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Prevention of progressive joint destruction in adjuvant induced arthritis in rats by a novel matrix metalloproteinase inhibitor, FR217840

Takeshi Ishikawa[a,*], Fusako Nishigaki[a], Susumu Miyata[a], Yoshitaka Hirayama[a], Kyoko Minoura[a], Junko Imanishi[a], Masahiro Neya[b], Tsuyoshi Mizutani[b], Yoshimasa Imamura[c], Yoshitaka Ohkubo[a], Seitaro Mutoh[a]

[a]*Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[b]*Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5-2-3, Tokodai, Tsukuba, Ibaraki 300-2698, Japan*
[c]*Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5-2-3, Tokodai, Tsukuba, Ibaraki 300-2698, Japan*

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Abstract

Matrix metalloproteinase (MMP) has been implicated in joint destruction of chronic arthritis diseases, such as rheumatoid arthritis. FR217840 (2*R*)-1-{[5-(4-fluorophenyl)-2-thienyl]sulfonyl}-*N*-hydroxy-4-(methylsulfonyl)-2-piperazinecarboxamide is a potent, orally active synthetic MMP inhibitor that inhibits human collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9) and membrane type MMP (MT-MMP) (MT1-MMP/MMP-14). FR217840 also inhibits rat collagenase and gelatinase. We studied the effect of FR217840 on a rat adjuvant induced arthritis model. Although oral administration (days 1–21) of FR217840 (3.2, 10, 32 mg/kg) to adjuvant injected Lewis rats did not affect inflammation, as indicated by both hind paw swelling and histological inflammatory infiltration, FR217840 suppressed both bone destruction and serum pyridinoline content in a dose-dependent manner. Also, FR217840 (32 mg/kg) reduced tartrate-resistant acid phosphatase (TRAP) cell number in the ankle joints of rats with arthritis. These results indicate that FR217840 successfully suppressed joint destruction and suggest that FR217840 may have potential as a novel anti-rheumatic drug.

Keywords: Matrix metalloproteinase; Arthritis, adjyuvant induced; Bone destruction; Osteoclast

1. Introduction

Rheumatoid arthritis is a systemic, immune/inflammatory disease characterized by joint swelling, synovial inflammation and joint destruction that leads to significant disability. The pathology of this joint destruction is characterized by destruction of articular cartilage and marginal and subchondral bone (Feldmann et al., 1996). Bone destruction is a critical feature of arthritis-affected joints, and in severe cases of rheumatoid arthritis, marked destruction of joints with focal erosion and juxtaarticular osteoporosis due to abnormal osteoclastic bone resorption is often observed (Kroger et al., 1994; Shimizu et al., 1985). Osteoclasts are large polynucleated cells that can be generated by fusion of osteoclast precursors of monocyte/macropharge lineage. It has been suggested that osteoclasts are involved not only in normal physiological bone remodeling but also in pathological articular bone resorption in rheumatoid arthritis. Osteoclast precursors and mature osteoclasts are abundant at sites of arthritic bone erosion (Suda et al., 1992; Udagawa et al., 2002).

Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, are also implicated in the pathogenesis of rheumatoid arthritis and animal arthritis models. Current evidence suggests that MMPs, including collagenases (MMP-1, MMP-8 and MMP-13), gelatinases

* Corresponding author. Tel.: +81 6 6390 1157; fax: +81 6 6304 5367.
E-mail address: takeshi_ishikawa@po.fujisawa.co.jp (T. Ishikawa).

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(MMP-2 and MMP-9), stromelysins (MMP-3 and MMP-10) and membrane type MMPs (MT-MMPs), play an important role in joint destruction. Degradation of articular cartilage collagen matrix appears to be mediated by infiltrating synovial fibroblasts and macrophages through the release of MMPs. A collagenase subtype, MMP-13, in concert with a gelatinase subtype, MMP-9, can solubilize cartilage collagen matrix in vitro (Engsig et al., 2000). In terms of bone resorption, in general, previous evidence suggests that cathepsin K is essential for degradation of organic components in bone matrix, but MMPs also contribute to bone matrix solubilization in some pathological situations, such as osteolysis due to bone metastasis in cancer (Delaisse et al., 2003). MMPs also might be involved in degradation of bone matrix in rheumatoid arthritis. Levels of cross-linked carboxyterminal telopeptide of type I collagen (ICTP), a bone resorption marker that reflects MMP-mediated type I collagen degradation, correlate with joint destruction in rheumatoid arthritis (Hakala et al., 1993; Sassi et al., 2003). Several MMP subtypes involved in collagen digestion have been identified in bone areas showing resorption. They include collagenases such as MMP-13, gelatinases such as MMP-2 and MMP-9, and other MMPs like MMP-12 and membrane type 1 MMP (MT1-MMP/MMP-14) (Bord et al., 1996; Delaisse et al., 1993; Dew et al., 2000; Hou et al., 2004; Okada et al., 1995; Sato et al., 1997, 1998). MMP-9 and MMP-14 are MMP subtypes that are extensively expressed in osteoclasts (Reponen et al., 1994; Sato et al., 1997; Tezuka et al., 1994; Wucherpfennig et al., 1994). Recently, involvement of MMPs such as MMP-13, that does not originate from osteoclasts but from osteoblasts, in bone collagen solubilization has been suggested. Calvariae of MMP-13 knockout mice resorbed more slowly compared to those from wild type mice (Delaisse et al., 2003).

FR217840 (2*R*)-1-{[5-(4-fluorophenyl)-2-thienyl]sulfonyl}-*N*-hydroxy-4-(methylsulfonyl)-2-piperazinecarboxamide is a novel synthetic MMP inhibitor that inhibits collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9) and MT-MMP (MMP-14) in vitro (Fig. 1). In the present study, we estimated the ability of FR217840 to prevent joint destruction in rat adjuvant induced arthritis. Adjuvant induced arthritis is a standard animal model of rheumatoid arthritis in humans (Joe and Wilder, 1999). This animal model has been widely used to investigate pathogenic mechanisms in rheumatoid arthritis, such as bone erosion, pannus formation and infiltration of inflammatory cells, and to evaluate potential new therapeutic agents. The adjuvant induced arthritis model is also characterized by increased numbers of osteoclasts, which is also a feature of human rheumatoid arthritis (Bolon et al., 2004).

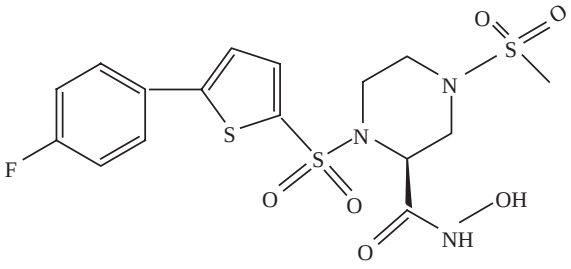

Fig. 1. Chemical structure of FR217840, a matrix metalloprotease inhibitor.

In this study, we demonstrated that FR217840 successfully inhibited joint destruction in a rat adjuvant induced arthritis model. Also, tartrate-resistant acid phosphatase (TRAP)-positive cell number infiltrating the right ankle joint (adjuvant injected sites) from FR217840-treated rats was significantly reduced. These results suggest that collagenases, gelatinases and MT-MMP might be involved in recruitment of TRAP-positive cells (a marker of osteoclasts) and in subsequent bone destruction in arthritic joints.

2. Materials and methods

2.1. Drugs

FR217840 was prepared at Fujisawa Pharmaceutical (Ibaraki, Japan).

2.2. Enzyme inhibition assays

Human MMP-1, MMP-2, MMP-3, MMP-8 and MMP-9 were obtained from Yagai (Yamagata, Japan). Culture media of rat synovial fibroblasts with collagenase and gelatinase activities were prepared as reported previously (Hashida et al., 1996). Briefly, synovial fibroblasts obtained from knee joints of normal female Lewis rats were stimulated with short-term culture media from casein-induced rat peritoneal polymorphonuclear leukocytes (PMNs). Rat synovial fibroblast culture media were then activated by incubation with 1 mM *p*-aminophenylmercuric acetate (Sigma, St. Louis, MO, USA) overnight at 4 °C before rat collagenase and gelatinase assay. MMP-1 and rat collagenase activities were estimated at 37 °C for 2 h using a Type I collagenase activity assay kit containing fluorescein-5-isothiocyanate (FITC)-labeled type I collagen (Yagai). MMP-8 activity was estimated at 37 °C for 2 h using a Type II collagenase activity assay kit containing FITC-labeled type II collagen (Yagai). MMP-2, MMP-3, MMP-9 and rat gelatinase activities were estimated at 42 °C for 2 h using a Type IV collagenase activity assay kit containing FITC-labeled type IV collagen (Yagai). MMP-13 activity was estimated at room temperature (RT) for 30 min using an Arthrogen-CIA MMP-13 kit obtained from Chondrex (Redmond, WA, USA). MMP-14 activity was estimated at 37 °C for 10 min using a MMP-14 colorimetric assay kit (Biomol, Plymouth Meeting, PA, USA). MMP activities except MMP-14 activity were measured with a spectrofluorophotometer (Spectrafluor plus, Tecan, Maennedorf, Switzerland). The reaction of MMP-14 was measured at 412 nm with a spectrophotometer (SpectraMax 250, Molecular Devices, Sunnyvale, CA, USA). All reactions for estimation of IC_{50}

values for MMP subtypes were performed according to the manufacture's instructions. Each IC_{50} value was estimated by Log-conversion following simple linear regression.

A disintegrin and metalloproteinase with thrombospondin repeats-4 (ADAMTS-4/Aggrecanase-1) assay was performed according to the method of Miller et al. with some modifications (Miller et al., 2003). Briefly, Microtiter plates were coated with streptavidin (Vector Laboratories, Burlingame, CA, USA). After the streptavidin coated plate was washed with phosphate buffered saline (PBS) containing 0.05% Tween-20, a biotinylated substrate peptide QTVTWPDMELPLPRNITEGEARGSVILTVKSVVYGLR (10 μg/ml), manufactured at Toray (kanagawa, Japan), was added to each well and incubated for 1 h at 37 °C. After blocking with PBS containing 1% bovine serum albumin, anti-substrate peptide antibody, which specifically recognizes SVVYGLR sequence, prepared in our laboratory, was added to each well and incubated for 1 h at 37 °C. After washing with PBS, 10 μg/ml of human ADAMTS-4 (Chemicon International, Temecula, CA, USA) was added to each well and incubated in the presence or absence of inhibitors for 7 h at 37 °C. After washing, horseradish peroxidase conjugated anti-human IgG (Zymed Laboratories, South San Francisco, CA, USA) was added to each well and incubated for 30 min at RT. Super sensitive TMB solution (Sigma) was added and incubated for 20 min at RT. The reaction was stopped by addition of 2 N H_2SO_4 and read at 450 nm.

2.3. Cell based tumor necrosis factor (TNF) inhibition assays

The cell based activities of FR255031 were evaluated in human THP-1 and rat splenocytes for inhibition of lipopolysaccarde (LPS) induced TNF-α secretion. Human THP-1 cells were treated with LPS (10 μg/ml) (serotype B5:055, Sigma) for 18 h in Dulbecco's modified eagle's medium (DMEM) (Sigma) supplemented with 10% fetal bovine serum (Moregate biothech, Bulimba, QLD, Australia). Rat splenocytes were treated with LPS (1 μg/ml) for 18 h in DMEM supplemented with 1% fetal bovine serum. At the end of incubation period, media were collected and frozen at −80 °C. The concentrations of human TNF-α were determined by an enzyme-linked immunosorbent assay (ELISA) using MAb1 as capture antibody, and MAb11 as detection antibody (Becton Dickinson, San Diego, CA, USA) according to the manufacture's instructions. The concentrations of rat TNF-α were determined by an ELISA using TN 3-19.12 as capture antibody, and polyclonal anti-Ms/Rt as detection antibody (Becton Dickinson) according to the manufacture's instructions.

2.4. Animals

Female, 7-week-old Lewis rats were purchased from Charles River Japan (Kanagawa, Japan) and bred in a clean atmosphere with 12 h light/dark cycles. Rats were fed standard rodent chow ad libitum and were free from infectious diseases. They were allowed 1 week to adapt to their environment and used at 8 weeks of age. All experimental procedures were performed according to guidelines of the Animal Experiment Committee of Fujisawa Pharmaceutical.

2.5. Induction of adjuvant arthritis

Arthritis was induced by injection of 0.5 mg of *Mycobacterium tuberculosis* (Difco Laboratories, Detroit, MI, USA) in 50 μl of liquid paraffin into the right hind footpad of Lewis rats (day 0). Normal non-treated rats were used as negative controls. The volume of both hind paws was measured on days −1 and 21 by a water displacement method using a plethysmometer for rats (MK-550; Muromachi Kikai Tokyo, Japan). Paw swelling was presented as a change in the hind paw volume.

2.6. Drug treatment

FR217840 was suspended in 0.5% methylcellulose. Animals were randomized and grouped (n=10) for drug treatment based on body weight on day −1. Vehicle (0.5% methylcellulose) or FR217840 (10, 32, 100 mg/kg) was orally administered twice a day from days 1 to 21.

2.7. Serum pyridinoline

For measurement of serum pyridinoline, peripheral blood was obtained from the abdominal artery of rats on day 22 after general anesthesia by inhalation of diethyl ether and serum was collected by centrifugation. Concentration of serum pyridinoline was measured by immunoassay using serum pyridinoline specific ELISA (Quidel, San Diego, CA, USA).

2.8. Radiological scoring

Both hind paws were removed for radiological scoring. All radiographs were taken with X-ray film (Kodak Diagnostic Film, Ready-Pack, X-OMAT™, Kodak, NY,

Table 1
Inhibitory activities of FR217840 various enzymes and TNF-α secretion in cell based assays

			IC50 (nM)
			FR217840
Human	Collagenase	MMP-1	6.94
		MMP-8	0.359
		MMP-13	1.39
	Gelatinase	MMP-2	10.4
		MMP-9	3.22
	Stromelysin	MMP-3	>10,000
	MT-MMP	MMP-14	0.729
	ADAMTS	Aggrecanase-1	>10,000
	TNF-α secretion		>10,000
Rat	Collagenase		16.8
	Gelatinase		37.5
	TNF-α secretion		2939

Table 2
Effect of FR217840 on paw swelling of arthritic joint on day 21

Treatment	Dose (mg/kg)	Right ankle	Left ankle
		Paw swelling (ml)	Paw swelling (ml)
NT	–	0.01±0.01	0.02±0.01
Control	–	2.82±0.14[a]	1.73±0.14[a]
	10	2.69±0.06	2.27±0.04
FR217840	32	2.50±0.10	1.97±0.08
	100	2.66±0.12	1.87±0.07

Paw swellings of normal non-treated (NT) group, adjuvant induced arthritis control (Control) group and FR217840-treated groups on day 21 are expressed as mean of change in right (injected site) or left (uninjected site) hind paw volume. Values are shown as the mean±S.E.M.

[a] $P<0.01$ compared to NT.

USA) using a MBR-1505R (Hitach Medical Corporation, Tokyo, Japan). The settings for radiographs were 5 mA, 50 kV and 1 min exposure. Films were placed 60 cm below the X-ray source. Radiological scoring was carried out on the basis of bone destruction (severity was classified into five grades: 0, normal; 1 to 4, slight to severe) or joint space narrowing (severity was classified into three grades: 0, normal; 1 to 2, slight to severe). Bone destruction was scored for each of the following features: calcaneous, talus, metatarsus and the distal tibia. The score for each specimen was represented as total grade of these features. Joint space narrowing was scored for each joint space among talus, naviculare and cuneiform.

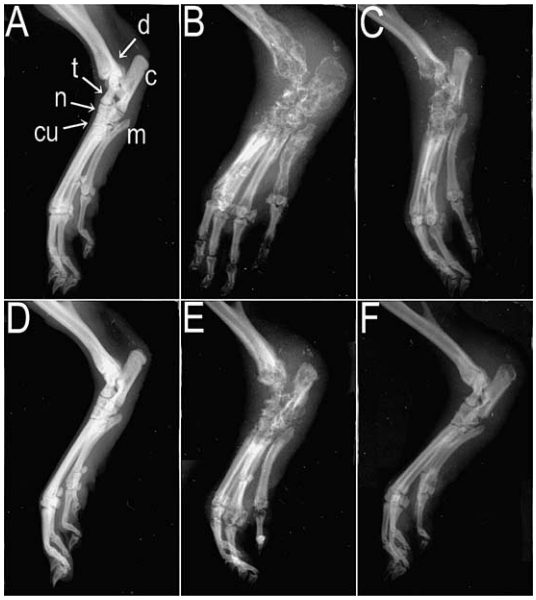

Fig. 2. Radiographic analysis of right ankle joints of normal non-treated (NT) rats (A), arthritis control (Control) rats (B) and FR217840 (32 mg/kg)-treated rats with arthritis (C), and left ankle joints of NT rats (D), control rats (E) and FR217840 (32 mg/kg)-treated rats with arthritis (F) (day 22). No joint damage was seen in the right and left hind paws of NT rats (A and D). Severe bone erosion and joint space narrowing was observed in arthritis control (Control) rats (B and E). Right joints (adjuvant injected site) were more severe than left joints. Arthritic changes were ameliorated in FR217840 (32 mg/kg)-treated rats with arthritis (C and F). c, calcaneous; cu, cuneiform; d, distal tibia; m, metatarsus; n, naviculare; t, talus.

2.9. Histological evaluation

For histological evaluation, both hind paws used for radiological scoring were used. Whole ankle joints were dissected and fixed for 3 days in 10% neutral buffered formalin. After decalcification in 10% formic acid, specimens were paraffin embedded. Tissue sections (2 μm) were stained with hematoxylin and eosin and for TRAP activity. Histological analysis, using hematoxylin and eosin sections, was carried out on the basis of synovial proliferation and inflammatory infiltration. Severity of lesions was classified into five grades: 0, no detectable change; 1 to 4, slight to severe. TRAP enzyme was detected in paraffin tissue sections using a commercial acid phosphatase leukocyte kit (Sigma). The number of TRAP-positive cells was calculated in talus bones from specimens (one tissue section per specimen) in each group using Eclipse E600 microscope (Nikon, Tokyo, Japan) at ×40 magnification. The area of talus bone, in which TRAP-positive cells were calculated, was measured using Image-Pro PULS software (Media Cybernetics, Silver Spring, MD, USA). The number of TRAP-positive cells in the talus was normalized by the area.

2.10. Statistical analysis

Data are expressed as mean±S.E.M. Statistical significance of differences was assessed by Dunnett's multiple

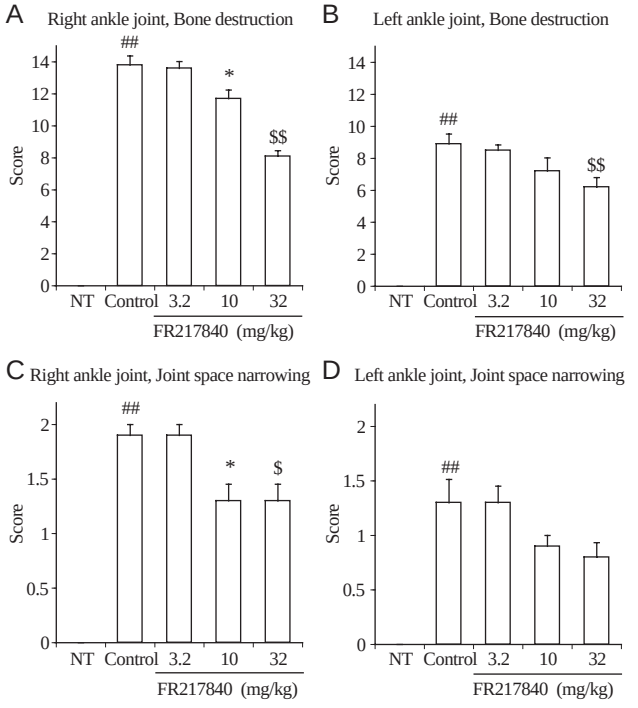

Fig. 3. Inhibitory effect of FR217840 on radiographic scores in right ankle joints (A and C) and left ankle joints (B and D) with arthritis. Normal non-treated (NT) rats were used as negative controls. Radiographic score was determined on the basis of bone destruction (A and B) or joint space narrowing (C and D), as described in Materials and methods. Values are shown as mean±S.E.M. ##; $P<0.01$ compared to NT. *$P<0.05$ compared to arthritis control (Control). $ or $$, $P<0.05$ or $P<0.01$ compared to control.

comparison test following one-way analysis of variance and Student's *t*-test for comparison of two samples. In the case of radiological or histological scoring, Wilcoxon's nonparametric analysis of variance for comparison of two samples was performed. $P<0.05$ was set as the level of significance.

3. Results

3.1. Inhibitory activity of FR217840 on various enzymes and on TNF-α secretion in cell based assays

Inhibitory effect of FR217840 on various enzymes and was examined (Table 1). FR217840 potently inhibited human collagenases (MMP-1, MMP-8 and MMP-13), human gelatinases (MMP-2 and MMP-9) and human MMP-14 with IC_{50} values of 6.94, 0.359, 1.39, 10.4, 3.22 and 0.729 nM, respectively. FR217840 did not inhibit human MMP-3 and human aggercanase-1 up to 10^{-5} M. FR255031 also inhibited both rat collagenase and rat gelatinase with similar potency (IC_{50} values of 16.8 and 37.5 nM, respectively) under our experimental conditions. In cell based assay, FR217840 did not inhibit human TNF-α secretion up to 10^{-5} M, but it only weakly inhibited rat TNF-α secretion with IC_{50} value of 2939 nM.

3.2. Evaluation of adjuvant induced arthritis

Plethysmographic estimation demonstrated that right hind paws (adjuvant injected sites) swelled markedly 1 day after injection and sustained until at least day 22, when the experiment was terminated. Swelling of left hind paws (uninjected sites) manifested from about day 10 and continued to day 22 (data not shown). FR217840 did not suppress both paws swelling on day 21 (Table 2).

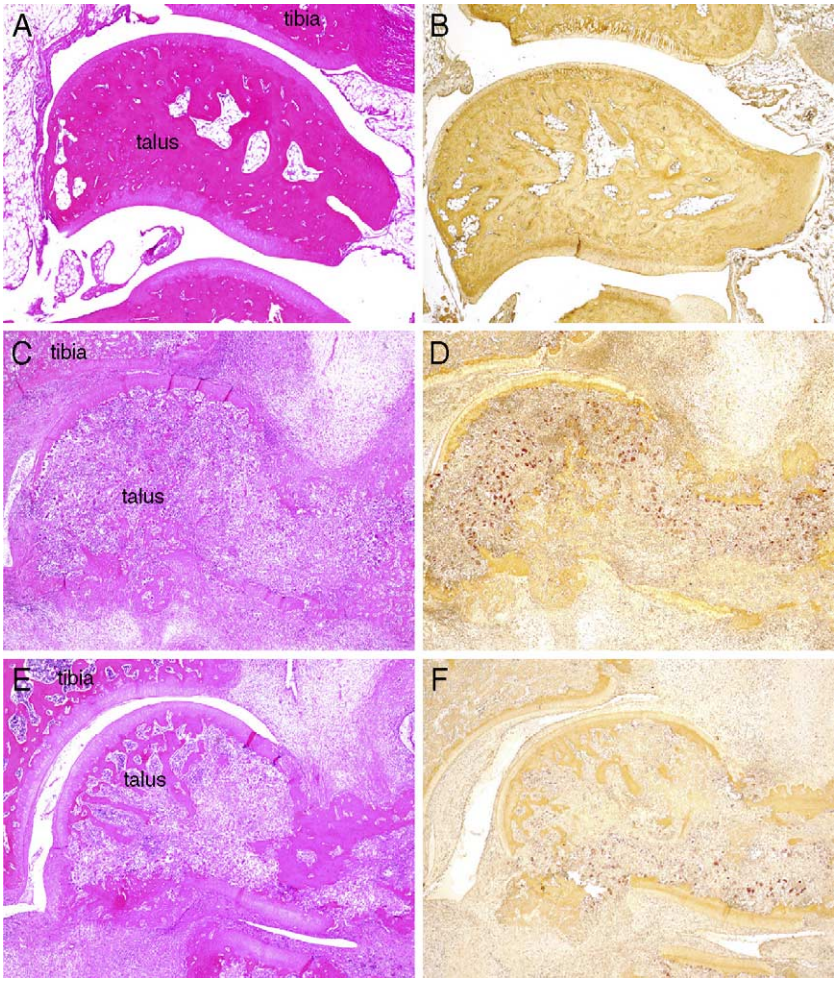

Fig. 4. Histological analysis of right ankle joints of normal non-treated (NT) rats (day 22) (A and B), arthritis control (Control) rats (C and D), FR217840 (32 mg/kg)-treated rats with arthritis (E and F). Tissue sections from each group were stained with hematoxylin and eosin (A, C and E), or for TRAP activity (B, D and F). Tissue sections stained with hematoxylin and eosin from an NT rats (A) exhibited normal histological features with no evidence of inflammation or erosion. Control rats showed severe joint damage, as evidenced by erosion, pannus formation and infiltration of inflammatory cells (C). FR217840 (32 mg/kg) relatively suppressed structural damage of right ankle joints, although severe hypertrophy of syonovium and inflammatory cells infiltration still remained (E). While a few TRAP-positive cells were detected in the NT ankle joints (B), a lot of TRAP-positive cells infiltrated into the talus in the Control ankle joints (D). FR217840-treated rats with arthritis decreased TRAP-positive cells number in the talus of ankle joints (F). (Original magnification ×40).

3.3. Radiological evaluation of adjuvant induced arthritis

Radiographic severity of joint destruction is shown in Fig. 2. In right and left ankle joints of arthritis control rats, severe bone destruction and deformation, plus joint space narrowing were detected (Fig. 2B and E). The destruction of right ankle joints was more severe than that of left joints. In both ankle joints, FR217840 (32 mg/kg) markedly inhibited bone destruction (Fig. 2C and F).

Scoring of radiological destruction in ankle joints was performed on the basis of bone destruction or joint space narrowing. Bone destruction of both ankle joints in FR217840-treated rats was significantly reduced in a dose-dependent manner when compared to arthritis control rats (Fig. 3A and B). Joint space narrowing of right ankle joints (adjuvant injected sites) in FR217840-treated rats was significantly reduced in dose-dependent manner (Fig. 3C). FR217840 also tended to reduce the scores of joint space narrowing in left ankle joints (adjuvant uninjected sites) (Fig. 3D). Both in bone destruction and in joint space narrowing, effects of FR217840 on right ankle joints (adjuvant injected sites) were more marked than left joints.

3.4. Histological evaluation of adjuvant induced arthritis

Photomicrographs of sections stained with hematoxylin and eosin, and for TRAP activity illustrate the disease severity and effect of compound treatment on joint histology (Fig. 4). No inflammation or tissue destruction was seen in hematoxylin and eosin sections from normal non-treated rats (Fig. 4A). In contrast, right ankle joints (adjuvant injected sites) of arthritis control rats showed severe joint destruction with extensive inflammation, and erosion of cartilage and bone. Enlarged cavities, as a result of the erosion, were filled with synovial fibroblasts and inflammatory cells (Fig. 4C). FR217840 (32 mg/kg) suppressed structural damage in right ankle joints, although severe hypertrophy of syonovium and inflammatory cell infiltration still remained (Table 3). Articular cartilage and bone trabeculae of the talus from FR217840-treated rats were relatively preserved compared to those from arthritis control rats (Fig. 4E).

In the talus of normal non-treated rats, about 5–10 TRAP-positive cells per section were detected (Fig. 4B). On the other hand, striking infiltration of numerous TRAP-positive cells (about 50–250 cells per section) was observed in the bone marrow space, as well as bone trabeculae of the talus for the right ankle (adjuvant injected site) in arthritis control rats (Fig. 4D). Some TRAP-positive cells were multinucleated (data not shown). FR217840 (32 mg/kg) appeared to decrease the number of TRAP-positive cells in the talus of the right ankle (Fig. 4F). Accordingly, the number of TRAP-positive cells was counted (Fig. 5) and found to be significantly reduced in the talus of the right ankle from FR217840 (32 mg/kg)-treated rats (Fig. 5A). In the talus of the left ankle (uninjected site), FR217840 also tended to reduce number of TRAP-positive cells, but the effect of the compound was more pronounced in the right talus (Fig. 5B).

Table 3
Effect of FR217840 (32 mg/kg) on the inflammatory scores of joint histology (adjuvant injected sites) from adjuvant induced arthritis rats

	Control	FR217840	
Inflammatory infiltration	3.4±0.2	3.8±0.2	n.s.
Synovial proliferation	3.8±0.2	3.2±0.4	n.s.

Inflammatory infiltration and synovial proliferation in right ankle joints (adjuvant injected sites) from adjuvant induced arthritis control (Control) group and FR217840 (32 mg/kg)-treated groups were graded as described in Materials and methods. Values are shown as the mean±S.E.M. n.s. means not significant.

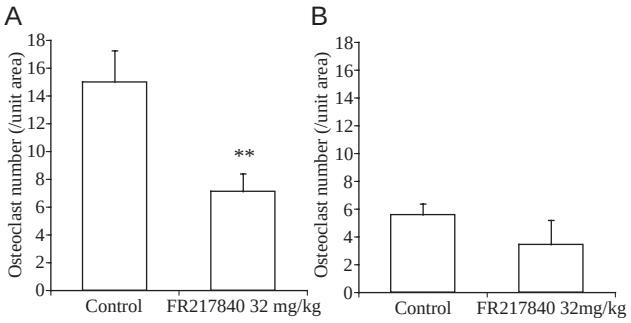

Fig. 5. Inhibitory effect of FR217840 (32 mg/kg) on TRAP positive-cell number in the talus of right ankle joints (A) and left ankle joints (B) with arthritis. TRAP positive-cell number was calculated as described in Materials and methods. Values are shown as mean±S.E.M. **P<0.01 compared to arthritis control (Control).

3.5. Serum pyridinoline levels

Levels of serum pyridinoline in arthritis control rats increased to twice that of normal non-treated rats on day 22, when the experiment was terminated. FR217840 (3.2–32 mg/kg) significantly suppressed the level of serum pyridinoline in a dose-dependent manner (Fig. 6).

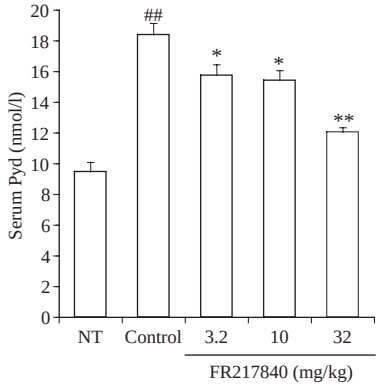

Fig. 6. Inhibitory effect of FR217840 on serum pyridinoline levels in rats with arthritis. Serum pyridinoline levels were determined as described in Materials and methods. Values are shown as mean±S.E.M. ##P<0.01 compared with normal non-treated (NT). * or **, P<0.05 or 0.01 compared to arthritis control (Control).

4. Discussion

In the present study, we evaluated the effect of MMP inhibition on joint destruction, in particular on bone destruction in a rat adjuvant induced arthritis model. Current evidence suggests that degradation of cartilage collagen matrix is primarily accomplished by MMPs in the pathogenesis of arthritis, but involvement of MMPs in bone destruction of arthritic joint in vivo is not fully understood.

We demonstrated that FR217840, an orally active inhibitor of collagenase, gelatinase and MT-MMP, suppressed joint destruction in rat adjuvant induced arthritis model, as indicated by radiological scores and serum pyridinoline level, without clear amelioration of inflammation, as indicated by paw swelling and inflammatory infiltration in joint histology. Also, we demonstrated that FR217840 decreased the number of TRAP-positive cells (a marker of osteoclasts) in ankle joint of adjuvant induced arthritis rats. Assessment of osteoclast populations in bones is an important step in understanding the pathogenesis of skeletal diseases and in developing new bone-sparing therapies (Bolon et al., 2004). As far as we know, this is the first report to indicate that an MMP inhibitor decreases TRAP-positive cell number in adjuvant induced arthritis rats.

Several studies have evaluated the effect of MMP inhibitors, such as BAY 12-9566 (Hamada et al., 2000) and GI168 (Conway et al., 1995), on rat adjuvant induced arthritis. The effect of Trocade, a collagenase selective inhibitor, on rat adjuvant induced arthritis has also been reported, but the authors simply indicated that the compound had no statistically significant effect on paw swelling and arthritic lesion score, and did not discuss the effect of the compound on joint destruction in rats with arthritis (Lewis et al., 1997). BAY 12-9566, which selectively inhibits MMP-2, MMP-3 and MMP-9, is effective against joint destruction in adjuvant induced arthritis rats, as assessed by both histological examination and measurement of collagen breakdown products. However, the compound does not inhibit osteoclast infiltration into the arthritic joints. In that report, Hamada et al. commented that BAY 12-9566 indirectly inhibits bone and cartilage matrix degradation in arthritic joints through its anti-inflammatory activities, as indicated by inhibition of PMN infiltration. BAY 12-9566 also inhibited paw swelling. GI168 is equipotent against collagenases and gelatinases, but much less active against stromelysin. Administered using osmotic mini-pumps, GI168 (12–25 mg/kg), as well as FR217840, is effective against bone destruction of arthritic joints, as assessed by radiological and histological examination. GI168 also had a statistically significant effect on the scores of adherent osteoclasts in arthritic joints. However, GI168, unlike FR217840, potently decreases ankle swelling (50% inhibition at a dose of 25 mg/kg). It is possible that both BAY 12-9566 and GI168 might suppress joint destruction in adjuvant induced arthritis through their anti-inflammatory activities. However, FR217840 did not affect inflammation in rat adjuvant induced arthritis. This suggests that FR217840 might be effective against bone destruction through another mechanism, that is, a decrease in TRAP positive-cell number in arthritic joint.

Considerable work has been done to define the relative roles of collagenases, gelatinases and MT-MMP in bone resorption. Initially it has been believed that MMPs from osteoclasts were associated with the degradation of the bone matrix. However, it is recently suggested that MMPs might act in the migratory/invasive activity of the osteoclast, rather than direct bone resorption activity (Blavier and Delaisse, 1995; Sato et al., 1998). Knockout mice of MMP-9 or MMP-14, each of which is abundantly expressed in osteoclast, exhibit a delay in osteoclast recruitment (Delaisse et al., 2003; Engsig et al., 2000). Collagenases like MMP-13, synthesized and secreted by osteoblast-lineage cells, might also contribute to osteoclast migration. Collagenases are responsible for degrading the non-mineralized osteoid layer covering bone surfaces, essential for exposing mineralized matrix to osteoclasts (Chambers et al., 1985; Holliday et al., 1997; Vaes et al., 1992). Calvariae of transgenic mice with type I collagen resistant to collagenase cleavage indicate a lack of bone resorption (Chiusaroli et al., 2003; Everts et al., 1999; Zhao et al., 1999).

In addition, several studies have evaluated the effect of MMP inhibitors on osteoclast migration in vitro. KB-R7785, a MMP inhibitor that inhibits MMP-1, MMP-3 and MMP-9 (IC_{50} values of 3, 1.9 and 3.9 nM, respectively) significantly affects the adhesion structure in osteoclasts and their movements to decreased osteoclast resorption activity in vitro (Goto et al., 2002). Also, RP59794, which is equipotent against MMP-1, MMP-3 and MMP-9, inhibits the migration of purified osteoclasts through collagen-coated membrane (Sato et al., 1998). These observations all support our results, indicating that FR217840, which potently inhibits collagenase (MMP-1 or MMP-13), gelatinase (MMP-9) and MT-MMP (MMP-14), suppresses infiltration of osteoclasts and subsequent bone destruction in arthritic joints.

FR217840 only weakly inhibits rat TNF-α secretion in cell-based assay. It is reported that anti-TNF agents inhibits ankle swelling as well as bone destruction in the rat adjuvant induced arthritis model (McComb et al., 1999). Although FR217840 shows its no clear anti-inflammatory activity, we cannot exclude the possibility that inhibition of TNF-α secretion by FR217840 might be effective against a decrease in TRAP positive-cell number and subsequent bone destruction in arthritic joint. TNF-α also has been shown to be able to induce osteoclast differentiation even in the absence of receptor activator of nuclear factor-κB ligand (RANKL) (Kobayashi et al., 2000).

Inhibitory activity of FR217840 on serum pyridinoline levels was more marked than that on radiological scores. Pyridinoline reflects the degradation of collagen, which is embedded in bone, articular cartilage and other connective

tissues. It is possible that a MMP inhibitor, FR217840 also could prevent articular cartilage or connective tissue degradation. Suppression of bone, cartilage and connective tissue collagen matrix degeneration in rats with arthritis may lead to synergistic reduction of serum pyridinoline levels.

Effects of FR217840 both on radiological scores and on TRAP-positive cell number in the right ankle (adjuvant injected site) were more remarkable than in the left ankle. Both severity of arthritic change in radiograph and TRAP-positive cell infiltration in the left ankle (uninjected site) were much more mild and varied widely. The exact explanation for this result was not elucidated, but the different effects of FR217840 between the right and left ankles might be, in part, due to mild arthritic changes in the left ankle joint. Previous reports indicated that histopathological effect of BAY 12-9566 obtained from adjuvant injected paws were also much clearer than that obtained from contralateral paws, supporting to some extent our findings (Hamada et al., 2000).

Although the potential utility of MMP inhibitors as therapeutic interventions in rheumatoid arthritis or osteoarthritis has led to an intense effort toward the development of such inhibitors, few MMP inhibitors have entered clinical trials and none have so far been successful (Jackson et al., 2001; Michaelides and Curtin, 1999). The first generation of MMPs inhibitors, such as Marimastat, which are peptide-like broad spectrum inhibitors, have been reported that the induction of musculoskeletal side effects was seen in clinical trials. These compounds, active against broad range of MMPs, might block normal matrix turnover and be more likely to cause side-effects and/or mask the beneficial effects of target MMP(s) inhibition. FR217840 is a small molecule, non-peptidic and relatively specific MMP inhibitor compared to Marimastat, but the side-effects of FR217840 will be a next problem to be elucidated.

In conclusion, FR217840 is an orally active MMP inhibitor that successfully improved joint destruction. Furthermore, it reduced TRAP-positive cell infiltration in arthritic joints. These results suggest that FR217840 may have potential as a novel anti-rheumatic drug.

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