concomitant bone damage [9]. There has also been some a speculation as to the prophylactic effects of plaster and cataplasm contained ketoprofen in suppressing the effects of adjuvant arthritis.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective depressors of bone resorption [10,18]; in particular the aryl propionic acid class seems to be the most effective class of NSAIDs at preventing bony loss in vivo osteopenic models with very little interruption of bone formation [21]. Ketoprofen is a potent non-steroidal anti-inflammatory drug that is used for the treatment of rheumatoid arthritis. However, the oral administration of ketoprofen can cause gastric irritation and renal adverse effects.

Earlier studies using this model for the study of arthritic bone erosion were limited to histological evaluations and measurements of the urinary excretion of pyridine and/or deoxypyrididine [3,15]. However, little information is available on the quantitative changes of cortical and trabecular bones during the development of adjuvant arthritis. The loss of trabecula bone is greater and faster that of the than cortical bone [16,19]. Therefore it is important to compare the response of a variety of skeletal sites. There are no data on the bony changes which occur during the development of adjuvant arthritis. The aim of this study was to investigate the changes in the cortical and trabecular bones during the development of adjuvant arthritis by X-ray.

Materials and Methods

Animals

Mature female Sprague-Dawley rats (KRICT, Taejon, Korea), 6 weeks of age at the time of adjuvant injection, were used for this study. Five rats were housed per cage (43 × 27 × 18 cm) in an air-conditioned environment (room temperature 23±2°C, humidity 55±5%) that was illuminated from 6:00 to 18:00. Animals were fed with a commercial diet (Samyang feed Co., Korea), and divided into three groups, plaster-treated (PT), cataplasm-treated (CT) and a non-treated control, each group was composed of ten animals. Adjuvant-arthritis was treated with plaster (X Co., Korea) and cataplasm (Y Co., Korea) containing 15 mg of ketoprofen at a site on the back twice a day (half day) for 5 days 1 hour prior to the adjuvant injection.
Induction of arthritis
On day 0, each rat was injected in the plantar region of the right hind limb with Freund's complete adjuvant (Gibco, USA, Lot No. 1020159) containing 0.6 mg of Mycobacterium butyricum (Difco, USA, Lot No. 138137LA) suspended in 0.1 ml of paraffin oil.

Measurements of foot size
Foot volumes of the injected (right hindpaw) and non-injected (left hindpaw) paws were measured up to a mark made on the tibiotarsal joint by using a messcylinder.

Clinical assessment of arthritis
Every week after the first immunization and during the entire following up period (28 days), clinical assessments were performed under blind conditions using the previously described scoring system [14]. The severity of arthritis was graded on a 0-4 scale as follows: 0 = normal; 1 = swelling and/or redness in 1 joint; 2 = swelling and/or redness in >1 joint; 3 = swelling and/or redness in the entire paw; and 4 = deformity and/or ankylosis. Each paw was graded, and the 4 grades were summed to a maximum possible score of 16.

Radiographic assessment of bony changes
Radiographic changes were assessed under blind conditions using the previously described scoring system [14]. Each limb was assessed for osteopenia and bony erosions and graded from 0 to 3 as follows: 0 = no change; 1 = slight change; 2 = moderate change; and 3 = severe change. Each paw was graded, and the 4 scores were summed up to a maximum possible score of 12.

This was performed using an X-ray unit (BLD-15RK, Dong-A X-ray, Co., Korea). Exposure was 51 kVp and 7.5 mAs with diagnostic film (medium speed, Kodak Co, USA) of 14” × 14”. Rats were anesthetized with xylazine HCl (0.1 mg/kg, i.m.) and ketamine HCl (0.2 mg/kg, i.m.) prior to X-raying. Whole bodies were X-rayed using a 90° projection from the dorsal-ventral aspect.

Changes of trabecular and cortical bone
Bony changes of the femur, tibia and vertebra (first sacral and third coccygeal vertebrae) were assessed blind using a previously described scoring system [11], and graded from 0 to 3 as follows: 0 = no lesions; 1 = slight osteoporosis; 2 = pronounced osteoporosis and slight bone erosion; 3 = massive osteoporosis and erosion.

We selected these bones is to reflect the differences in the change of the arthritis process in the trabecular and cortical bones. Femur and tibia are composed of trabecular and cortical bones, whereas the vertebrae are mainly composed of trabecular bone [16].

Statistical analysis
All results were expressed as the mean ± standard error of the mean. The statistical significance of differences was assessed by the unpaired two-tailed Student’s t-test; P values of <0.05 were considered significant.

Results
In the control animals, the adjuvant-injected foot showed primary inflammation on day 3 after adjuvant injection. On day 21, the increase of paw volume peaked 250%, which persisted until the end of experiment. On the other hand, the non-injected foot showed a 200% increased paw volume on day 13 after injection (Fig. 1). The PT and the CT groups showed reduced primary paw swelling but secondary paw swelling was unaffected.

As shown in Fig. 2, the clinical arthritis score of the control rats increased until the end of experiment. Little difference was evident between the effect of CT and PT until day 7 after the adjuvant injection. After day 7, the CT seemed to be the more effective in the non-injected paw.

![Fig. 1. Changes of paw edema by injection of adjuvant in the control (●), plaster- (■) and cataplasm-treated (▲) groups (n = 10). *p<0.05, compared to the control group.](image-url)
Prophylactic effect of plaster and cataplasm contained ketoprofen in rats with adjuvant arthritis 67

Radiographic assessment of bone changes

As shown in Fig 3, the X-ray score of the PT and CT rats were significantly lower than the controls rat from day 0 to 7 of the experimental period (p<0.05, p<0.01) and CT showed a significant difference compared with the control until the end of the experiment (p<0.05). The CT reduced the development poly-arthritis more so than PT.

Changes of trabecular and corical bones

During the experimental period, there were bony changes were observed in all the regions of the femur and tibia of the non-adjuvant-injected leg as well as the adjuvant-injected leg, especially in the distal region.

Femur

A significant decrease was observed on days 21 and 28 in the adjuvant-injected leg of the PT and the CT groups (p<0.05). The non-injected leg than the injected leg showed a smaller and a more delayed decrease in the development of bone loss and erosion than the injected leg. Only the distal region of femur in the PT group was significantly lower than the control in the development of bone loss and erosion on day 28 (p<0.01, Fig 4).

Tibia

The distal tibia in both injected (p<0.01) and non-injected legs (p<0.05) showed a significant decrease in the PT and the CT groups after day 28 (Fig 5).

Vertebrae

The first sacral vertebra showed a significant decrease in the development of bone loss and erosion in the PT and the CT groups after day 21 compared with the control. The third coccygeal vertebra showed decrease an adjuvant arthritis in PT and CT when compared with the control in the development of bone loss and erosion, they was not significant (Fig 6).

Discussion

Adjuvant-induced arthritis in rats has been widely used as a model for polyarthritis such as rheumatoid arthritis [6]. To evaluate the anti-arthritic activities, we investigated the prophylactic effects of plaster and cataplasm contained ketoprofen in rats with adjuvant arthritis.
ketoprofen on adjuvant-arthritis. In the present study, CT and PT compared with the control prominently suppressed swelling and bony changes in the arthritic limbs. It has been reported that ketoprofen has a potent anti-inflammatory action, but oral administration causes gastric mucous membrane disturbances [5].

Recently, plaster and cataplasm contained ketoprofen have been evaluated to determine their clinical effects on anti-arthritis because topical ketoprofen treatment circumvented gastrointestinal disturbances. In an attempt to improve the skin penetration of ketoprofen, various transdermal formulations have been prepared [8].

The PT and CT rats showed less primary paw swelling in the adjuvant injected and non-injected paws than the control, but secondary paw swelling was unaffected. The score of clinical arthritis in the controls rat showed increases until the end of the experiment. However, the mean scores of the PT and CT rats were lower than the controls rat during the experimental period.

The radiological scores of the PT and CT rats were significantly lower than the control rats from day 0 to 7. The CT reduced the development of poly-arthritis more so than PT. During the experimental period, the main radiological changes were osteoporosis, erosion and osteal reaction in the tarsal, metatarsal, distal regions of the tibia and the femur, which were visible after day 14, especially in the injected hind paw, as mentioned Blackham et al [2]. In the case of CT, the mean arthritis score was shown to significantly reduce from day 7 to the end of experiment. The PT, however, showed no significant bone changes except at day 7, which means that the CT is more effective than PT.

Bony changes were observed in all regions of the femur and the tibia of the nonadjuvant-injected legs as well as the adjuvant-injected legs. In the femur, a significant decrease occurred on days 21 and 28 in the adjuvant-injected leg of the CT and the PT rats. Only the distal region of the femurs in the PT group were significantly decreased compared with the controls on day 28. The distal tibia from both injected and non-injected legs showed significant decrease in the CT and PT groups on day 28 when compared with the control. Trabecular bone has a larger surface area and a faster turnover than cortical bone, which could account for the present observation [12,17,19]. We hypothesize that the proximal and distal regions of femur and tibia are com-
posed primarily of trabecular bone.

Our findings indicate that the bony changes occurred after paw volumes had increase. In general, increased paw volume in the injected hind paw represents a primary inflammatory phase, which appeared from day 3 to 10 after adjuvant injection [16]. In the non-injected hind paw, the inflammation is of the later chronic nature [1,20]. The late-phase inflammation is associated with decreased bone mineral density induced by physiological response regulated by the parathyroid hormones, calcitonin, or vitamin D. However, recently it was reported that generalized bone loss associated with inflammation occurred independently of these hormones in rat [7]. Another factor is the inflammatory cytokines, which have been found at elevated levels in arthritic patients [13]. Inflammatory cytokines such as IL-1, IL-6, and TNF-α are potent inducers of bone resorption [4]. It is possible that these cytokines mediate both the increase of paw volume and the reduced bone density [16].

We observed significant decreases in the development of bone loss and erosion in CT and the PT after day 21 in the first sacral compared with the control. However, the third coccygeal vertebra showed decrease adjuvant arthritis in the CT and the PT when compared with the control, but this was not significant. Takagi et al [16] reported that the sacral and coccygeal vertebrae showed significant decreases in bone mineral density on day 21. In the present study, trabecular bone changes were shown to be detected as minor bone changes because we only measured bony changes in the first sacral and third coccygeal vertebrae body. This method is unsuitable for measuring minor bony changes. Radiographic assessment of bony changes is more suitable for measuring changes in long bones such as the femur or the tibia than in vertebrae like structures, Takagi et al [16] mentioned.

In summary, the prophylactic effect of the CT and PT prominently suppressed swelling and bony changes in all regions of the femur, tibia and paw of the non-adjuvant-injected leg and the adjuvant-injected leg. The CT may be more useful clinically than the PT in the rheumatoid arthritis.

Acknowledgment

This project was financially supported by grant from the

---

**Fig. 7.** Radiographs of the hind limbs of rats with adjuvant arthritis on day 28. A. Normal rat. B. Control rat; showing organized and widespread cystic degeneration, joint subluxation and large areas of erosion with persistent severe periarticular soft tissue swelling. C. Plaster-treated rat; showing periarticular soft tissue swelling, bony erosion, osteoporosis, periosteal reaction, and severe cystic degeneration of the tarsals and metatarsals. D. Cataplasm-treated rat; showing periarticular soft tissue swelling, erosion and osteoporosis of the tarsals and metatarsals, and periosteal reaction of the metatarsals. The tarsals showed mild cystic bone degeneration compare with that of PT (C) and Control (B).
health and medical life science project (2000R-P-5). Also, the authors thank to Sanga Pharm. Co., Ltd. for their material assistance.

References

1. Baumgartner, W. A., Beck, F. W., Lorber, A., Person, C. M. and Whitehouse, W. A. Adjuvant disease in rats: Biochemical criteria for distinguishing several phase of inflammation and arthritis. Proc. Soc. Exp. Biol. Med. 1974, 45, 625-630.

2. Blackham, A., Burns, J. W., Farmer, J. B., Radzivonik, H. and Westwick, J. An X-ray analysis of adjuvant arthritis in the rat. The effect of prednisolone and indomethacin. Agents Actions 1977, 7, 145-151.

3. Bonnet, J., Zerath, E., Picud, N., Lesur, C., Mattio, A., Tordjman, C., Hott, M. and Marie, P. J. Bone morphometric changes in adjuvant-induced polyarticular osteopenia in rats: Evidence for an early bone formation defect. Bone Miner. Res. 1995, 8, 659-668.

4. Dinarello, C. A. Interleukin-1 and its biologically related cytokine. Adv. Immunol. 1989, 14, 153-205.

5. Gurol, Z., Hekimoglu, S., Demirdamar, R. and Sumnu, M. Percutaneous absorption of ketoprofen. I. In vitro release and percutaneous absorption of ketoprofen from different ointment bases. Pharm. Acta. Helv. 1996, 71, 205-212.

6. Masaki, M., Matsushita, M. and Wakitani, S. Change in bone minerals in adjuvant-induced arthritis in interleukin-6-deficient mice. Arthritis Rheum. 1999, 42, 1635-1643.

7. Segawa, Y., Nakamura, T., Aota, S., Tanaka, Y., Yoshida, K., Tsuzuike, N. and Matuda, K. Change in urinary deoxypyridinoline level and vertebral bone mass in the development of adjuvant-induced arthritis in rats. Bone 1995, 17, 57-62.

8. Takagi, T., Tsao, P.W., Totsuka, R., Suzuki, T., Murata, T. and Takata, I. Change in bone mineral density in rat adjuvant arthritis. Clin. Immun. Immunopathol. 1997, 84, 166-170.

9. Takagi, T., Yamamoto, T. and Asano, S. Effect of prostaglandin D2 on the femoral bone mineral density in ovariectomized rat. Calcif. Tissue. Int. 1993, 52, 442-446.

10. Thompson, D. D. and Rodan, G. A. Indomethacin inhibition of tenotomy-induced bone resorption in rats. J. Bone. Miner. Res. 1988, 10, 187-194.

11. Vanderschueren, D., Herck, E. and Suiker, A. M. H. Bone and mineral metabolism in aged male rats: Short and long terms effects of androgen deficiency. Endocrinol. 1992, 130, 2906-2916.

12. Welchman, B. M., Chau, T. T. and Rona, G. Histopathologic evaluation of the effects of ectolac in established adjuvant arthritis in rats: Evidence for reversal of joint damage. Arthritis Rheum. 1987, 30, 466-470.

13. Williams, R. C., Offenbacher, S., Jeffcoat, M. K. and Wechter, W. J. Indomethacin or flurbiprofen treatment of periodontal disease in the beagle: Comparison of the effect on alveolar bone loss with effect on crevicular fluid arachidonate metabolism. J. Periodont Res. 1988, 23, 134-138.

14. Winder, C. V., Lembke, L. A. and Stephens, M. D. Comparative bioassay of drugs in adjuvant-induced arthritis in rats: Flufenamic acid, mefenamic acid, and phenylbutazone. Arthritis Rheum. 1969, 12, 472-482.