Inhibition of Cartilage and Bone Destruction in Adjuvant Arthritis in the Rat by a Matrix Metalloproteinase Inhibitor

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Summary

Considerable evidence has associated the expression of matrix metalloproteinases (MMPs) with the degradation of cartilage and bone in chronic conditions such as arthritis. Direct evaluation of MMPs' role in vivo has awaited the development of MMP inhibitors with appropriate pharmacological properties. We have identified butanediamide, N4-hydroxy-2-(2-methylpropyl)-N1-[2-[[2-(morpholinyl)ethyl]-,[S-(R*,S*)] (GI168) as a potent MMP inhibitor with sufficient solubility and stability to permit evaluation in an experimental model of chronic destructive arthritis (adjuvant-induced arthritis) in rats. In this model, pronounced acute and chronic synovial inflammation, distal tibia and metatarsal marrow hyperplasia associated with osteoclasia, severe bone and cartilage destruction, and ectopic new bone growth are well developed by 3 wk after adjuvant injection. Rats were injected with Freund's adjuvant on day 0. GI168 was was administered systemically from days 8 to 21 by osmotic minipumps implanted subcutaneously. GI168 at 6, 12, and 25 mg/kg per d reduced ankle swelling in a dose-related fashion. Radiological and histological ankle joint evaluation on day 22 revealed a profound dose related inhibition of bone and cartilage destruction in treated rats relative to rats receiving vehicle alone. A significant reduction in edema, pannus formation, periosteal new bone growth and the numbers of adherent marrow osteoclasts was also noted. However, no significant decrease in polymorphonuclear and mononuclear leukocyte infiltration of synovium and marrow hematopoietic cellularity was seen. This unique profile of antiarthritic activity indicates that GI168 is osteo- and chondro-protective, and it supports a direct role for MMP in cartilage and bone damage and pannus formation in adjuvant-induced arthritis.

In rheumatoid arthritis, pain and swelling can generally be controlled by currently available drugs, but it has been difficult to halt the progressive joint destruction associated with this disease. Therefore, much effort has been directed at more specific inhibition of the biochemical mechanisms underlying joint destruction. The enzymes most often implicated in the joint destruction are the matrix metalloproteinases (MMPs).[1] MMPs are a family of zinc-dependent endopeptidases that degrade components of the extracellular matrix (1). MMPs fall into three classes based on their substrate

specificity. Interstitial (MMP-1) and neutrophil collagenase (MMP-8) are the only enzymes known to degrade collagen types I and II at neutral pH. Collagen type I is the major structural protein of bone whereas collagen type II is a major component of cartilage. Gelatinases (MMP-2 and 9) degrade collagen types IV and V and denatured protein. Gelatinase works in concert with collagenase in that collagen type I fragmented by collagenase can unwind and become a substrate for gelatinase. Stromelysin (MMP-3) degrades fibronectin and glycoproteins and proteoglycans, a key component of cartilage.

There has been considerable work in in vitro systems implicating MMPs in connective tissue breakdown. Treatment

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[1] *Abbreviation used in this paper:* MMP, matrix metalloproteinase.

of cartilage with phorbol myristate acetate or IL-1 in vitro stimulates tissue degradation and MMP secretion into the media (2). Treatment of bone with parathyroid hormone or 1,25-dihydroxy vitamin D3 in vitro causes bone resorption and MMP secretion into the media (3, 4). Stimulation of proteoglycan release from explants of rabbit articular cartilage stimulated with retinoid, IL-1 or LPS in vitro (5) is blocked by synthetic MMP inhibitors. MMP inhibitors block proteoglycan release from cartilage explants isolated from rabbits that had undergone prior meniscectomy (6). MMP inhibitors reduce the loss of collagen and proteoglycan from IL-1–treated bovine nasal cartilage (7) and human articular cartilage (8). Parathyroid hormone–induced resorption of bone in the mouse calvaria was also inhibited by a synthetic MMP inhibitor (9).

In contrast to the in vitro situation, there are only indirect lines of evidence supporting a destructive role for MMPs in vivo. It is clear that the mRNA and protein levels of collagenase (10–13), gelatinase (2, 13), and stromelysin (2, 10–14) are elevated in the arthritic joints of both animals and humans. However, mRNA and protein level measurements don't distinguish between active and inactive forms of MMPs. MMPs are synthesized and secreted as latent zymogens and must be cleaved to the active form outside the cell (1). MMP activity is also regulated by the endogenous inhibitors α2-macroglobulin and tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) (1). Thus, both the degree of zymogen activation and inhibitor concentrations can impact MMP activity in vivo. It is also possible that other enzymes might play significant roles in joint destruction. For example, possible low pH microenvironments in the arthritic joint would allow cysteine proteinases to operate in a collagenolytic mode (15–17). Besides consideration of the enzymes degrading the matrix, one must consider that products of matrix degradation could influence the inflammation process in vivo. For example, peptide products from the degradation of collagen, gelatin, and fibronectin are chemotactic for a variety of inflammatory cells (18–21).

Here we describe an MMP inhibitor with sufficient solubility to permit continuous systemic administration in vivo. Administration of this inhibitor showed dramatic inhibition of connective tissue destruction in the joints of rats with adjuvant-induced arthritis. These data imply that MMP inhibitors could be developed for therapeutic use to halt destruction in rheumatoid arthritis.

Materials and Methods

Animals. Male Lewis rats (160–170 g) from Charles River Laboratories (Raleigh, NC) grew to ~240–250 g before the injection of adjuvant (see below). The animals were free of pathogenic viruses as determined by a standard viral titer screen (Microbiological Associates, Bethesda, MD).

Induction and Measurement of Adjuvant Arthritis. Freund's complete adjuvant was prepared by adding 100 mg *Mycobacterium tuberculosis* (Difco Laboratories, Detroit, MI) to 15.6 ml heavy paraffin oil followed by addition of 1 ml saline. The mixture was then

emulsified by pulsing for 90 s with a polytron (Brinkmann Instruments, Inc., Westbury, NY). Each rat was injected intradermally at the tail base with 300 μg of *Mycobacterium* in a 0.05-ml vol. Paw inflammation was quantitated by measuring paw diameter with calipers.

MMP Inhibitor and Minipump Implantation. The MMP inhibitor butanediamide, N4-hydroxy-2-(2-methylpropyl)-N1-[2-[[2-(4-morpholinyl)ethyl]amino]2-oxo-1-(phenylmethyl)ethyl]-,[S-(R*,S*)] (GI168), was synthesized in the Department of Medicinal Chemistry at Glaxo Inc. GI168 was dissolved in DMSO and added to 50 mM sodium citrate buffer, pH 4.0, giving a final DMSO concentration of 20%. Rats were anesthetized with a constant flow of 2.5% isoflurane, their backs were shaved, cleaned with Betadine™, two 14-d minipumps (model 2ML2; Alza Corp., Palo Alto, CA) were inserted subcutaneously into their backs, and the insertion sites were closed with wound clips. These pumps deliver 5 μl/h, so at the highest soluble drug concentration of 25 mg/ml, the pumps delivered 25 mg/kg/d. Controls received 50 mM sodium citrate, pH 4.0, containing 20% DMSO.

Radiological and Histological Scoring. At the end of the experiment, paws were fixed in 10% buffered formalin and radiological plates were prepared. A section was then taken through the middle of the paw and stained with hematoxylin and eosin for histological analysis. The radiological plates were scored in a blind fashion by Dr. Richard L. Clark (University of North Carolina, Chapel Hill, NC) using a previously described scale from 0 (no damage) to 4 (severe damage) for the parameters defined in Table 1 (22). Histological effects were also scored in a blind fashion from 0 (no damage) to 5 (very severe damage) by two independent observers for the parameters defined in Table 2. Differences between the radiological and histological scores of vehicle-treated and drug-treated rats were assessed using a one degree of freedom chi-square test. Type I error rates of all tests were maintained at 5% using Sidak's method (23).

MMP Assays. Recombinant human collagenase (MMP-1) and stromelysin (MMP-3) were expressed in *Escherichia coli*, purified from inclusion bodies, and refolded at room temperature for 1 h before use. Human 92-kD gelatinase (MMP-9) was purified as described previously (24). Rat collagenase was purified from rat uterus (25). Collagenase assays were conducted in a buffer (200 mM NaCl, 50 mM Tris, 5 mM $CaCl_2$, 20 M $ZnSO_4$, pH 7.6) containing 0.05% Brij 35, 3 nM enzyme, and 100 μM of the chromogenic substrate Ac-Pro-Leu-Gly-SCH[$CH_2CH(CH_3)_2$] CO-Leu-Gly-OC$_2H_5$ (Bachem California, Torrance, CA) (26). Human 92-kD gelatinase was measured in assay buffer containing 0.01% Brij 35, 1 nM enzyme, and 10 μM of the fluorogenic substrate Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(Nma)-NH$_2$ (24). Human stromelysin was measured in assay buffer containing 0.05% Brij 35, 10 nM enzyme, and 10 μM of the fluorogenic substrate Dnp-Pro-Gln-Gln-Phe-Lys-Arg-Lys(Nma)-NH$_2$. In all the assays described above, substrate concentrations are well below the K_m so the IC$_{50}$ ~ Ki for the compounds.

Plasma Assay of GI168. GI168 was administered to untreated rats at 25 mg/kg per d by two 14-d minipumps implanted subcutaneously (see above). On day 4 and 12 of drug infusion, rats were killed by CO_2 asphyxiation, blood was taken from the inferior vena cava, and plasma prepared and frozen at −20°C. GI168 was separated from plasma by centrifugation through a 30,000-mol wt cut-off filter (model YMT-30; Amicon Corp., Beverly, MA) for 3 min at 1,500 g. Less than 20% of the plasma appeared as filtrate under these conditions. The plasma filtrate was then serially diluted and tested for inhibition of human collagenase (see MMP assays above). The filtrate dilution necessary for 50% inhibition of human collagenase activity was multiplied by the known IC$_{50}$

concentration to calculate initial plasma drug concentrations. Control experiments showed no effect of filtrate alone on the collagenase assay and <10% binding of GI168 to plasma proteins or the filter apparatus.

Results

MMP Inhibition In Vitro and GI168 Delivery In Vivo. GI168 inhibited rat collagenase with an IC_{50} of 15 nM. Against human enzymes, GI168 was equipotent against collagenase and gelatinase (IC_{50} = 3 nM) but much less active against stromelysin (IC_{50} = 225 nM). To check for inhibition of gelatinase activity across species, zymograms were run using serum-free media from cell cultures of human LOX melanoma, rat Mat Ly Lu prostate tumor, and mouse M27 Lewis lung carcinoma. Zymograms were renatured and developed in the presence of 50 nM GI168. Under these conditions, gelatinases from all three cell lines were inactive (data not shown), indicating cross-species inhibition of gelatinase at 50 nM GI168.

GI168 has poor oral bioavailability and a short plasma $t_{1/2}$ after subcutaneous injection (data not shown), so minipump delivery was evaluated. At its maximum solubility of 25 mg/ml, GI168 was shown to be stable after storage for 2 wk at 37°C. GI168 was then continuously administered to untreated rats at 25 mg/kg per d by implanting two 14-d minipumps subcutaneously. On days 4 and 12 after minipump implantation, the plasma levels of GI168 were 223 ± 11 (*n* = 4) and 113 ± 7 (*n* = 4) nM, respectively. Since GI168 shows <10% binding to plasma proteins (see Materials and Methods), GI168 in the plasma is immediately available for inhibition of MMPs in vivo. Thus with minipump infusion, the steady-state plasma levels of unbound GI168 were 7- to 15-fold above the IC_{50} for inhibition of rat collagenase in vitro.

Effect of GI168 on Ankle Swelling. In the adjuvant arthritis model, the adjuvant was injected on day 0. Ankles started to swell on days 9–10, and cartilage and bone damage becomes progressive from day 15 onward. The inflammation and connective tissue damage is constant up to ~ day 30, resulting in severe systemic disease and severe joint deformity (reviewed in references 27, 28). Given the constraints of the 14-d minipump delivery system, two 14-d pumps were implanted on day 8 and the experiment terminated on day 21. By day 21, there had been 10 d of ankle swelling, and the essential features of cartilage and bone damage were clearly evident. Oral indomethacin (1 mg/kg) once daily was used as a positive control for an antiinflammatory response. The effect of GI168 on ankle swelling is shown in Fig. 1. In rats with vehicle-filled pumps, ankle diameter increased in a linear fashion from days 8–15, with a plateau between days 15 and 20. GI168 decreased ankle swelling in a dose-response manner with 25, 12, and 6 mg/kg per d, giving 50, 38, and 16% inhibition of ankle swelling on day 20, respectively. The indomethacin control gave 54% inhibition of ankle swelling on day 20, as expected (Fig. 1). This experiment confirmed an initial experiment in which 25 mg/kg per d GI168 and
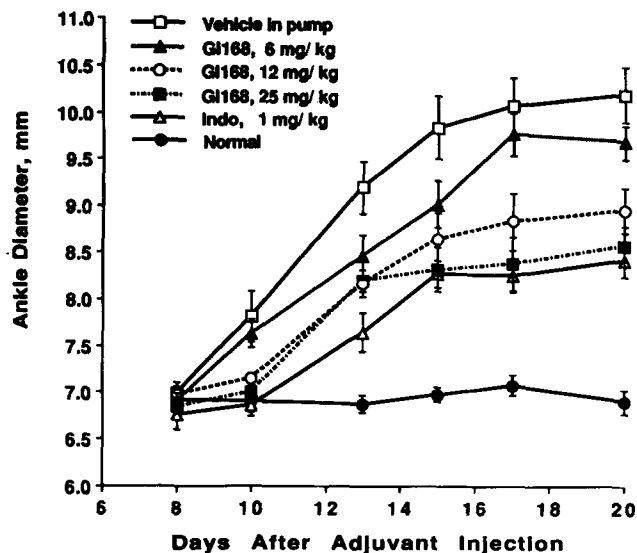

Figure 1. Effect of GI168 on adjuvant arthritis–induced ankle swelling in rats. Rats were implanted subcutaneously with two 14-d minipumps on day 8 to continuously deliver the cited daily doses of GI168. Indomethacin (*indo*) was given orally daily. Normal rats received no treatment. Data represent mean ± SEM with 5–10 rats per group.

1 mg/kg per d indomethacin inhibited ankle swelling by 50 and 79%, respectively (data not shown).

Effect of GI168 on Radiological Parameters of Joint Damage. Representative radiographs of ankle joints from normal rats and rats treated with vehicle or GI168 after adjuvant injection are shown in Fig. 2. Compared to the vehicle-treated control, the ankle from the rat treated with GI168 had greater bone density, lacked focal areas of severe bone loss, and showed less soft tissue swelling.

Since both experiments with GI168 had similar joint swelling, the ankles were combined for radiological and histological scoring. Radiological scoring of day 21 ankles is shown in Table 1. GI168 caused a dose-dependent inhibition of pathology in all five radiologic parameters, with even the lowest dose of 6 mg/kg per d causing a shift to less pathology in all parameters except edema (Table 1). The inhibition at 25 and 12 mg/kg per d GI168 was statistically significant in all five parameters, whereas at 6 mg/kg per d, the inhibition was only statistically significant against bone marrow erosion and bone demineralization.

Effect of GI168 on Histological Parameters of Joint Damage. Representative photomicrographs of ankle joints from normal rats and rats treated with vehicle or GI168 after adjuvant injection are shown in Fig. 3. Joints of vehicle-treated arthritic rats showed acute articular distension and swelling. There was increased synovial fluid that contained sloughed type A synovial cells, neutrophils, and fibrin strands. The joint capsule and subsynovial tissue were distended with edema and characterized by serous degeneration of the subsynovial adipose tissue with an infiltration of neutrophils with fewer lymphocytes and histiocytes.

Figure 2. Radiographic evidence of the protective effect of GI168 in adjuvant arthritis. Shown are radiographs of ankle joints from normal rats that did not receive adjuvant (*normal*) and adjuvant-injected rats treated with either vehicle alone (*vehicle*) or treated with GI168 at 25 mg/kg per (*GI168*). A joint that is representative of the group and not an extreme case is shown for each group. These are the same joints from which the histology photomicrographs were prepared. Note the higher bone density, the lack of focal areas of severe bone loss, and lesser soft tissue changes in the GI168-treated compared to the vehicle-treated rat joint (×3).

Joints of vehicle-treated arthritic rats showed a marked loss of trabecular bone with the resultant enlarged medullary cavities filled with hematopoietic precursors and inflammatory cells (Fig. 3). This ongoing remodeling of trabecular bone was associated with marked periosteal, endosteal, and trabecular new bone formation. In the more severely affected joints, there was also osteoclastic resorption of epiphyseal bone and the subchondral bone plate. In these joints, cartilage resorption by chondroclasts from the epiphyseal medullary cavity was also common. Cartilage loss was commonly seen at the margin of the joint in association with progressive pannus formation.

Several parameters of connective tissue and inflammatory cellular changes were scored (Table 2). GI168 caused a dose-dependent inhibition of the events directly associated with bone destruction, even with the lowest dose of 6 mg/kg per d, causing a shift to less pathology in bone erosion (both marginal erosions and trabecular osteoclasia), the number of adherent osteoclasts, and periosteal new bone formation and remodeling (Table 2) consistent with the results of the radiological scoring (Table 1). There was statistically significant inhibition of the bone destruction parameters at 12 and 25 mg/kg per d, as well as significant protection against cartilage destruction and pannus formation. With the low dose of GI168, only inhibition of periosteal new bone growth and remodeling was statistically significant.

Though GI168 significantly inhibited edema at the 25 and 12 mg/kg per d dose, it was not uniformly successful in inhibiting all the events associated with soft tissue swelling (Table 2). Neither dose of GI168 significantly reduced synovial lining necrosis, and only the high dose reduced synovial lining distension. GI168 had no obvious effect on the degree of infiltration of mononuclear leukocytes and PMNs into the joint space or bone marrow cavities. In addition, instead of the serous degeneration of subsynovial and periarticular fat observed in the vehicle-treated arthritic joints, a marked fibroblastic invasion of the subsynovial space and joint capsule was seen in the joints of rats receiving 25 mg/kg per d GI168.

Effects on Body Weight. During the 2-wk treatment window, vehicle-treated arthritis rats lost 1% of their body weight, whereas normal (no arthritis or treatment) rats increased their body weight by 29%. Arthritic rats receiving GI168 at 25 or 12 mg/kg per d had a 13% increase in body weight over the 2 wk of treatment. Indomethacin-treated arthritic rats had a 5% increase in body weight (Table 3).

Discussion

This report shows that an MMP inhibitor is effective against erosive arthritis in vivo. GI168 provided protection against a wide spectrum of effects including tissue edema, cartilage and bone destruction, abnormal bone deposition, and pannus formation. GI168 also had beneficial systemic effects, as indicated by its positive effects on body weight gain (Table 3).

Comparison of plasma concentrations and IC$_{50}$s for inhibition of purified MMPs indicates that the GI168 doses used here should inhibit collagenase and gelatinase in vivo. At the

Table 1. *Effect of GI168 on the Radiological Parameters of Adjuvant Arthritis*

Parameter	Radiologic score[*]	Percent of group with each score			
		Vehicle-treated ($n = 19$)	GI168 6 mg/kg ($n = 6$)	GI168 12 mg/kg ($n = 5$)	GI168 26 mg/kg ($n = 10$)
Edema	0.0–0.5	0	0	0	40
	1.0–1.5	5	17	80	50
	2.0–2.5	58	66	20	10
	3.0–4.0	37	17	0	0
Mean score[‡]		2.6	2.2	1.6 ($P < 0.01$)[§]	1.0 ($P < 0.001$)
Bone erosion	0.0–0.5	21	100	100	100
	1.0–1.5	42	0	0	0
	2.0–2.5	32	0	0	0
	3.0–4.0	5	0	0	0
Mean score		1.4	0.04 ($P < 0.05$)	0 ($P < 0.05$)	0.1 ($P < 0.01$)
Bone demineralization	0.0–0.5	11	100	100	100
	1.0–1.5	63	0	0	0
	2.0–2.5	21	0	0	0
	3.0–4.0	5	0	0	0
Mean score		1.5	0.5 ($P < 0.05$)	0.5 ($P < 0.05$)	0.4 ($P < 0.001$)
Abnormal bone growth	0.0–0.5	21	83	100	100
	1.0–1.5	63	17	0	0
	2.0–2.5	16	0	0	0
	3.0–4.0	0	0	0	0
Mean score		1.2	0.6	0.5 ($P < 0.05$)	0.4 ($P < 0.01$)
Joint space narrowing	0.0–0.5	21	17	100	100
	1.0–1.5	42	83	0	0
	2.0–2.5	32	0	0	0
	3.0–4.0	5	0	0	0
Mean score		1.4	0.9	0.5 ($P < 0.05$)	0.3 ($P < 0.05$)

[*] Radiologic plates were scored in a blinded fashion from 0 (normal) to 4 (very severe pathology). See Materials and Methods for details.
[‡] Scores for individual animals in each group were averaged.
[§] Significantly different from vehicle-treated group using Cochran-Mantel Haenszel statistics.
n = Number of rats in each group.
Dose is mg/kg per d. GI168 was delivered by subcutaneous minipumps. Details are described in Materials and Methods.

high dose of 25 mg/kg per d, the steady-state concentration of unbound GI168 in the plasma was 100–200 nM, values several-fold above that needed to inhibit rat collagenase (IC_{50} = 15 nM), human collagenase (IC_{50} = 3 nM), human gelatinase (IC_{50} = 3 nM), and rat gelatinase (100% inhibition at 50 nM). Even though the plasma levels of GI168 are near the IC_{50} for inhibition of human stromelysin (IC_{50} = 225 nM), inhibition of this enzyme in vivo cannot be predicted since the potency of GI168 against rat stromelysin is not known. Use of more selective inhibitors and measurements of inhibitor concentrations in the joint in vivo should be helpful

in further defining which class of MMP is most important in protecting against arthritis.

Considerable work has been done to define the relative roles of cysteine proteases and MMPs in bone and cartilage resorption. Its well established that lysosomal cysteine proteases can degrade collagen at low pH (15–17) and that osteoclasts use the coordinated secretion of H^+ and cysteine proteases as a mechanism to degrade mineralized bone (29). MMPs are apparently used by osteoblasts to remove demineralized bone (osteoid) from the surface of growing bone, thus preparing the surface for osteoclast-mediated degradation (29). In the
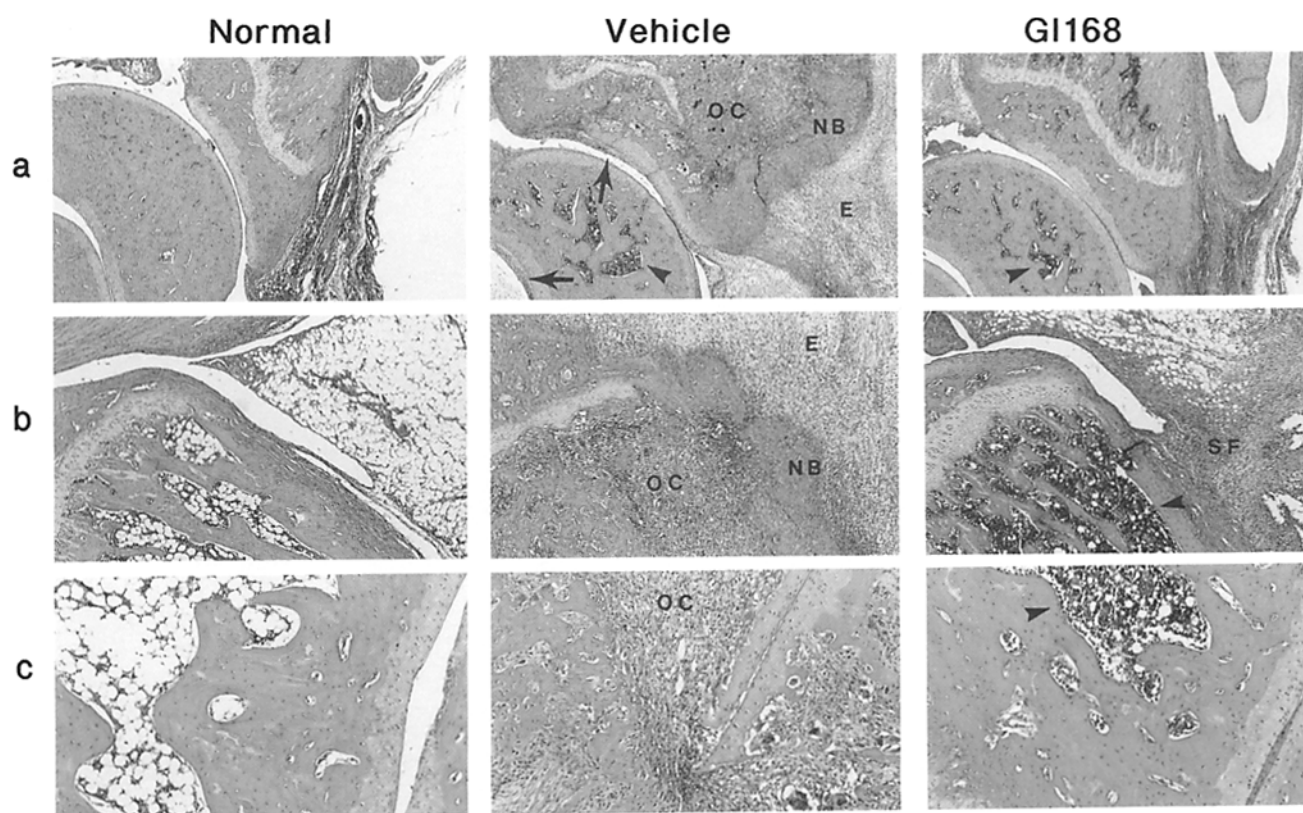
Figure 3. Histologic evidence of the protective effect of GI168 in adjuvant arthritis. Shown are photomicrographs of histologic sections of three regions within ankle joints from normal rats that did not receive adjuvant (*normal*) and adjuvant-injected rats treated with either vehicle alone (*vehicle*) or treated with GI168 at 25 mg/kg per d (GI168). A joint that is representative of the group, and not an extreme case, is shown for each group. These are the same joints from which the radiographs were prepared (Fig. 1). Regions shown: (*a*) distal tibia articulation with talus (×11); (*b*) proximal calcaneus with synovium, which is located behind the Achilles tendon (×22); (*c*) a tarsal joint (×44). Note the decrease in edema (*E*), pannus (*large arrows*), osteoclastic activity in marrow cavities (*OC*), and ectopic new bone growth (*NB*) in the GI168-treated compared to the analogously labeled regions in the vehicle-treated arthritic control rat joint. Note also the synovial fibrosis (*SF*) in the GI168-treated joint, as well as the increased marrow cellularity (small arrowheads) in both vehicle- and GI168-treated joints.

Table 2. *Effect of GI168 on the Histological Parameters of Adjuvant Arthritis*

Parameter	Histologic score	Vehicle-treated ($n = 19$)	GI168 6 mg/kg ($n = 6$)	GI168 12 mg/kg ($n = 5$)	GI168 25 mg/kg ($n = 10$)
			Percent of group with each score		
Bone erosion	0–1	11	17	40	70
	2–3	11	50	60	30
	4–5	78	33	0	0
Mean score[‡]		4.2	3.0	1.8 ($P < 0.01$)[§]	1.4 ($P < 0.01$)
Adherent	0–1	11	0	20	40
osteoclasts	2–3	5	83	80	60
	4–5	84	17	0	0
Mean score		4.3	3.0	2.0 ($P < 0.05$)	1.8 ($P < 0.01$)
Periosteal	0–1	0	0	0	0
new bone growth	2–3	11	83	100	100
and remodeling	4–5	89	17	0	0

continued

Table 2. *(continued)*

Parameter	Histologic score	Percent of group with each score			
		Vehicle-treated (n = 19)	GI168 6 mg/kg (n = 6)	GI168 12 mg/kg (n = 5)	GI168 25 mg/kg (n = 10)
Mean score		4.2	3.2 (P <0.05)	2.4 (P <0.01)	2.7 (P <0.01)
Cartilage	0–1	21	67	80	70
destruction	2–3	53	33	20	30
	4–5	26	0	0	0
Mean score		2.7	1.2	1.0 (P <0.05)	0.8 (P <0.01)
Pannus	0–1	16	0	60	30
formation	2–3	0	83	40	60
	4–5	84	17	0	10
Mean score		3.9	2.5	1.4 (P <0.01)	2.1 (P <0.01)
Edema	0–1	0	0	0	20
	2–3	21	33	100	80
	4–5	79	67	0	0
Mean score		4.4	3.7	2.8 (P <0.05)	2.3 (P <0.05)
Synovial	0–1	26	67	100	100
lining	2–3	53	0	0	0
distension	4–5	21	33	0	0
Mean score		2.3	1.5	0.6	0.2 (P <0.01)
Synovial	0–1	0	0	0	20
lining	2–3	63	33	60	70
necrosis	4–5	37	77	40	10
Mean score		3.4	3.7	3.2	2.7
PMN	0–1	0	0	0	0
infiltration	2–3	16	0	0	40
	4–5	84	100	100	60
Mean score		4.3	4.8	4.8	3.7
Lymphocytic	0–1	0	0	0	70
infiltration	2–3	100	100	100	30
	4–5	0	0	0	0
Mean score		2.8	3.0	2.8	2.8
Subsynovial and	0–1	0	0	0	0
capsular fibrosis	2–3	95	100	80	40
	4–5	5	0	20	60
Mean score		2.4	2.3	3.0	3.7 (P <0.01)

* Slides were scored in a blinded fashion. 0–1, normal to very slight pathology; 2–3, slight to moderate pathology; 4–5, severe to very severe pathology.
‡ Scores within each group were averaged.
§ Significantly different from vehicle-treated group using Cochran-Mantel Haenszel statistics.
n = Number of rats in each group.
Dose is mg/kg per d. GI168 was delivered by subcutaneous minipumps. Details are described in Materials and Methods.

Table 3. *Effect of GI168 and Indomethacin on Body Weight Gain*

Group*	Percent body weight gain†
Normal	29.4 ± 1.3§ (n = 12)
AA-vehicle treated	−1.3 ± 1.3 (n = 19)
AA-indomethacin (1 mg/kg po)	5.1 ± 1.9 (n = 12)
AA-GI168 (6 mg/kg sc)	5.1 ± 2.2 (n = 5)
AA-GI168 (12 mg/kg sc)	13.8 ± 4.6§ (n = 5)
AA-GI168 (25 mg/kg sc)	12.4 ± 3.2§ (n = 10)

* Normals were untreated. AA groups were injected to induce adjuvant arthritis. Daily drug doses for days 8–21 are shown in parentheses.
† Rats were weighed 8 and 21 d after adjuvant injection and percent body weight gain was calculated. Data represents mean and SEM of n rats per group.
§ Significantly (P <0.05) different from AA-vehicle–treated controls using Dunnett's multiple comparison test.
po, orally; sc, subcutaneously.

case of cartilage, use of inhibitors suggests that both cysteine proteases and MMPs are involved in matrix degradation in vitro (30). The fact that GI168 dramatically inhibits bone and cartilage degradation indicates that MMPs play a central role in erosive arthritis in vivo. Given that many cell types produce MMPs (fibroblasts, synovial cells, neutrophils), it is possible that MMPs are in great excess in the arthritic joint relative to cysteine proteases, and are thus responsible for the bulk of the tissue degradation. Cysteine proteases may still be important in the cleavage of MMP zymogens to active forms (30).

In the present study, the histopathological evaluation did not show a significant decrease in infiltrating PMNs or mononuclear leukocytes in response to GI168. In addition, synovial fibrosis was not suppressed. The apparent lack of effect on these inflammation parameters despite the profound inhibition of bone and cartilage destruction represents a unique antiinflammatory profile. One would expect suppression of these parameters if inflammatory cells require MMPs to mi-

grate into tissue. The observation that MMP inhibition has no effect on cellular infiltration into the joint is consistent with a non-MMP recruitment mechanism, such as the production and action of cytokines.

With regard to inflammatory cytokines, we recently discovered that the MMP inhibitor GI129471 inhibits LPS-induced TNF release from inflammatory cells with an IC₅₀ of ~50 nM (31). This data opened up the possibility that the efficacy of GI168 against adjuvant arthritis may also involve inhibition of TNF production. However, GI168 is 15- to 20-fold less potent that GI129471 in inhibiting LPS-induced TNF release from human and mouse inflammatory cells in vitro (Conway, J. G., and G. M. McGeehan, unpublished observation). Thus, at steady-state plasma concentrations of 100–200 nM, one would not expect GI168 to inhibit TNF production from inflammatory cells in the arthritic rats. It is interesting to speculate that an MMP inhibitor with more potency against TNF production will additionally block inflammatory cell influx and synovial fibrosis. Recent clinical reports of the beneficial effect of anti-TNF antibody therapy in rheumatoid arthritis support this view (32). The test of whether MMP inhibitors can prevent trabecular bone loss in models that do not have a major inflammatory component, such as ovariectomy-induced osteoporosis in rats, will also be enlightening.

In summary, the present studies show that administration of an MMP inhibitor during 2 wk dramatically suppresses cartilage and bone destruction and pannus formation in adjuvant arthritis in rats, suggesting that MMPs mediate joint damage in this chronic inflammatory arthritis model. Development of better long-term delivery methods, preferably orally active inhibitors, will be necessary to investigate the efficacy of MMP inhibitors during the complete time frame of adjuvant-induced early inflammatory changes to severe joint deformity beyond 30 d (27, 28). Orally active inhibitors with known specificities against MMPs, including TNF convertase (31), would greatly facilitate further studies of these enzymes' roles in arthritis and other pathologies.

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