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Disease-Modifying Activity of SB 273005, an Orally Active, Nonpeptide $\alpha_v\beta_3$ (Vitronectin Receptor) Antagonist, in Rat Adjuvant-Induced Arthritis

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Objective. **To evaluate the effects of SB 273005, a potent, orally active nonpeptide antagonist of the integrin $\alpha_v\beta_3$ vitronectin receptor, on joint integrity in rats with adjuvant-induced arthritis (AIA).**

Methods. **Male Lewis rats with AIA were orally dosed either prophylactically (days 0–20) or therapeutically (days 10–20) with SB 273005. Efficacy was determined by measurement of paw inflammation, assessment of bone mineral density using dual-energy x-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), and histologic evaluation.**

Results. **SB 273005 is a potent antagonist of the closely related integrins, $\alpha_v\beta_3$ (Ki = 1.2 n*M*) and $\alpha_v\beta_5$ (Ki = 0.3 n*M*). When SB 273005 was administered prophylactically to AIA rats twice per day, it inhibited paw edema at doses of 10, 30, and 60 mg/kg, by 40%, 50%, and 52%, respectively. Therapeutic administration twice daily was also effective, and a reduction in paw edema was observed at 30 mg/kg and 60 mg/kg of the antagonist (by 36% and 48%, respectively). SB 273005 was also effective when administered once per day, both prophylactically and therapeutically. Significant improvement in joint integrity in treated rats was shown using DEXA and MRI analyses. These findings were confirmed histologically, and significant protection of bone, cartilage, and soft tissue was observed within the joint.**

Conclusion. **Symptoms of AIA in rats were significantly reduced by either prophylactic or therapeutic treatment with the $\alpha_v\beta_3$ antagonist, SB 273005. Measurements of paw inflammation and of bone, cartilage, and soft tissue structure indicated that this compound exerts a protective effect on joint integrity and thus appears to have disease-modifying properties.**

Alpha$_v$beta$_3$ is a member of the integrin family of adhesion molecules. It is a 160/85-kd heterodimer that mediates cell adhesion to extracellular matrix and serum proteins by recognition of the arg-gly-asp (RGD) sequence. This integrin is expressed by proliferating and migrating endothelial cells (1) and has been proposed to play a role in angiogenesis and neovascularization (2,3). Alpha$_v$beta$_3$ is also expressed by malignant melanoma cells and its expression appears to correlate with tumor progression (4). Antagonists of this receptor have been shown to promote tumor regression by inducing apoptosis of angiogenic blood vessels (5–7), and antibodies generated against the receptor block human breast cancer growth and angiogenesis in human skin (8).

High-level expression of $\alpha_v\beta_3$ has also been shown on bone-resorbing osteoclasts, and this appears to play an essential role in mediating the attachment of these cells to bone matrix to allow active bone resorption (9–12). Antagonism of the receptor with neutralizing antibodies (13) or with small peptide inhibitors (14–16) has proven effective in inhibiting bone resorption in animal models of osteopenia. Unlike $\alpha_v\beta_3$, which is expressed on mature osteoclasts, $\alpha_v\beta_5$ is expressed on osteoclast precursors and has been proposed to play a role in osteoclast differentation (17). It was recently

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reported that small nonpeptide $\alpha_v\beta_3/\alpha_v\beta_5$ inhibitors are effective in the ovariectomized (ovx) rat model of bone resorption (18,19). Several studies from our laboratories have demonstrated that orally available antagonists prevent bone loss in both in vitro and in vivo models of bone resorption (19–22), and these agents show great promise as therapeutic agents for the treatment of osteoporosis.

In the therapeutic area of rheumatoid arthritis (RA), compounds with the ability to inhibit osteoclast-mediated bone resorption as well as angiogenesis have shown high potential. An angiogenesis inhibitor, AGM-1470, has been shown to inhibit joint inflammation and disease severity in the adjuvant-induced arthritis (AIA) rat model (23) and in a rat model of collagen-induced arthritis, when used both alone and in combination with cyclosporin A (24). In addition, it has recently been shown that an $\alpha_v\beta_3$ antagonist has beneficial effects in a rabbit model of inflammatory arthritis (25). In these studies, intraarticular administration of a cyclic peptide antagonist of $\alpha_v\beta_3$ to rabbits with antigen-induced arthritis, early in disease, inhibited synovial angiogenesis and reduced inflammatory cell infiltration, pannus formation, and cartilage erosions. These results, along with the studies showing protection of bone in the ovx rat, suggested to us that these molecules might be effective in AIA in the rat. This animal model of RA displays an aggressive inflammatory component and destructive joint disease. In the studies reported herein we show that SB 273005, a potent antagonist of the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, inhibits both paw edema and destruction of bone and cartilage in AIA.

MATERIALS AND METHODS

Animals. Male, inbred Lewis rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). Within any given experiment, only animals of the same age were used. All experimental procedures were in accordance with protocols approved by the SmithKline Beecham Institutional Animal Care and Use Committee, and met or exceeded the standards of the American Association for the Accreditation of Laboratory Animal Care, the United States Department of Health and Human Services, and all local and federal animal welfare laws.

Materials. SB 273005 (Figure 1) was synthesized at SmithKline Beecham Pharmaceuticals (20). For in vivo experiments, SB 273005 was administered orally in 1% methyl cellulose, adjusted to pH 3.5 (Sigma, St. Louis, MO).

Induction of arthritis. AIA was induced by a single injection of 0.75 mg of *Mycobacterium butyricum* (Difco, Detroit, MI), suspended in paraffin oil, into the base of the tail of male Lewis rats, ages 6–8 weeks and weighing 160–180 gm. Hind-paw volumes were measured by a water displacement method on day 20 (26). Test compounds were homogenized in 1% methyl cellulose and administered orally in a volume of 10 ml/kg. Control animals were administered vehicle alone. Two dosing protocols were used: prophylactic dosing (1, 3, 10, 30, and 60 mg/kg/day either once or twice daily) initiated on the day of adjuvant injection, and therapeutic administration initiated on day 10 after injection.

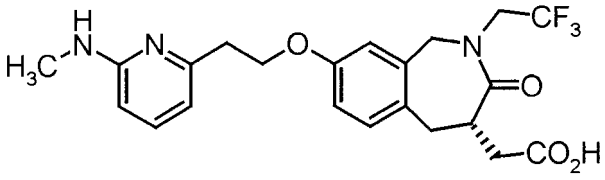

Figure 1. Structure of SB 273005.

Change in paw volume is presented as the mean and SEM percentage inhibition of hind-paw edema in 10–12 animals per group, which was calculated as follows:

$$\%\ \text{inhibition} = 1 - \left[\frac{\text{AIA (treated)} - \text{non-AIA control}}{\text{AIA (control)} - \text{non-AIA control}}\right] \times 100.$$

For statistical analysis, the paw volumes of AIA rats treated with SB 273005 were compared with those of the untreated controls (AIA and non-AIA) by Student's *t*-test.

Bone mineral density (BMD) measurement. Animals were killed on day 21 and the hind limbs were removed and fixed in 70% ethanol. The BMD of the distal tibia was determined by dual-energy x-ray absorptiometry (DEXA) using the Hologic QDR-1000 equipped with high-resolution scanning software. Quality control of the instrument was carried out each day prior to sample analysis by scanning both a human anthropomorphic spine phantom (low resolution) and the lumbar portion of a rat spine (high resolution), both of which were embedded in methylmethacrylate. All high-resolution scans were carried out with the sample placed on top of an acrylic block, 1.5 inches deep. The x-ray beam was collimated to a diameter of 1.27 mm and line spacing and point resolution were 0.25 mm and 0.127 mm, respectively.

Scans were made of the distal tibia region of bones that had been excised and stored in 70% ethanol in a square plastic container. The depth of the liquid was constant for all samples and was sufficient to cover the limbs by ~0.5 inches. BMD, as well as bone mineral content (BMC) and bone area, were determined for the distal tibia. The region of interest was defined as the area between a line drawn parallel to the proximal edge of the calcaneus and a second line drawn perpendicular to the long axis of the tibia, midway between the first line and the point where the tibia meets the fibula. The point of connection between the fibula and tibia had to be approximated in some samples with low BMD. The width of the region of interest was kept constant between samples.

Magnetic resonance imaging (MRI). All MRI studies were carried out on a 9.4-Tesla AMX spectrometer with microimaging accessories (Bruker Instruments, Billerica, MA). Coronal sections (250-μm thick) of rat tibiotarsal joints were imaged with an in-plane resolution of 70×70 μm. A repetition time of 1 second and an echo time of 7.5 ms were used, with a data matrix of 256×256. The morphologic changes in the joint architecture of the AIA rats were compared with those in the normal (non-AIA) controls and with those in the AIA rats

treated with SB 273005. The joints were graded by a blinded observer (RK), and joint integrity was expressed as the percentage of joints with 1) no protection (i.e., severe disease similar to that in the AIA controls), 2) moderate protection, or 3) significant protection (i.e., similar to that in the non-AIA control animals).

Histology. Hind-limb tibiotalus joints from the following groups of rats were evaluated and scored for histologic changes: 4 normal rats, 11 rats with AIA, and 2 groups of 12 rats with AIA treated with SB 273005 at either 60 mg/kg or 30 mg/kg once per day. Rats were killed on day 21 by CO_2 administration, and the hind legs were fixed in formalin and decalcified in Cal-Rite (Richard-Allen Scientific, Kalamazoo, MI). The paws were then removed from the legs at the distal tibial diaphysis. After routine processing, the samples were embedded and coronal sections were cut in the plane midway through the tibiotalus and talartarsal joints. Sections were stained with Safranin O and counterstained with fast green.

The histologic characteristics of the bone and articular cartilage/periarticular soft tissue were scored separately by a blinded observer (SB). The bone was graded as follows: 0 = normal, 1 = subperiosteal fibrosis with periosteal woven bone formation, 2 = definite marrow inflammation, endosteal and trabecular bone resorption, and periosteal woven bone formation with osteophytes, 3 = extensive marrow inflammation with concomitant endosteal and trabecular bone resorption, and extensive periosteal woven bone formation with osteophytes, and 4 = marrow replaced by granulation tissue, little or no trabecular bone remaining, woven bone in marrow cavity, extensive obliteration of cortical contours by osteoclastic resorption, and cortex usually breached at the metaphysis. The cartilage/synovium was graded as follows: 0 = normal, 1 = mild lymphocytic infiltration of the synovium and surrounding tissues, 2 = synovial fibrosis and edema, partial lymphocytic infiltration of the joint space, and minor pannus erosion of the cartilage, and 3 = extensive infiltration of the joint space, fibrous ankylosis of the joints, peripheral and subchondral cartilage erosion, and extensive fibrosis of soft tissue with regional necrotic liquefaction.

RESULTS

Effect of prophylactic and therapeutic treatment with SB 273005 in AIA rats. *Paw inflammation.* The effect of SB 273005 was evaluated using the prophylactic dosing protocol, in which compound was administered to male Lewis rats starting on the day of adjuvant injection. SB 273005 was administered twice daily in acidified 1% methyl cellulose, and paw inflammation was measured on day 20 after adjuvant injection. Hind-paw inflammation was effectively inhibited at SB 273005 dose levels of 60 mg/kg (by 52%), 30 mg/kg (by 50%), and 10 mg/kg (by 40%) (all $P < 0.001$ versus controls) (Figure 2A). Although no clear dose-response curve was observed in this experiment, there was a statistically significant difference between the 52% inhibition in the group treated prophylactically with 60 mg/kg and the 40% inhibition achieved with 10 mg/kg ($P < 0.05$).

SB 273005 was also tested in the AIA rat using a therapeutic dosing protocol, which was used to more closely represent the clinical setting. In these experiments, rats were immunized with adjuvant on day 0 and treated with compound twice daily on days 10–20. Using this protocol, hind-paw inflammation was reduced at 60 mg/kg (by 48%) and 30 mg/kg (by 36%) of SB 273005 (both $P < 0.001$ versus controls), but not at 10 mg/kg (13% reduction) (P not significant versus controls) (Figure 2B).

Inhibition of hind-paw inflammation was also

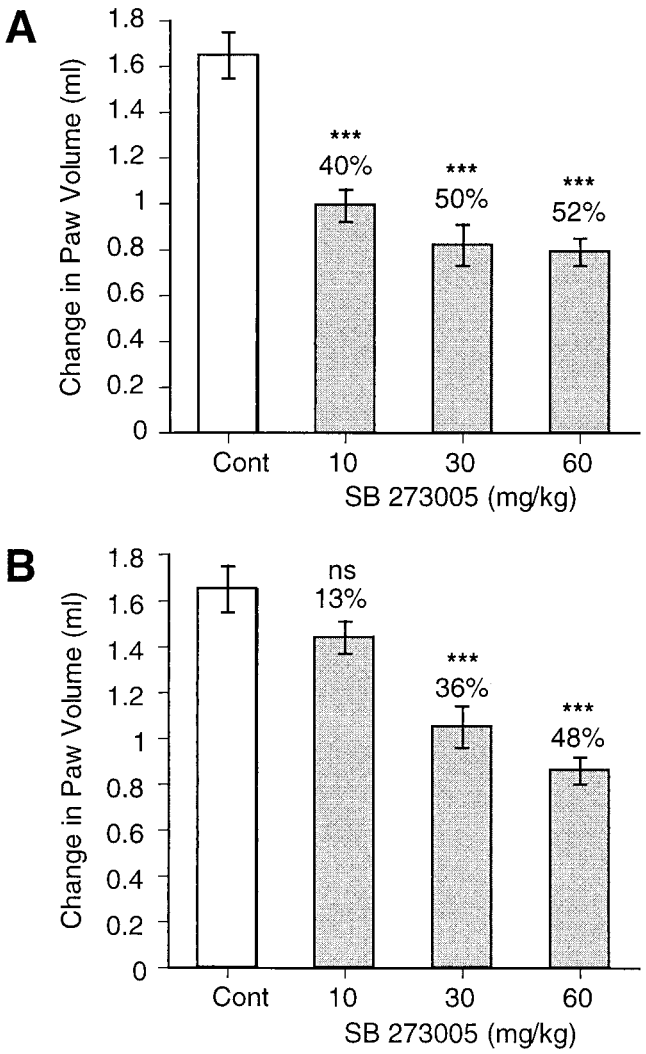

Figure 2. Inhibition of paw edema in Lewis rats with adjuvant-induced arthritis (AIA), in which rats were orally dosed with SB 273005 at 10, 30, and 60 mg/kg, twice daily, in 1% methyl cellulose, during days 0–20 in the prophylactic protocol **(A)** or days 10–20 in the therapeutic protocol **(B)**. Bars show the mean and SEM of 12 rats per group. Treated groups were compared with the untreated AIA controls (Cont) by Student's t test. *** = $P < 0.001$. ns = not significant.

observed when SB 273005 was administered prophylactically and therapeutically only once per day, although this was somewhat less effective than the twice-daily administration. With the prophylactic protocol, inhibition was observed at 60 mg/kg (by 40%), 30 mg/kg (by 28%), 10 mg/kg (by 30%) (all $P < 0.001$ versus controls), and 3 mg/kg (by 18%) ($P < 0.01$ versus controls) (Figure 3A). Inhibition following therapeutic administration was observed at 60 mg/kg (by 22%), 30 mg/kg (by 24%) (both $P < 0.001$ versus controls), and 10 mg/kg (by 19%) ($P < 0.05$ versus controls) (Figure 3B).

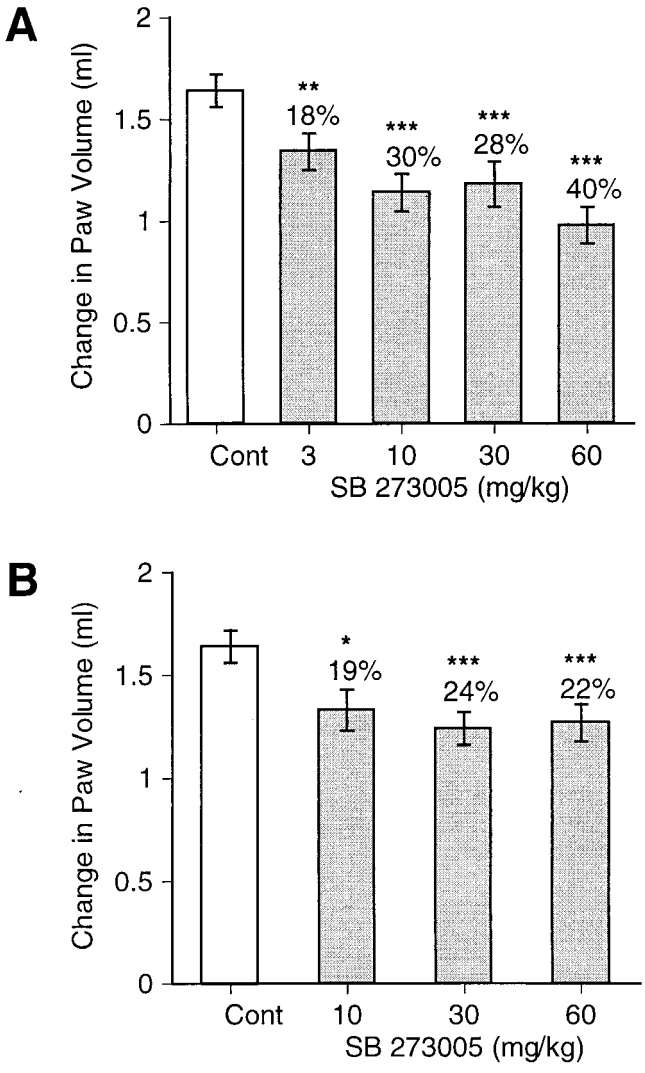

Figure 3. Inhibition of paw edema in Lewis rats with adjuvant-induced arthritis (AIA) using a prophylactic dosing protocol once per day. Rats were treated with SB 273005 at 3, 10, 30, and 60 mg/kg orally in 1% methyl cellulose, during days 0–20 in the prophylactic protocol **(A)** or days 10–20 in the therapeutic protocol **(B)**. Bars show the mean and SEM of 12 rats per group. Treated groups were compared with the untreated AIA controls (Cont) by Student's *t*-test. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

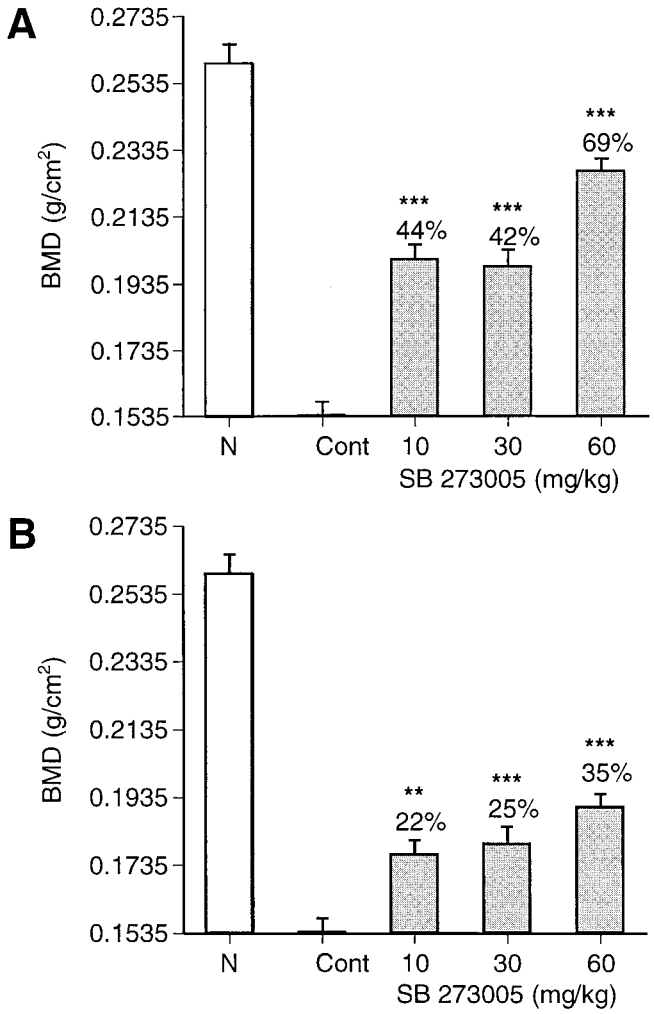

Figure 4. Bone densitometry evaluation of the distal tibia in adjuvant-induced arthritis (AIA) rats treated with SB 273005. Rats were treated with various doses of compound twice daily during days 0–20 (prophylactic) **(A)** or days 10–20 (therapeutic) **(B)**. Values are the percentage change in bone mineral density (BMD) relative to the BMD in non-AIA normal controls (N; assigned a value of 100%). Bars show the mean and SEM of 12 animals per group. Treated groups were compared with the untreated AIA controls (Cont) by Student's *t*-test. ** = $P < 0.01$; *** = $P < 0.001$.

BMD changes. BMD was monitored by DEXA using the Hologic QDR-1000 equipped with high-resolution scanning software. Scans were made of the distal tibia obtained from the animals on day 21. The BMD, as well as the BMC and bone area, were determined. However, because the improvements in the BMC

values were identical to those in the BMD, we have chosen to report only the BMD data. The bone integrity of normal control rats was assigned a value of 100%, and that of AIA rats, a value of 0%. Compared with the AIA control rats, animals treated prophylactically with SB 273005 at 60, 30, and 10 mg/kg twice daily showed a significant normalization of BMD. There was 69%, 42%, and 44% normalization of BMD (all $P < 0.001$ versus controls) at 60, 30, and 10 mg/kg twice daily, respectively (Figure 4A).

Following therapeutic treatment, in which animals were dosed starting on day 10 following adjuvant injection and then dosed daily until day 20, there was also a significant normalization of BMD. There was 35%, 25% (both $P < 0.001$ versus controls), and 22% ($P < 0.01$ versus controls) normalization of BMD at 60, 30, and 10 mg/kg twice daily, respectively (Figure 4B). Following the single daily dose of SB 273005, the only significant improvement in BMD was observed when SB 273005 was administered prophylactically at 60 mg/kg (24% normalization; $P < 0.05$ versus controls) (results not shown).

MRI findings. Using MRI, the morphologic changes in the joint architecture of AIA rats were compared with those in normal (non-AIA) control rats and with those in AIA rats treated twice daily prophylactically or therapeutically with 60, 30, and 10 mg/kg SB 273005. Ex vivo MR images were obtained on day 21 from the intact left and right tibiotalar joints, and a total of 21–24 joints per group were evaluated. The images from AIA control rats (Figure 5A) were interpreted as demonstrating no protection against AIA, in which significant damage as well as swelling and marked loss in cortical as well as trabecular bone were observed. In comparison, in treated AIA rats, either moderate protection (Figure 5B) or significant protection (Figure 5C) was evident, in which the distal tibia, fibula, and talus were well defined and edema was minor (similar to that in a non-AIA normal rat). Following prophylactic treatment, 80% of the joints showed significant protection at the 60 mg/kg twice-daily dose, whereas ~40% of the joints from the animals dosed with either 30 or 10 mg/kg twice daily showed significant protection (Figure 6A). In the rats treated therapeutically with SB 273005, there was significant protection in 65% of the joints from the 60 mg/kg group and in 20% of the joints from the 30 mg/kg group (Figure 6B).

Histologic features. The histologic changes occurring in the bone and soft tissue compartments of AIA rats treated with SB 273005 at 60, 30, and 10 mg/kg (once daily) were compared with those in the vehicle-treated AIA controls. Histologic changes to the bone (the tibia and the talus) were scored 0–4 (normal to severely affected), whereas cartilage/soft tissue changes were scored on a scale of 0–3 depending on the severity, as described in Materials and Methods. Normal, non-AIA rats were scored 0 for all components. The mean severity scores for the lesions in the tibia, talus, and cartilage/soft tissue in AIA rats were determined separately, and the mean histologic scores for these regions are shown in Figure 7.

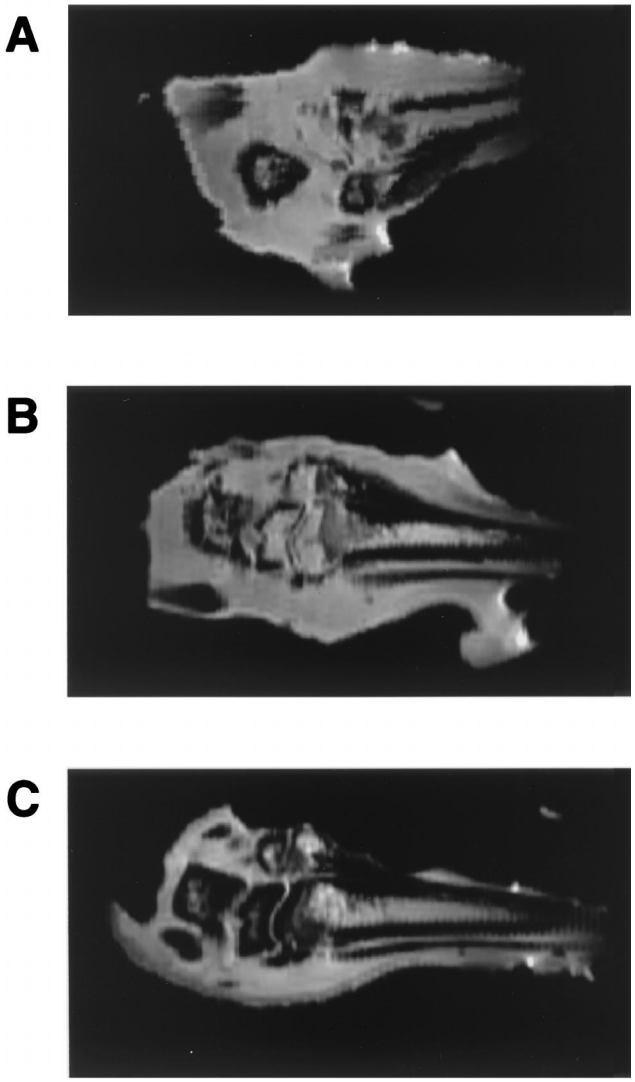

Figure 5. Representative magnetic resonance images on day 21 of a joint from an untreated rat with adjuvant-induced arthritis (AIA), in which no protection of joint integrity is evident **(A),** a joint from an AIA rat treated with SB 273005, showing moderate protection **(B),** or a joint from a treated AIA rat, showing significant protection **(C)**. White areas indicate soft tissue and inflammation and the black areas indicate the bone.

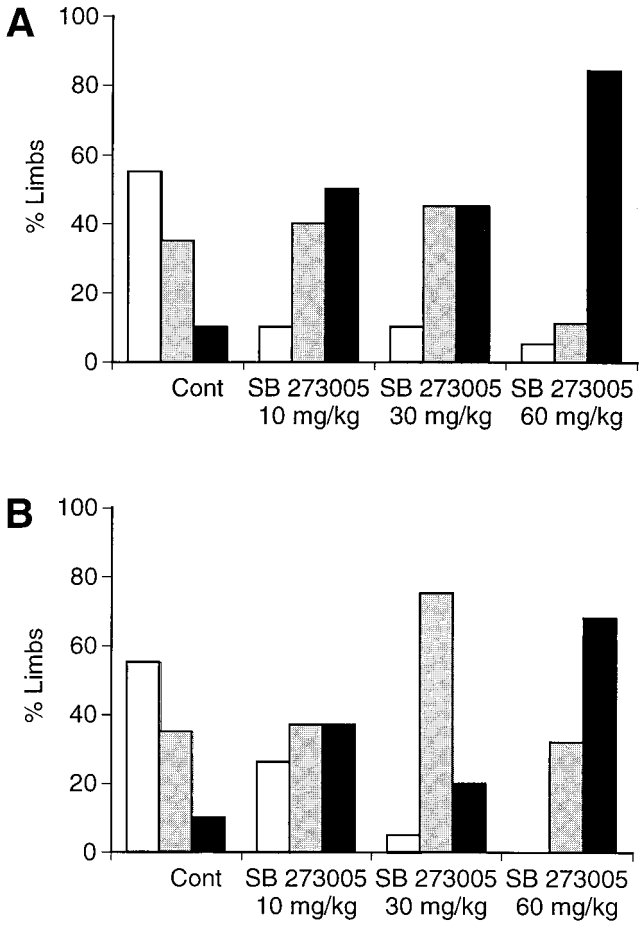

Figure 6. Evaluation of treatment effects based on magnetic resonance imaging of the tibiotarsal joints of adjuvant-induced arthritis (AIA) rats treated prophylactically **(A)** or therapeutically **(B)** with SB 273005 (twice daily). Values are expressed as the percentage of joints showing no protection (open bars), moderate protection (shaded bars), or significant protection (black bars). There were 20–24 limbs imaged in each group. Cont = untreated AIA control rats.

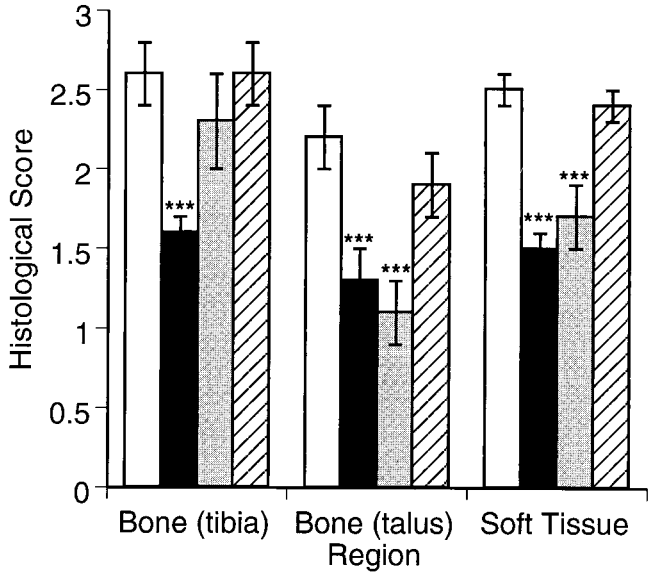

Figure 7. Histologic evaluation of the tibiotalus joints of adjuvant-induced arthritis (AIA) rats treated with SB 273005 once per day. Open bars = control AIA rats; black bars = AIA rats treated with 60 mg/kg; shaded bars = AIA rats treated with 30 mg/kg; hatched bars = AIA rats treated with 10 mg/kg. Bars show the mean ± SEM histologic score of 22–24 limbs for each group. Treated groups were compared with the untreated AIA controls by Mann-Whitney U test. *** = $P <$ 0.001 versus controls.

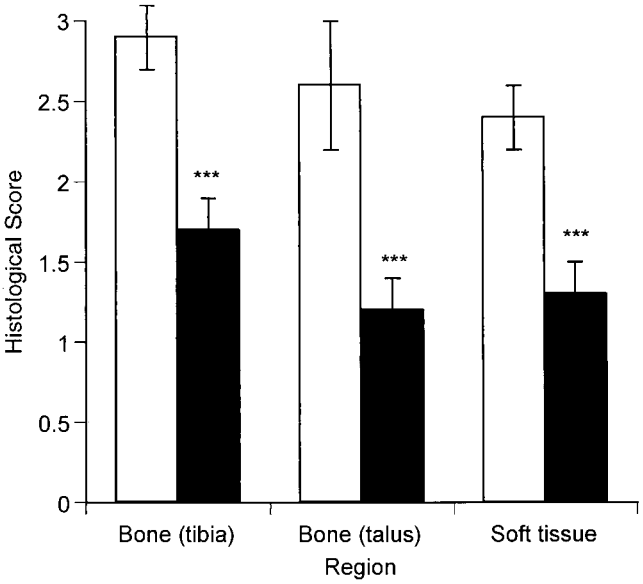

Figure 8. Histologic evaluation of the tibiotalus joints of rats treated with SB 273005 twice per day. Open bars = control adjuvant-induced arthritis (AIA) rats; black bars = AIA rats treated with 10 mg/kg twice daily. Bars show the mean ± SEM histologic score of 22–24 limbs for each group. The treated group was compared with the untreated AIA controls by Mann-Whitney U test. *** = $P <$ 0.001 versus controls.

Of the 24 vehicle-treated AIA rat joints evaluated, all but 2 showed moderate-to–extremely severe changes in the tibia, talus, and cartilage/soft tissue components. The mean scores for the vehicle-treated groups were 2.6 for the tibia, 2.2 for the talus region, and 2.5 for the soft tissue. Administration of 60 mg/kg SB 273005 to AIA rats resulted in significant protection in both the bone and cartilage/soft tissue. None of these compound-treated animals was scored extremely severe for any component, and only 2 animals were scored severe (3.0) for bone lesions and 1 animal was scored 3.0 for cartilage/soft tissue damage. The mean scores for the tibia, talus, and cartilage for the 60 mg/kg group were 1.6, 1.3, and 1.5, respectively. In AIA rats treated with 30

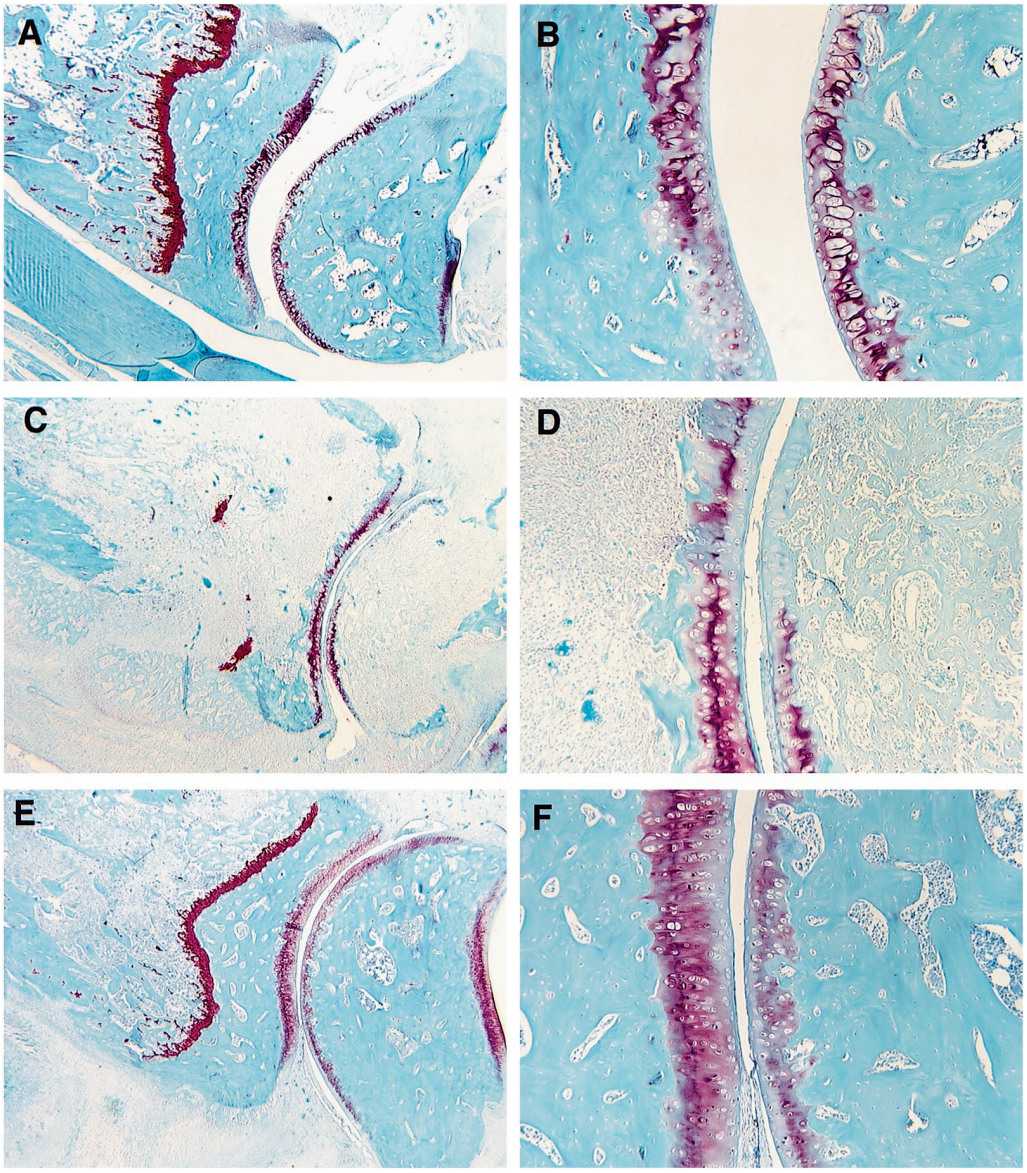

Figure 9. Photomicrographs of coronal sections of rat tibiotalus joints. **A** and **B,** normal tibiotalus joints from a normal, non–adjuvant-induced arthritis (AIA) rat. Articular cartilage is stained with Safranin O (pink), and other tissues are stained blue/green. The articulation of the distal tibia and proximal talus runs vertically through the image. **C** and **D,** Section of a tibiotalus joint from a vehicle-treated AIA rat. **E** and **F,** Tibiotalus joint from an AIA rat administered 60 mg/kg SB 273005 once per day on days 0–20. Note that compared with the joint from the AIA control rat, joint integrity has been significantly maintained with SB 273005. (Original magnification × 5 in **A**, **C**, and **E**; × 25 in **B**, **D**, and **F**.)

mg/kg SB 273005, the lesions in the talus and the cartilage, but not the tibia, were protected, with mean scores being 1.1, 1.7, and 2.3, respectively (Figure 7).

There was no significant protection observed on any parameter at the 10 mg/kg dose administered once per day. To determine whether a 10 mg/kg dose of SB 273005 administered twice daily would protect the joint integrity of AIA rats, joints from these animals were examined histologically and compared with those of vehicle-treated controls. The mean scores for the vehicle-treated groups were 2.9 for the tibia, 2.6 for the talus region, and 2.4 for the soft tissue. Administration of 10 mg/kg SB 273005 twice daily resulted in significant protection in all tissues. The mean scores for these

groups were 1.7 for the tibia, 1.2 for the talus, and 1.3 for the soft tissue (Figure 8).

Representative photomicrographs of the joints from a normal rat, a vehicle-treated rat with AIA, and a rat with AIA treated prophylactically with 60 mg/kg SB 273005 once per day are shown in Figure 9. The joint architecture of the normal, non-AIA joint (Figures 9A and B) was scored 0 for all 3 components. In the AIA animals dosed with vehicle, inflammation was both extensive and severe (Figures 9C and D). The majority of the original bone in both the tibia and talus was lost. The marrow cavities were replaced with granulation tissue and reactive woven bone. As a result, a score of 3.0 was given for both of these bone components. Articular cartilage showed full-depth loss of proteoglycan (as indicated by reduced Safranin O staining) and the joint space had been infiltrated with granulation tissue. There was also extensive fibrosis of the surrounding soft tissues (not shown in this photomicrograph) and as a result, a score of 3.0 was given for this component. Compared with the joint from the vehicle-treated control rat, inflammation was markedly attenuated in the joints of animals treated with SB 273005 at 60 mg/kg once daily (Figures 9E and F). Although some inflammation was apparent in the marrow of the tibia, the talus appeared histologically normal. Therefore, the tibia and talus components were scored 1.0 and 0, respectively. The articular cartilage demonstrated some surface loss of proteoglycan, and the surrounding soft tissue contained a lymphocytic infiltrate and was edematous. This resulted in a score of 2.0 being assigned to the treated joints.

DISCUSSION

SB 273005 is an orally active, nonpeptide, RGD-mimetic $\alpha_v\beta_3$ antagonist. This compound binds $\alpha_v\beta_3$ and the closely related integrin, $\alpha_v\beta_5$, with low n*M* affinity, but binds only weakly to the related integrins $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$ (20). The compound also inhibits $\alpha_v\beta_3$-mediated cell adhesion (at a 50% inhibition concentration [IC_{50}] of 3 n*M*) (20), endothelial cell migration (IC_{50} 1.8 n*M*), and osteoclast-mediated bone resorption in vitro (IC_{50} 11 n*M*). Studies in animal models of bone resorption have shown that this compound, as well as other small-molecule nonpeptide vitronectin receptor antagonists, inhibit bone loss in the thyroparathyroidectomized and chronic ovx rat models of bone resorption in vivo (18–20).

In addition to the effects observed with vitronectin receptor antagonists in animal models of bone resorption, a cyclic peptide $\alpha_v\beta_3$ antagonist was shown to decrease synovial vascularity and swelling in antigen-induced arthritis in rabbits (25). Angiogenesis is one of the earliest histopathologic findings in RA and appears to play a significant role in pannus formation. Suppression of angiogenesis, therefore, may impact disease progression and this has been observed in animal models of both adjuvant- and collagen-induced arthritis (23,24). In the rabbit model of antigen-induced arthritis, the angiogenic basic fibroblast growth factor was utilized to increase angiogenesis and this, in turn, increased the arthritic response to antigen in the animals (25). Treatment with the cyclic-peptide $\alpha_v\beta_3$ antagonist inhibited synovial angiogenesis, and reduced synovial cell infiltrate, pannus formation, and cartilage erosions.

The beneficial effects of antagonizing $\alpha_v\beta_3$ that were observed in the rabbit model of arthritis (25) and in the bone resorption models (18–20) encouraged us to examine the effects of SB 273005 in the rat AIA model. Inhibition of paw edema was observed in AIA in Lewis rats when SB 273005 was administered orally either twice or once per day. The lowest effective dose following prophylactic twice-daily dosing was 10 mg/kg, in which a 40% inhibition of inflammation was observed; lower doses were not tested in this experiment. However, in another experiment, 3 mg/kg twice daily was shown to be inactive (Badger AM: unpublished observations). The minimum effective dose using therapeutic twice-daily dosing was 30 mg/kg. Using a once-per-day dosing protocol, prophylactic dosing was clearly superior to therapeutic dosing. The effect of SB 273005 on paw edema is unlikely to be a direct antiinflammatory effect, which would resemble that of a nonsteroidal antiinflammatory drug, since studies in a classic model of carrageenan-induced inflammation, in which indomethacin showed strong activity, demonstrated that SB 273005 was inactive at 30 mg/kg and showed only 25% inhibition of inflammation at 60 mg/kg (Griswold D: unpublished observations).

It has previously been reported that SB 273005 potently inhibits both human endothelial cell migration and osteoclast-mediated bone resorption (19–22). The compound could have beneficial effects in inflammatory arthritis by preventing new vessel formation in the inflamed synovium as well as osteoclast-mediated bone resorption. It is possible that in adjuvant-driven disease, there is significant release of proinflammatory factors due to the significant destruction of bone and cartilage. Degraded matrix components may drive the inflammatory response and once this matrix degradation is blocked, inflammation subsides. In addition, the combi-

nation of inhibition of angiogenesis and proinflammatory products released from the bone could result in a significant reduction in pannus formation. An additional potential mechanism for the efficacy of SB 273005 in the AIA rat is that of inhibiting leukocyte migration to the joints. For example, CD31/platelet endothelial cell adhesion molecule 1 is a ligand for $\alpha_v\beta_3$ that is involved in the adhesion of leukocytes to endothelium (27,28), and interference with this interaction by the vitronectin receptor antagonist could reduce cell trafficking into the joint.

Evidence of the protection of bone integrity by SB 273005 was provided by DEXA, in which there was a remarkable 69% normalization of BMD at the 60 mg/kg twice-daily dose. Additional evidence of the disease-modifying activity of the compound was provided by the obvious improvement observed in the MR images, in which 80% of the joints were significantly protected by prophylactic treatment at the 60 mg/kg twice daily dose, and >60% of the joints were protected at this dose administered therapeutically. Of particular interest was the finding that in the groups treated therapeutically, the MR images indicated better protection than would have been suggested by the effect on inflammation. Histologic evaluation of the joints also showed that treatment with SB 273005 resulted in significant protective effects on both the bone and cartilage/soft tissue. Again, these data are consistent with those generated for the $\alpha_v\beta_3$ antagonist cyclic peptide (25), which prevented cartilage erosions in the rabbit model of inflammatory arthritis.

The profile of activity described here for SB 273005 suggests strongly that orally active, small-molecule vitronectin receptor antagonists could provide significant benefit in the treatment of inflammatory diseases such as RA. These compounds may prevent not only the swelling associated with this disease, but also the loss of bone, cartilage, and soft tissue structure within articulating joints.

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