Effects of YM529, a novel minodronic acid, on adjuvant arthritis in rats

S. Masuda-Aiba, T. Shinozaki, K. Takagishi

Department of Orthopaedic Surgery, Gunma University Faculty of Medicine

Abstract

Objective

The effects of a new third-generation bisphosphonate, YM529, on both the development and treatment of adjuvant arthritis were investigated in rats.

Methods

Five-week-old Lewis rats with adjuvant arthritis were prophylactically and therapeutically treated with 0.001, 0.01 and 0.05 mg/kg/day of YM529 and the arthritis scores were measured. Soft X-ray and histological findings were compared to those of the control group. Body weights were also measured.

Results

YM529 suppressed the severity of adjuvant arthritis in a dose-dependent manner when used as either a prophylactic or therapeutic drug. Administration of the drug had little effect on body weight.

Conclusion

YM529 may act on arthritic joints locally to prevent inflammation. However, further experiments are necessary to elucidate the underlying mechanisms.

Key words

Adjuvant arthritis, bisphosphonates, YM529, cytokines.

Sachiko Masuda-Aiba, MD, Post-Graduate Student; Tetsuya Shinozaki, MD, PhD, Assistant Professor; Kenji Takagishi, MD, PhD, Professor.

Please address correspondence and reprint requests to: T. Shinozaki, MD, PhD, Department of Orthopaedic Surgery, Gunma University Faculty of Medicine, 3-39-22 Showa, Maebashi, Gunma 371-8511 Japan.
E-mail: tshinoza@showa.gunma-u.ac.jp

Received on May 19, 2003; accepted in revised form on October 14, 2003.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease involving chronic joint inflammation and destruction. Current therapy comprises a combination of disease-modifying anti-rheumatic drugs (DMARDs) or methotrexate, and biological anti-cytokine agents such as etanercept, infliximab and anakinra (1-4). However, even such combination therapies have not yielded satisfactory results in terms of lower disability and a higher proportion of remission in RA patients.

Bisphosphonates generally inhibit bone resorption by suppressing osteoclasts; they are currently being developed as potential drugs for osteoporosis (5), multiple myeloma (6), tumor-induced hypercalcemia, and metastatic bone diseases (7). Some bisphosphonates exert inhibitory effects on adjuvant arthritis (AA) and antigen-induced arthritis, which include the inhibition of interleukin-1 (IL-1) production by the murine macrophage cell line J774-1 (8, 9). Rat adjuvant arthritis has been used in pre-clinical studies as a standard animal model for chronic inflammation occurring in human RA (10). Following the promising results in animal studies, it was found that pamidronate clinically suppressed bone resorption and reduced disease activity in a dose-dependent manner without serious side effects in patients with RA(11).

Minodronic acid, 1-hydroxy-2-(imidazo [1,2-a]pyridin-3-yl)ethane-1,1-bisphosphonic acid monohydrate (YM529) is a novel bisphosphonate that shows 100-fold greater potency against bone resorption than pamidronate, a second-generation bisphosphonate in animal models (12,13). In rats with collagen-induced arthritis, minodronic acid suppresses the decrease in bone mineral density and deterioration of the bone microstructure, and reduces inflammation (14). These results suggest that minodronic acid may constitute an effective agent in RA treatment. The current study examines the preventive and therapeutic effects of YM529 on AArats.

Materials and methods

Animals

Inbred 5-week-old female Lewis rats were purchased from Charles River Japan, Inc. (Kanagawa, Japan), and housed in specifically prepared pathogen-free rooms for the duration of the experiment. They consumed standard rat chow and water *ad libitum* for one week while becoming acclimated to their new environment.

This study was performed at the Animal Experiment Facility, Gunma University Faculty of Medicine. The Gunma University, Showa Campus Animal Care and Experimentation Committee approved the protocol.

Induction of adjuvant arthritis

Freund's Complete Adjuvant (FCA) was prepared by mixing heat-killed *Mycobacterium butyricum* (Difco Laboratories, MI, USA) with paraffin oil. Adjuvant arthritis was induced by intradermal injection of 0.1 ml FCAcontaining 12 mg/ml *Mycobacterium butyricum* at the base of the animal's tail.

Administration protocol

Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan) kindly provided the YM529, which we dissolved in 1 N of sodium hydroxide solution and then compensated to pH 6.3 with hydrochloric acid at concentrations of 0.05, 0.01, and 0.001 mg/kg/0.1 ml.

Prophylactic administration. Rats were divided into four groups (n = 10 each). From the day when the adjuvant was given to day 14 of the experiment, rats in each group were injected once daily subcutaneously with the different concentrations of YM529 described above. Identical volumes of physiological saline were given as a control.

Therapeutic administration. After the 14-day injection period the rats with AA were divided into four groups (n = 10), based on their arthritis score. The procedure for therapeutic administration was identical to that of preventive administration.

Arthritis evaluation

During injection periods of YM529, arthritis was evaluated on the basis of the arthritis score according to Cannon *et al.* with some modification (15). In brief, the severity of arthritis in the mid-forepaw and mid-hindpaw was

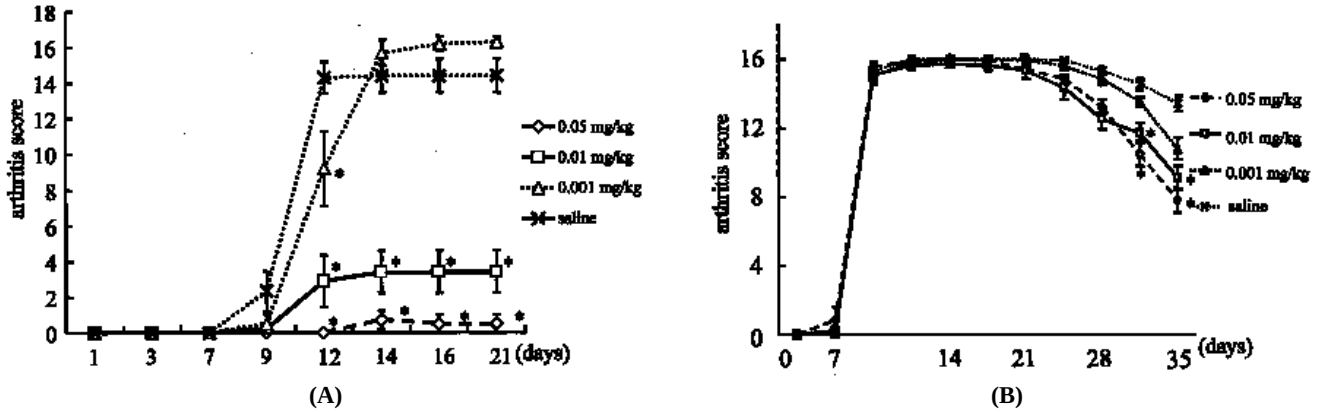

Fig. 1. Mean and standard error of arthritis scores when YM529 was administered asa (**A**) prophylactic or (**B**) therapeutic agent.
*Scores showing statistically significant differences between the control and YM529 treated groups.

scored as: 0 = no arthritis; 1 = extreme redness and minimal swelling; 2 = medium swelling; 3 = severe swelling; 4 = severe swelling and non-weight bearing. The presence/absence of arthritis in each digit in the interphalangeal, metacarpophalangeal, and metatarsophalangeal joints of the paws was scored as 0 = no arthritis or 1 = arthritis present. The presence/absence of tail swelling was scored as 0 = no swelling or 1 = swelling present. A maximum score of 18 was possible for each animal.

Evaluation of bone destruction

Rats were sacrificed after one week following completion of the YM529 injections. Both hind paws were dissected and immediately fixed in 10% neutral buffered formalin. They were imaged using a soft X-ray instrument (CMB-2; Softex Co., Ltd., Tokyo, Japan) and soft X-ray film (Fujicolor Trading Co., Ltd., Japan). One day after the fixation, bone destruction, soft part swelling, osteoporosis, cartilage loss, and erosion were evaluated without knowledge of the treatment using the radiological index described by Cannon *et al.* (15). A subjective 0–3 grading scale was used, with 0 indicating negative or normal, 1 indicating mildly affected, 2 moderately affected, and 3 severely affected.

Histopathological evaluation

Hematoxylin and eosin staining were performed on the sagittal section of the foot. The extent of changes in synovial lining cells (hyperplasia, hypertrophic shape), the degree of inflammatory infiltration of subsynovial tissue was graded using a semiquantitative score from 0–3, with 0 indicating normal and 3 indicating severe damage, as described by Oelzner *et al.* (16).

Statistical analysis

Fischer's protected least significant difference and the Bonferroni/Dunn test were used for comparative analysis of data from different rat groups. A probability value of less than 0.05 was assumed to indicate significant difference.

Results

Prophylactic effects of YM529

Arthritis evaluation. Rats in the control group and those treated with 0.001 mg/kg developed AA at day 12 and were little changed at day 14; those treated with 0.01 and 0.05 mg/kg showed less severe AA (Fig. 1A). The mean arthritis scores on day 14 were 14.7 ± 0.92 in the control group, and

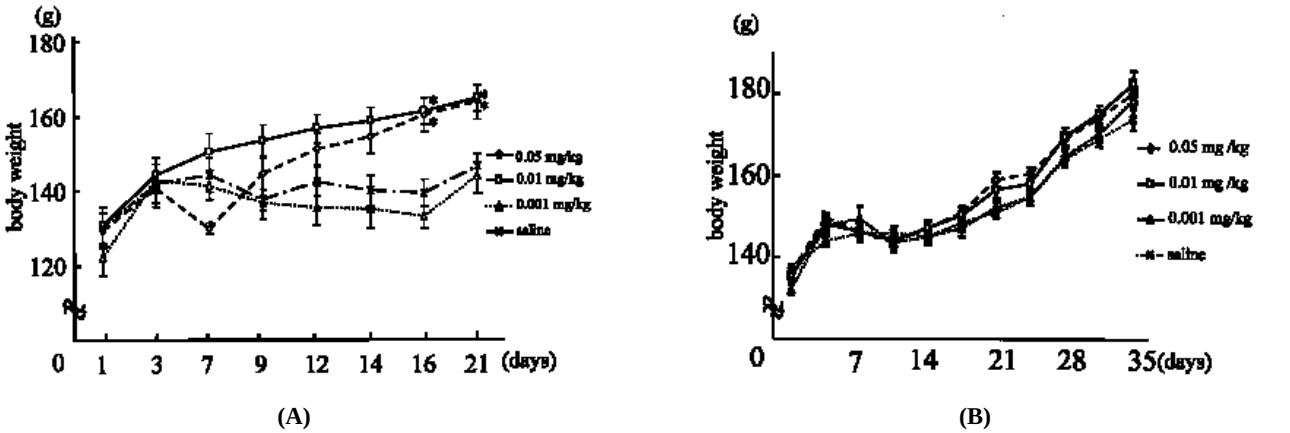

Fig. 2. Weight change in adjuvant arthritis rats when YM529 was administered as a (**A**) prophylactic or (**B**) therapeutic agent. Data expressed as the mean ± standard error.
*Note the statistically significant differences between the control and YM529 treated groups.

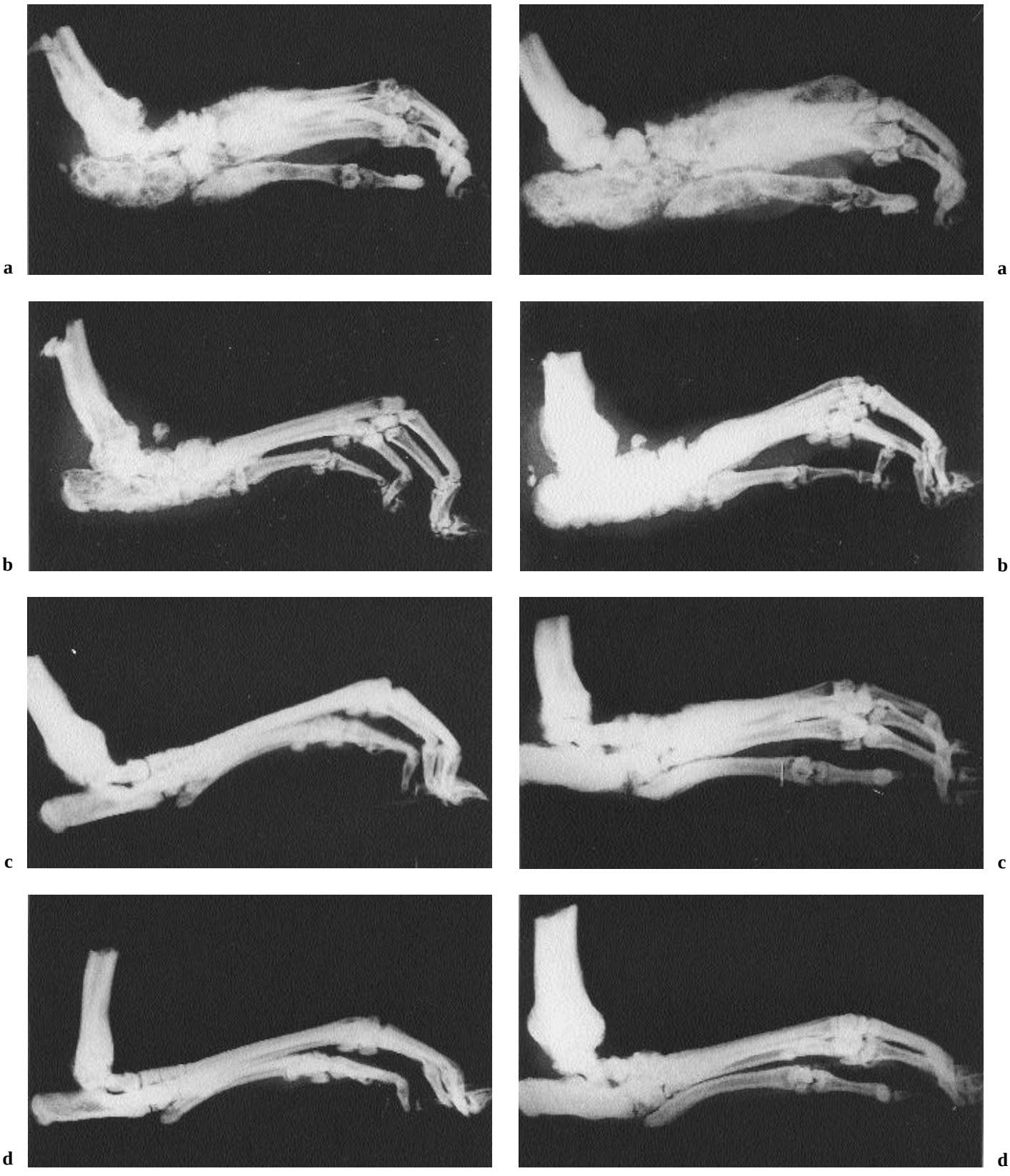

Fig. 3. Soft tissue X-ray findings when YM529 was administered as a prophylactic (**A**) or therapeutic (**B**) agent.
Soft X-rays showed severe hind footpad swelling, demineralization, and joint destruction of rats in the control and 0.001 mg/kg/day YM529 treated groups, but the extent of those features decreased with increased concentration of YM529.
(**a**) control, (**b**) 0.001 mg/kg/day YM529, (**c**) 0.01 mg/kg/day YM529, (**d**) 0.05 mg/kg/day YM529

16.2 ± 0.32, 3.4 ± 1.19 and 0.8 ± 0.42 in the 0.001, 0.01 and 0.05 mg/kg treated groups, respectively. A statistically significant decrease in the arthritic score was observed in rats treated with 0.001 mg/kg on day 12, and for those treated with 0.01 and 0.05 mg/kg at days 12, 14, 16, and 21 (Fig. 1A).

Weight change in rats administered YM529. Rats treated with 0.05 mg/kg showed temporarily reduced scores on day 7. However, the scores recovered on day 9. Weight gain occurred in rats given 0.01 and 0.05 mg/kg after day 9. Statistically significant weight loss was apparent on days 16 and 21 in rats given 0.001 mg/kg and the control rats (Fig. 2A).

Radiological change in rats adminis - tered YM529. Soft X-rays showed severe hind footpad swelling, demineralization, and joint destruction in rats in the control and 0.001 mg/kg treated groups. However, the extent of those features decreased with th eYM529 concentration: some of the rats which received 0.01 or 0.05 mg/kg had an almost normal appearance (Fig. 3A). The mean radiological indexes on day 21 were 2.7 ± 0.15 in the control group, and 2.7 ± 0.15, 0.6 ± 0.22 and 0.2 ± 0.13 in the 0.001, 0.01 and 0.05 mg/kg groups, respectively. Statistically significant decreases were observed for both the 0.01 and 0.05 mg/kg treated groups (Fig. 4A).

Histological findings. Histopathological assessment of the foot pads of rats in the control and 0.001 mg/kg YM529 groups revealed marked infiltration of neutrophils and lymphocytes with disruption and loss of articular cartilage. However, inflammatory cell invasion and articular cartilage destruction were less prominent with increasing YM529 concentrations (Fig. 5A).

Therapeutic effects of YM529

Arthritis evaluation. After day 21, YM529 improved the arthritis score in a dose-dependent manner (Fig. 1B). The mean arthritis scores on day 35 were 13.4 ± 0.452 in the control group, and 10.1 ± 0.53, 9.1 ± 0.67 and 7.8 ± 0.71 in 0.001, 0.01 and 0.05 mg/kg treated groups, respectively. A statistically significant decrease in arthritis scores was observed in rats treated with 0.01 and 0.05 mg/kg at days 30and 35 (Fig. 1B).

Weight change in rats administered YM529. Weight gain occurred in rats from each group after day 14. The present study found no statistically significant difference (Fig. 2B).

Radiological change in rats adminis - tered YM529. Soft X-rays showed improvement of hind footpad swelling, demineralization, and joint destruction in the rats in the 0.01 and 0.05 mg/kg/day YM529 treated groups. However, rats treated with 0.001 mg/kg showed only a slight increase of those features (Fig. 3B). Mean radiological indexes on day 35 were 2.65 ± 0.13 in the control group, and 2.22 ± 0.17, 1.75 ± 0.08 and 1.30 ± 0.11 in the 0.001, 0.01 and 0.05 mg/kg YM529 groups, respectively. Statistically significant decreases were observed for both the 0.01 and 0.05 mg/kg treated groups (Fig. 4B).

Histological findings. Histopathological assessment of the foot pads of rats in the control and 0.001 mg/kg YM529 groups revealed marked infiltration of neutrophils and lymphocytes with disruption and loss of articular cartilage. However, inflammatory cell invasion and articular cartilage destruction were less prominent in the rats in the 0.01 and 0.05 mg/kg YM529 groups (Fig. 5B).

Discussion

Bisphosphonate is reported to induce anti-inflammatory effects in AArats by inhibiting IL-1-like activity of resident peritoneal macrophages and decreasing concentrations of cytokine-induced neutrophil chemoattractant-1 and TNF-α in bone marrow (8,9). Clodronate and pamidronate, some types of bisphosphonates, inhibit secretion of pro-inflammatory cytokines from macrophage-like RAW 264 cells stimulated by lipopolysaccharides (17). Liposomal clodronate is reported to induce macrophage elimination. Consequently, it inhibits local production of IL-1β, IL-6, TNFα, and MMP-9 (18). Except

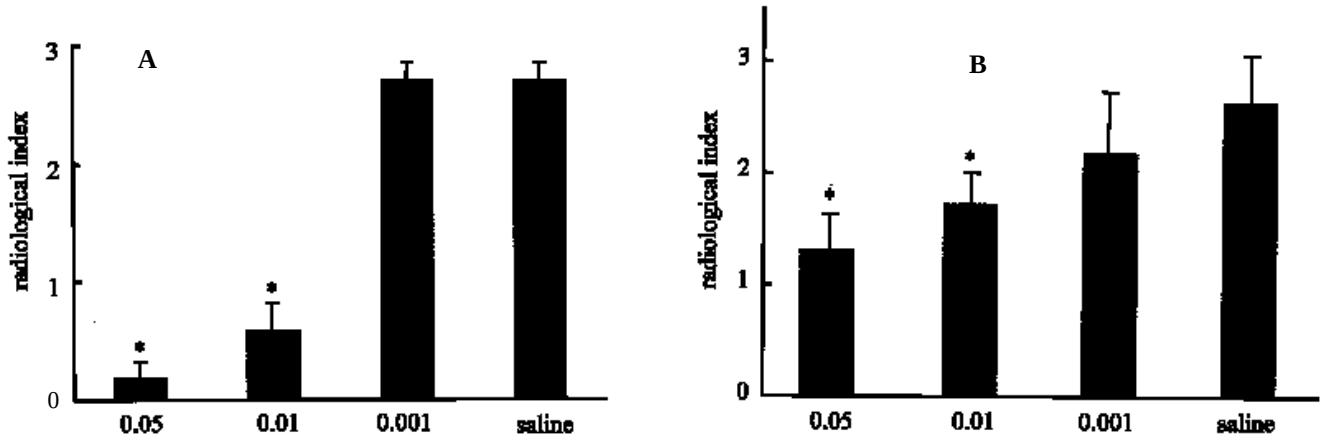

Fig. 4. Radiological index when YM529 was administered as a (**A**) prophylactic or (**B**) therapeutic agent. Bars above symbols indicate mean ± standard error.
*Scores showing statistically significant differences between the control and YM529 treated groups.

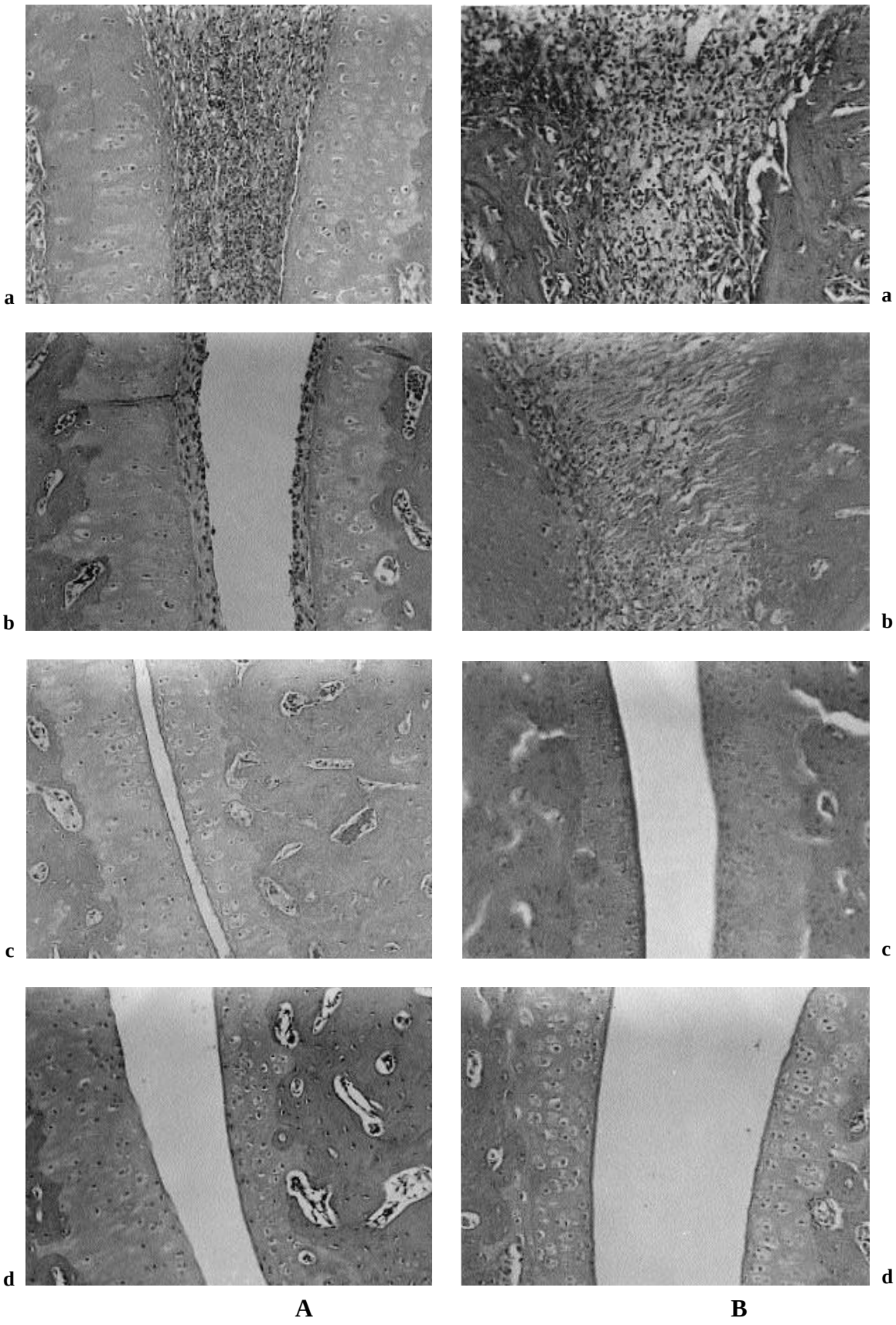

Fig. 5. Histological findings when YM529 was administered as a (**A**) prophylactic or (B) therapeutic agent.
Histology of control (**a**) and 0.001 mg/kg/day (**b**) footpads shows infiltration of neutrophils and lymphocytes with disruption and loss of articular cartilage. However, alterations are less pronounced with greater concentrations of YM529 (**c**: 0.01 mg/kg/day, **d**: 0.05 mg/kg/day) (HE, x79).

for these experimental reports, bisphosphonate clinically suppressed bone resorption and disease activity in patients with RA (11).

Only recently developed as a new third-generation bisphosphonate, YM529 has a heterocyclic R2 side chain and shows a 100-fold greater potency in animal models against bone resorption than pamidronate, a second generation bisphosphonate (12,13). The R2 side chain, and especially the basic nitrogen group, appears to play an important role in the interaction of bisphosphonates with a pharmacological target because minor modification to the structure or conformation of the R2 side chain can affect the anti-resorptive potency dramatically (19-22).

In the present study, YM529 suppressed rat AAdevelopment with a significant reduction of arthritic changes at the high concentrations (Fig. 1A). In addition, when administered as therapy it improved the arthritic condition of rates in a dose-dependent manner (Fig. 1B). Soft X-ray and histological findings provided further evidence of improvement of the arthritic condition when YM529 was used both as a prophylactic (Figs. 3A, 4A, 5A) and as a therapeutic agent (Figs. 3B, 4B, 5B) in rat AA. Weight loss is a marker for toxicity in animals (23). However, weight loss also occurred with progression of arthritis resulting from a poor general condition (Fig. 2A). Body weight increased concomitant with an improvement in the arthritic condition (Fig. 2A). Moreover, no weight loss was observed when YM529 was administered therapeutically (Fig. 2B). However, YM529 at a dose of 0.1 mg/kg/day, although it prevented AA, caused diarrhea and gastric ulcer with weight loss and consequent death (data not shown). This result suggests that YM529, at too high a dose, might induce fatal adverse effects even though it suppresses inflammation. When used at appropriate concentration, YM529 effectively suppressed AAin rats with little toxicity.

Synovial tissues from patients with rheumatoid arthritis produced cytokines such as TNFα, IL-1β (24-26), and matrix metalloproteinase-3 (24). Local joints of adjuvant arthritis rats are also known to have elevated levels of TNFα, IL-1β and IL-6 (27, 28). Clodronate inhibited the local production of TNFα, IL-1β and IL-6 (18). Minodronic acid prevented arthritis by suppressing bone resorption in rats with collagen-induced arthritis (14). In that study, minodronic acid might have suppressed arthritis by inhibiting the increase in inflammatory cytokine level. However, no study has addressed how minodronates such as YM529 affect the production of these cytokines. The present study showed little effect on TNFα, IL-1β and IL-6 in rat serum when YM529 was administered at different concentrations (data not shown). That relative lack of effect implies that YM529 inhibits the production of TNFα, IL-1β and IL-6 in local joints of AA in rats. If this is the case, measurement of local cytokines such as TNFα, IL-1β and IL-6 might be appropriate. On the other hand, it is possible that this drug has anti-inflammatory effects which are unrelated to these functions or that do not inhibit synthesis of these cytokines under certain conditions. In fact, other investigators have demonstrated that aminobisphosphonates were ineffective against inflammation in collagen-induced arthritis in rats and mice (29-31). These contrasting findings could be attributable to differences in the molecular structure of the agents, the method of administration, or the animal models used. In this sense, the exact mechanism of bisphosphonate toward inflammation remains partially unclear. Zoledronic acid, another type of third generation bisphosphonate, is 850-fold stronger than pamidronate as an inhibitor of resorption. It is another candidate with a comparable mechanism to that of minodronic acid (6). Further studies should be conducted to elucidate its mechanism.

In conclusion, YM529 may exert its anti-inflammatory effect by modulating some cytokines. However, further studies are necessary to determine the optimal effect profile of various therapy regimens with YM529 to reduce disease activity without adverse effects.

Acknowledgements

The authors would like to thank Mr. Kiichi Osawa for creating the soft X-ray images and Ms. Hiroe Miyahata for preparing the histological sections.

References

1. KREMER JM, WEINBLATTME, BANKHURST AD *et al*.: Etanercept added to background methotrexate therapy in patients with rheumatoid arthritis: continued observations. *Arthritis Rheum* 2003; 48: 1493–9.
2. FLEISHCHMANN RM, BAUMGARTNER SW, TINDALL EA *et al*.: Response to etanercept (Enbrel) in elderly patients with rheumatoid arthritis: a retrospective analysis of clinical trial results. *J Rheumatol* 2003; 30: 691–6.
3. NAHAR IK, SHOJANIA K, MARRA CA, ALAMGIR AH, ANIS AH: Infliximab treatment of rheumatoid arthritis and Crohn's disease. *Ann Pharmacother* 2002; 37: 1256–65.
4. FLEISHMANN RM, SCHECHTMAN J, BENNETT R *et al*.: Anakinra, a recombinant human interleukin-1 receptor antagonist (r-metHuIL-1ra), in patients with rheumatoid arthritis: A large, international, multicenter, placebo-controlled trial. *Arthritis Rheum* 2003; 48: 927–34.
5. ROSENCJ: A tale of two worlds in prescribing etidronate for osteoporosis. *Lancet* 1997; 350: 1340.
6. KRAJ M, POGLOD R, MAJ S, PAWLIKOWSKI J, SOKOLOWSKA U, SZCZEPANIK J: Comparative evaluation of safety and efficacy of pamidronate and zoledronic acid in multiple myeloma patients (single center experience). *Acta Pol Pharm* 2002; 59: 478–82.
7. COLEMAN RE: Should bisphosphonates be the treatment of choice for metastatic bone disease ? *Semin Oncol* 2001; 28: 35–41.
8. TANAHASHI M, KOIKE J, KAWABE N, NAKADATE-MATSUSHITA T: Inhibitory effect of TRK-530 on inflammatory cytokines in bone marrow of rats with adjuvant arthritis. *Pharmacology* 1998; 56: 237–41.
9. TANAHASHI M, FUNABA Y, ITOH M, KAWABE N, NAKADATE-MATSUSHITA T: Inhibitory effects of TRK-530 on rat adjuvant arthritis. *Pharmacol* 1998; 56: 242–51.
10. ROUBENOFF R, FREEMAN LM, SMITH DE, ABAD LW, DINARELLO CA, KEHAYIAS JJ: Adjuvant arthritis as a model of inflammatory cachexia. *Arthritis Rheum* 1997; 40: 534–9.
11. EGGELMEIJER F, PAPAPOULOS SE, van PAASSEN HC, DIJKMANS BAC, BREEDVELDFC: Clinical and biochemical response to single infusion of pamidronate in patients with active rheumatoid arthritis: A double blind placebo controlled study. *J Rheumatol* 1994; 21: 2016–20.
12. DUNFORD JE, THOMPSON K, COXON FP *et al*.: Structure-activity relationships for inhibition of farnesyl diphosphonate synthase *in vitro* and inhibition of bone resorption *in vivo* by nitrogen-containing bisphosphonates. *J Pharm Exp Therap* 2001; 296: 235–42.
13. TAKEUCHI M, SAKAMOTO S, KAWAMUKI K, KURIHARA H, NAKAHARA H, ISOMURA Y: Studies on novel bone resorption inhibitors. 2. Synthesis and pharmacological activities of fused Aza-heteroarylbisphosphonate derivatives. *Chem Pharm Bull* 1998;

46: 1703–9.

14. YAMANE I, HAGINO H, OKANO T, ENOKIDA M, YAMASAKI D, TESHIMA R: Effect of minodronic acid (Ono-5920) on bone mineral density and arthritis in adult rats with collagen-induced arthritis. *Arthritis Rheum* 2003; 48: 1732–41.
15. CANNON GW, WOODS ML, CLAYTON F, GRIFFITHS MM: Induction of arthritis in DA rats by incomplete Freund's adjuvant. *J Rheumatol* 1993; 20: 7–11.
16. OELZNER P, KUNZE A, HENZGEN S *et al.*: High-dose clodronate therapy prevents joint destruction in chronic antigen-induced arthritis of the rat but inhibits bone formation at the axial skeleton. *Inflamm Res* 2000; 49: 424–33.
17. PENNANEN N, LAPINJOKI S, URTTI A *et al.*: Effect of liposomal and free bisphosphonates on the IL-1 beta, IL-6 and TNF alpha secretion from RAW 264 cells *in vitro*. *Pharm Res* 1995; 12: 916–22.
18. RICHARDS PJ, WILLIAMS BD, WILLIAMS AS: Suppression of chronic streptococcal cell wall-induced arthritis in Lewis rats by liposomal clodronate. *Rheumatology* 2001; 40: 978–87.
19. SIETSEMA WK, EBETIONO FH, SALVAGNO AM, BEVAN JA: Antiresorptive dose-response relationships across three generations of bisphosphonates. *Drugs Exp Clin Res* 1989; 15: 389–96.
20. EBETINO FH, DANSEREAU SM: Bisphosphonate antiresorptive structure-activity relationships, *In* BIJVOET OLM, FLEISCH HA, CANFIELD RE, RUSSELL RGG (Eds.): *Bisphosphonate on Bones*. Amsterdam, Elsevier Publishing Co. 1995: 139–53.
21. ROGERS MJ, GORDON S, BONFORD HL *et al.*: Cellular and molecular mechanisms of action of bisphosphonates. *Cancer* 2000; 88: 2961-78.
22. EBETINO FH, BAYLESS AV, AMBURGEY J, IBBOTSON KJ, DANSEREAU S, EBRAHIMPOUR A: Elucidation of pharmacophore for the bisphosphonate mechanism of bone resorptive activity. *Phosphorus Sulfur Silicon* 1996; 109–110: 217–220.
23. ROUBENOFF R, FREEMAN LM, SMITH DE, ABAD LW, DINARELLO CA, KEHAYIAS JJ: Adjuvant arthritis as a model of inflammatory cachexia. *Arthritis Rheum* 1997; 40: 534–9.
24. ELKAYAM O, YARON I, SHIRAZI I, JUDOVITCH R, CAPSI D, YARON M: Active lefunomide metabolite inhibits interleukin 1beta, tumor necrosis factor, nitric oxide, and metalloproteinase-3 production in activated human synovial tissue cultures. *Ann Rheum Dis* 2003; 62: 440–3.
25. OHTA S, HARIGAI M, TANAKA M *et al.*: Tumor necrosis factor–alpha (TNF-alpha) converting enzyme contributes to production of TNF-alpha in synovial tissues from patients with rheumatoid arthritis. *J Rheumatol* 2001; 28: 1756–63.
26. BRENNAN FM, HAYES AL, CIESIELSKI CJ, GREEN P, FOXWELL BM, FELDMANN M: Evidence that rheumatoid arthritis synovial T cells are similar to cytokine-activated T cells: involvement of phosphatidylinositol 3-kinase and nuclear factor kappaB pathways in tumor necrosis factor alpha production in rheumatoid arthritis. *Arthritis Rheum* 2002; 46: 31–41.
27. WANG B, CHEN M-Z: Effects of indomethacin on secretory function of synoviocytes from adjuvant arthritis rats. *Int J Tissue React* 1998; 20: 91–4.
28. SMITH-OLIVER T, NOEL LS, STIMPSON SS, YARNALL DP, CONNOLLY KM: Elevated levels of TNF in the joints of adjuvant arthritic rats. *Cytokines* 1993; 5: 298–304.
29. MARKUSSE HM, LAFEBER GJ, BREEDVELD FC: Bisphosphonate in collagen arthritis. *Rheumatol Int* 1990; 9: 9–14.
30. NAKAMURAM, ANDO T, ABE M *et al.*: Contrast between effects of aminobisphosphonates and non-aminobisphosphonates on collagen-induced arthritis in mice. *Br J Pharmacol* 1996; 119: 205–12.
31. ZHAO H, SHUTO T, HIRATA G, IWAMOTO Y: Aminobisphosphonate (YM175) inhibits bone destruction in rat adjuvant arthritis. *J Orthop Sci* 2000; 5: 397–403.