Inhibition of Disease Progression by a Novel Retinoid Antagonist in Animal Models of Arthritis

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ABSTRACT. ***Objective***. To investigate the usefulness of a novel retinoic acid receptor (RAR) antagonist (BMS-189453) in animal models of arthritis.

Methods. BMS-189453 was tested in HIG-82 rabbit synovial fibroblasts to determine its ability to repress collagenase (matrix metalloproteinase-1, MMP-1) mRNA expression *in vitro*. Cells were stimulated with phorbol myristate acetate or interleukin 1ß and mRNA quantified by slot-blot analysis. *In vivo*, BMS-189453 was evaluated in 2 animal models of arthritis: collagen induced arthritis (CIA) in mice and streptococcal cell wall induced arthritis (SCWA) in rats. Clinical scores for arthritis were recorded weekly. At the end of each study, limbs were evaluated histologically. In CIA, these results were correlated with mRNA levels for collagenase-3 (MMP-13) and stromelysin-1 (MMP-3) as determined by Northern blot.

Results. BMS-189453 reduced MMP-1 expression in HIG-82 synovial fibroblasts in culture. BMS-189453 treatment blocked the clinical progression of arthritis beyond soft tissue inflammation in the CIA model. In the SCWA model, BMS-189453 treatment resulted in significantly reduced swelling with no notable progression to joint distortion/destruction. Histological evaluation of the joints from animals in both models confirmed this result. Analysis of mRNA from the CIA paws showed that BMS-189453 prevented the overexpression of MMP-13 and MMP-3 in arthritic joints.

Conclusion. Improvement in clinical and histologic variables in 2 separate animal models, along with simultaneous reduction in MMP expression in the affected joint, suggests that RAR antagonists such as BMS-189453 may be useful as agents to treat rheumatoid arthritis and for determining the role of MMP in disease progression. This is the first study to show the clinical potential of RAR antagonists in arthritis. (J Rheumatol 2003;30:355–63)

Key Indexing Terms:
RETINOID ANTAGONIST RETINOIC ACID RECEPTOR AP-1
MATRIX METALLOPROTEINASE COLLAGEN INDUCED ARTHRITIS
STREPTOCOCCAL CELL WALL INDUCED ARTHRITIS

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic inflammation of the synovial joint. The observed synovial inflammation is associated with the destruction of articular cartilage and bone erosions leading to the loss of joint function. While the exact molecular mechanisms responsible for cartilage destruction have not been elucidated, proteinases produced by macrophages, neutrophils, synovial cells, and chondrocytes are believed to play a critical role in the degradation of the cartilage matrix. In particular, there is good evidence that the matrix metalloproteinase (MMP) family of proteolytic enzymes plays an important role in the pathological process of arthritis. Expression levels for several distinct MMP are found to be elevated in arthritic tissues, and high levels of MMP are found in synovial fluid taken from arthritic joints[1,2].

Retinoids, analogs of vitamin A, play an important role in a wide variety of biological functions including cell proliferation, differentiation, and morphogenesis. Retinoids exert their effects largely through their interaction with the retinoic acid receptors (RAR-α, -ß, -γ). These nuclear receptors function as ligand dependent transcription factors that bind to retinoic acid response elements in the promoter region of target genes leading to the modulation of transcription of these genes[3-5]. An alternative mechanism of action by retinoids is gene repression involving the ligand dependent interaction of RAR with the transcription factor AP-1, a protein heterodimer comprising c-jun and c-fos[6,7]. One important target gene for retinoic acid via AP-1 is fibroblast interstitial collagenase

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(MMP-1), which degrades interstitial collagen and is thought to play a critical role in the degradation of the cartilage matrix in arthritis[8]. Retinoic acid was shown to suppress collagenase gene expression through interactions with AP-1 *in vitro*[9], and the DNA binding activity of AP-1 was shown to be increased in collagen induced arthritis (CIA) in mice and human RA[10]. In CIA, the activation of AP-1 precedes clinical arthritis and MMP gene expression[10]. Injection of CIA mice with short double stranded AP-1 DNA oligonucleotides that compete for the binding of AP-1 inhibited arthritic joint destruction[11]. Compounds possessing anti-AP-1 activity therefore have potential for the treatment of arthritis by suppressing collagenase and other AP-1 regulated MMP, thus blocking the erosive progression of the disease.

BMS-189453 (Figure 1) is an RAR-α, -ß, -γ antagonist shown to compete with retinoic acid for gene transactivation through the RAR while maintaining AP-1 transrepressive activity in *in vitro* systems[12,13]. To investigate the usefulness of BMS-189453 in arthritis, we evaluated the inhibitory effect of BMS-189453 on collagenase induction in HIG-82 cells. This cell culture system provides a well documented model for the induction of MMP-1 expression involving an AP-1 mechanism[14]. Since MMP-1 expression was found to be reduced in a dose dependent manner in these cells, it was reasonable to test the effects of BMS-189453 on arthritis *in vivo* using 2 well characterized animal models: the mouse CIA model and the rat streptococcal cell wall induced arthritis (SCWA) model.

CIA is a chronic autoimmune polyarthritis that resembles human RA in terms of genetic linkage, T cell dependency, tendency to affect peripheral joints, pannus formation, cartilage erosion, and joint destruction[15,16]. Agents used to treat human RA such as corticosteroids, nonsteroidal antiinflammatory drugs (NSAID), methotrexate, and penicillamine are active in this model[17,18]. Since CIA does not perfectly mimic human RA and the effects of retinoids in this model have been inconsistent[19-21], we also evaluated BMS-189453 in the SCWA model in rats. While the precise relationship to human RA is not known, the clinical, pathological, radiological, and immunological features of SCWA resemble that seen in human arthritis[22,23]. Unlike CIA, the serum of SCWA rats contains rheumatoid factor, and the disease severity is remitting and relapsing, much like the course of disease progression in humans[22,24].

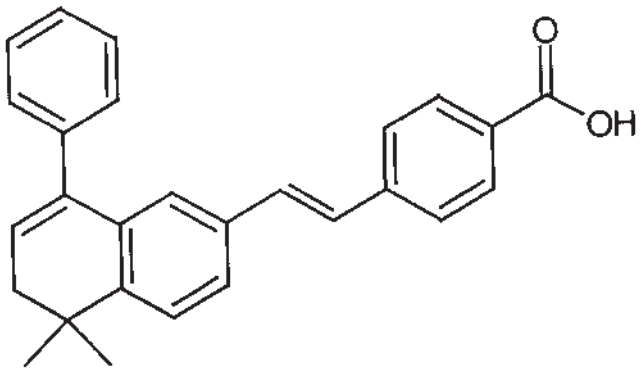

Figure 1. The chemical structure of RAR pan-antagonist BMS-189453: 4-[[(E)-[5,6-Dihydro-5,5-dimethyl-8-phenyl]-2-naphthalenyl]ethenyl]benzoic acid.

The effects of BMS-189453 in these models revealed that BMS-189453 modulates the clinical and histological features of arthritis in both mouse CIA and rat SCWA. Gene expression studies using mRNA from mouse CIA limbs show that BMS-189453 suppresses both collagenase-3 (MMP-13) and stromelysin-1 (MMP-3), which correlates well with results from the HIG-82 experiments. These results suggest that retinoid antagonists such as BMS-189453 may be useful for the treatment of human RA.

MATERIALS AND METHODS

Compounds tested. BMS-189453 (Figure 1), synthesized by Medicinal Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, was used as the sodium salt. Prednisolone and *all-trans* retinoic acid (tRA) were purchased through Sigma-Aldrich, St. Louis, MO, USA.

Suppression of collagenase expression in HIG-82 synovial fibroblasts. The ability of BMS-189453 to suppress MMP-1 in HIG-82 rabbit synovial fibroblasts was evaluated in a described assay[14]. In short, early passage HIG-82 cells were treated for 7.5 h with 7.5 nM phorbol myristate acetate (PMA) or for 12 h with 10 ng/ml of IL-1ß to induce MMP-1 expression. Suppression of MMP-1 expression was measured by cotreatment with BMS-189453 and compared with the RAR agonist tRA in the concentration range from 0.001 to 10 µM. Total RNA was isolated from these cells and blotted onto nitrocellulose membranes using the slot-blot method. The resulting blots were probed with a ^{32}P-labeled cDNA for MMP-1 and GAPDH. Hybridization was quantified by phosphorimagery.

Animal models. All animal test methods reported here were approved by the Institutional Animal Care and Use Committee. The animals were cared for according to American Association for the Accreditation of Laboratory Care guidelines and given standard laboratory chow and water *ad libitum*.

CIA was induced in 6–8 week old female DBA/1 Lac J mice (Charles River Laboratories, Wilmington, MA, USA) by intradermal injections of type II bovine collagen (provided by Dr. John Stuart, Memphis, TN, USA) emulsified in complete Freund's adjuvant (Difco) on days 1 and 7. Using the preventive dosing protocol, treatment with intraperitoneal (IP) injections of BMS-189453 (15 mg/kg), tRA (5 mg/kg), prednisolone (3 mg/kg), or vehicle (water) was given once daily throughout the 70 day study beginning 3 days prior to the first injection of collagen. The dose of BMS-189453 (15 mg/kg) was chosen based upon its effectiveness in preliminary dose-response experiments. Sixteen animals were used for each treatment group (N = 16). Mice were inspected daily for the onset of arthritis characterized by edema and/or erythema in the limbs. In the established arthritis dosing protocol, treatment with BMS-189453 (15 mg/kg), prednisolone (3 mg/kg), or vehicle was initiated when the first visual signs of arthritis were observed and continued once daily for 8 weeks. The mice were evenly distributed among test groups as arthritic symptoms first appeared, with a final population size of 12 animals (n = 12) and a total of 18–24 first affected limbs. For both dosing protocols, the limbs were scored at least weekly using clinical scores defined as: 0 = normal; 1 = joint inflammation presented as erythema and edema; 2 = joint distortion with or without joint inflammation; 3 = immobilization and ankylosis of the joints.

SCWA was induced in female Lewis rats, 100–150 g each, obtained from Charles River Laboratories. To induce the development of arthritis, each animal was given an IP injection in the lower abdomen with peptidoglycan-polysaccharide polymers from group A streptococci (Lee Laboratories) at a rham-

nose equivalent of 15 µg/g body weight. Three days prior to arthritis induction, treatment with BMS-189453 (15 mg/kg IP), prednisolone (3 mg/kg IP), or vehicle (water) was initiated and continued daily throughout the 42 day study. Eight animals were used for each treatment group (n = 8). Rats were inspected daily for the onset of arthritis characterized by edema and/or erythema in the limbs. Each hind paw was measured at least weekly with a constant tension caliper.

Histology. At the end of the experiment, the animals were sacrificed by carbon dioxide asphyxiation and their limbs removed and placed in fixative. Both hind limbs and forelimbs were analyzed histologically in the mouse CIA model, whereas hind limbs alone were analyzed in the rat SCWA model to correlate with the limbs clinically graded or paws measured from each model, respectively. The joints were then decalcified, processed, and embedded in paraffin with an orientation to allow a lateral section from the ankle to the digits. Serial sections were made 4-8 µm thick, mounted on glass slides, and stained with safrinin O and fast green stain. The following scoring system was used to assess the arthritis: 0 = normal joints, absence of inflammation; 1 = evidence of soft tissue inflammation: synovial hyperplasia and/or cell infiltration into the synovial space (pannus); 2 = Grade 1 and definite erosion of articular cartilage; 3 = Grade 2 and definite erosion of articular bone, but joint architecture mostly intact; 4 = Grade 3 with bone erosion resulting in a major loss of joint architecture.

Northern blot. Total RNA (30 µg) was prepared from CIA (affected limbs only) that were pooled from each test group at day 40 of the preventive dosing protocol. RNA was prepared using the Trizol method (Gibco BRL), electrophoresed through a 0.9% (w/v) agarose/2.2 M formaldehyde gel, and vacuum transferred to a Zeta-Probe (Bio-Rad) blotting membrane. Following ultraviolet crosslinking of the RNA to the membrane, cDNA probes for mouse collagenase-3 (MMP-13), stromelysin-1 (MMP-3), and GAPDH were labeled with ^{32}P-dCTP (3000 Ci/mmol; Dupont-NEN) using an Amersham Megaprime DNA labeling system. The MMP-13 and MMP-3 probes were kindly provided by Dr. Karen Hasty (Memphis, TN, USA) and the GAPDH probe was purchased from Clontech. The membranes were hybridized overnight and washed before signal quantitation by PhosphoImager analysis. Transcript sizes of mRNA were estimated by comparison with mRNA markers and the expression of MMP-13 and MMP-3 was normalized by the expression of GAPDH.

RESULTS

Inhibition of PMA and IL-1ß induced collagenase (MMP-1) expression by retinoic acid and BMS-189453 in HIG-82 cells. The rabbit synovial fibroblast cell line, HIG-82, is commonly used as a model for the induction of MMP-1 expression involving an AP-1 mechanism[14]. This culture model was chosen as representative of human synovial fibroblasts because they express MMP-1, while both mice and rats express MMP-13. Figure 2 shows that a reduction in MMP-1 expression was seen when cells were induced with either PMA or IL-1ß and cotreated with either the RAR panagonist tRA or the RAR antagonist BMS-189453. A 50% reduction in MMP-1 expression (IC_{50}) was measured at 40 nM for tRA cotreated HIG-82 cells, while treatment at 1 µM reduced expression to basal levels. To suppress IL-1ß induced MMP-1 expression, higher concentrations of tRA were required (IC_{50} = 500 nM with 80% maximal inhibition at 10 µM). The RAR antagonist, BMS-189453, also suppressed PMA induced MMP-1 expression, but maximum inhibition was 45% at 100 nM. Similar results were obtained using IL-1ß as an inducer, with IC_{50} values 10-fold higher than tRA (IC_{50} = 5 µM).

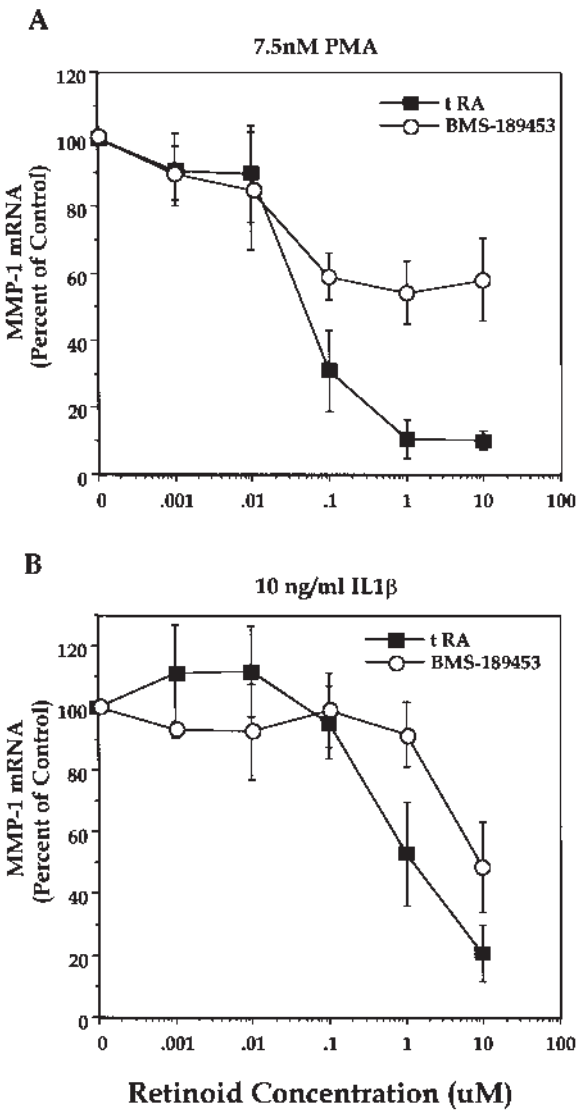

Figure 2. Retinoic acid (tRA) and BMS-189453 inhibition of MMP-1 induction by PMA (A) and IL-1ß (B) in HIG-82 rabbit synovial fibroblasts in culture. The standard deviation is indicated by the vertical bar (n = 4).

Activity of BMS-189453 in CIA in mice. A preventive protocol of drug dosing was initially used where the drug was administered IP beginning 3 days prior to collagen injection and continuing daily throughout the experiment. Clinical signs of arthritis first appeared around day 20 in animals treated with vehicle or tRA, while the first onset of the clinical signs of arthritis did not occur until day 32 in the BMS-189453 or prednisolone treated groups (Figure 3). The total penetrance of arthritis was 100% in the vehicle and tRA treated groups by day 50, but only reached about 75% in the BMS-189453 treated group and 25% in the prednisolone treated group at the end of the 70 day study (data not shown). The overall effects of each treatment on CIA can be represented by the mean clinical scores, which were derived by adding the clinical scores from all the limbs in each group of animals and dividing the

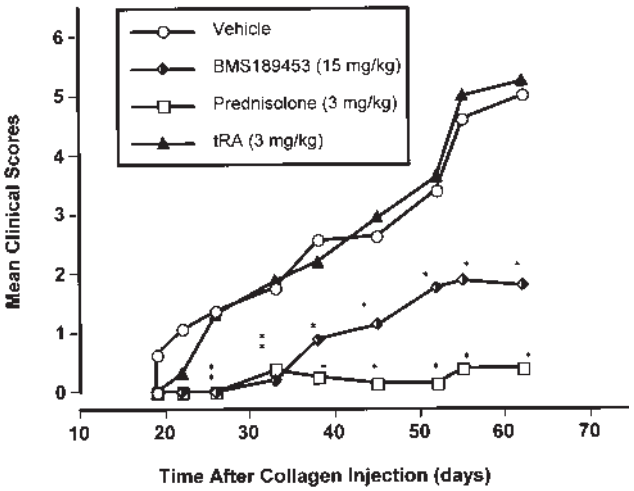

Figure 3. BMS-189453 significantly reduced the mean clinical scores of CIA mice throughout the time course of disease progression. Clinical scores were recorded as described in Materials and Methods. *Statistically significant differences ($p < 0.01$) were achieved for BMS-189453 and prednisolone compared to vehicle beginning on day 26 of the study as determined by ANOVA (Tukey procedure).

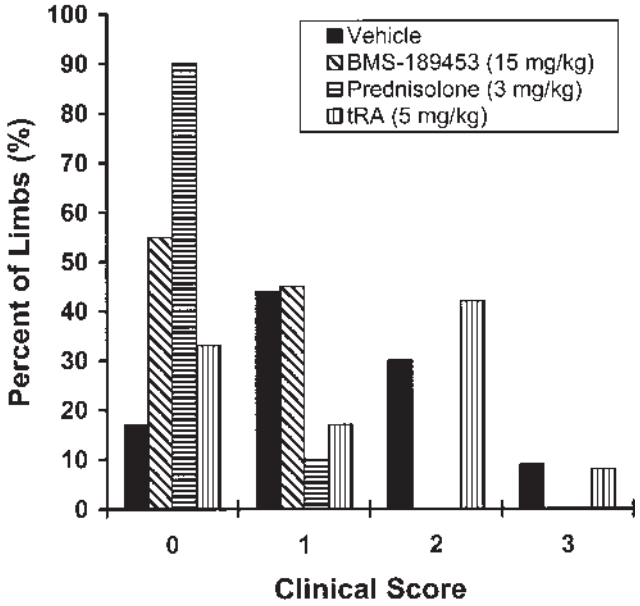

Figure 4. The distribution of clinical scores for limbs in CIA mice at the end of the 70 day study. Clinical scores were given to each limb using the following definitions: 0 = normal; 1 = joint inflammation presented as erythema and edema; 2 = joint distortion with or without joint inflammation; 3 = immobilization and ankylosis of the joints. Compared to vehicle, BMS-189453 shifted the distribution of clinical scores to lower grades limited to soft tissue inflammation.

sum by the number of animals. As shown in Figure 3, both the RAR antagonist BMS-189453 and prednisolone (the positive control for this model) significantly reduced the mean clinical scores in the CIA mice when compared with the vehicle or tRA treated mice. By the end of the study, the mean clinical scores of the mice in the vehicle and tRA treated groups approached 6, while those in the BMS-189453 treated group had a mean clinical score less than 2. Statistically significant differences ($p < 0.01$) were achieved for BMS-189453 and prednisolone compared to the vehicle beginning on day 26 of the study, as determined by analysis of variance (ANOVA, Tukey procedure).

Examination of the distribution of clinical scores for all limbs in each group of mice (Figure 4) shows that 55% of the limbs from the BMS-189453 treated mice appeared normal and the other 45% of the limbs had clinical scores of 1 (soft tissue inflammation). In contrast, greater than 80% of the limbs in the vehicle treated group had clinical scores of 1 or higher. Nearly 40% of the limbs in the vehicle treated group had scores of 2 or higher (joint distortion or immobilization). Prednisolone showed the greatest benefit, with 90% of the limbs appearing normal, while the clinical score distribution of the tRA treated mice closely resembled that of the vehicle treatment.

At the end of the study, all limbs were processed for histological analysis. The slides were read by a blinded investigator using the scoring system described in Materials and Methods. Representative histological sections of mouse joints from normal (grade 0), vehicle treated (grade 3), and BMS-189453 treated (grade 1) mice are shown in Figure 5. The histological score distribution is illustrated in Figure 6A. Nearly 80% of the limbs from mice in the vehicle treated group had histological grades of 3 and 4, indicating significant erosion of cartilage and bone. Similar results were recorded for the tRA treated group. In BMS-189453 treated mice, no limbs were scored a grade 4 and only 5% were scored grade 3, while 58% of the limbs were scored grade 1 or 2. Greater than 30% of the limbs in the BMS-189453 group were normal. The results with prednisolone were similar to those observed for BMS-189453.

A second protocol was developed to mimic the efficacy of BMS-189453 in established arthritis. Treatment with BMS-189453 or prednisolone was initiated when the clinical signs of arthritis first appeared, typically between 20 and 25 days following the first injection with collagen II. Figure 7 illustrates the mean clinical score of the first affected limb(s) from CIA mice produced in this way and treated with BMS-189453, prednisolone, or vehicle throughout the 8 week treatment period. The mean clinical score for all first affected limbs within the BMS-189453 and prednisolone treated groups never exceeded a score of 1 (soft tissue inflammation), while the clinical score severity for the first affected limbs in the vehicle treated group progressed throughout the duration of the study. Prednisolone differs from BMS-189453 in that it lowers the mean clinical score from baseline due to its antiinflammatory properties. Statistically significant differences ($p < 0.05$) were achieved for BMS-189453 and prednisolone compared to the vehicle as determined by ANOVA (Tukey procedure). Examination of the distribution of clinical scores

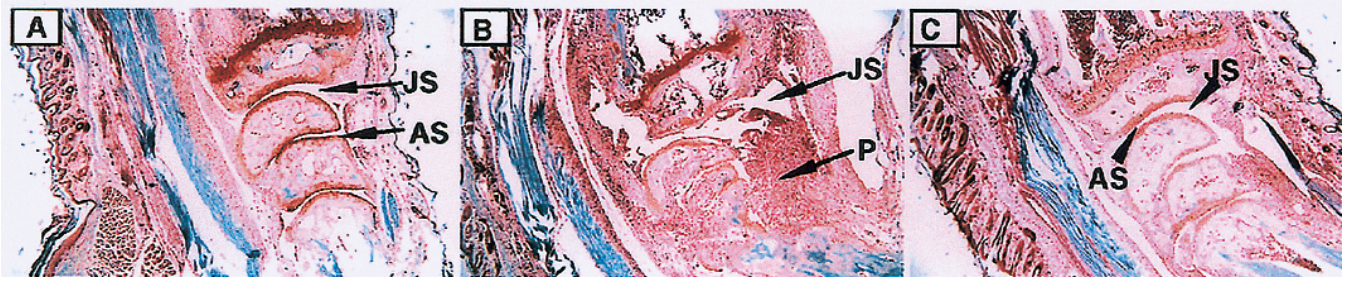

Figure 5. Representative histology from mouse paws from normal (A), arthritic (B), and BMS-189453 treated (C) mouse paws. At the end of the study, limbs from all animals were removed for sectioning and staining with safrinin O and fast green. Note the normal joint architecture with a thin synovial membrane, narrow joint space (JS), and smooth articular surface (AS) in the normal mouse limb. The vehicle treated CIA mouse shows joint swelling with cellular infiltration, synovial membrane hyperplasia, pannus (P) formation, and cartilage and bone erosion. These pathological changes were markedly reduced in the BMS-189453 treated mouse.

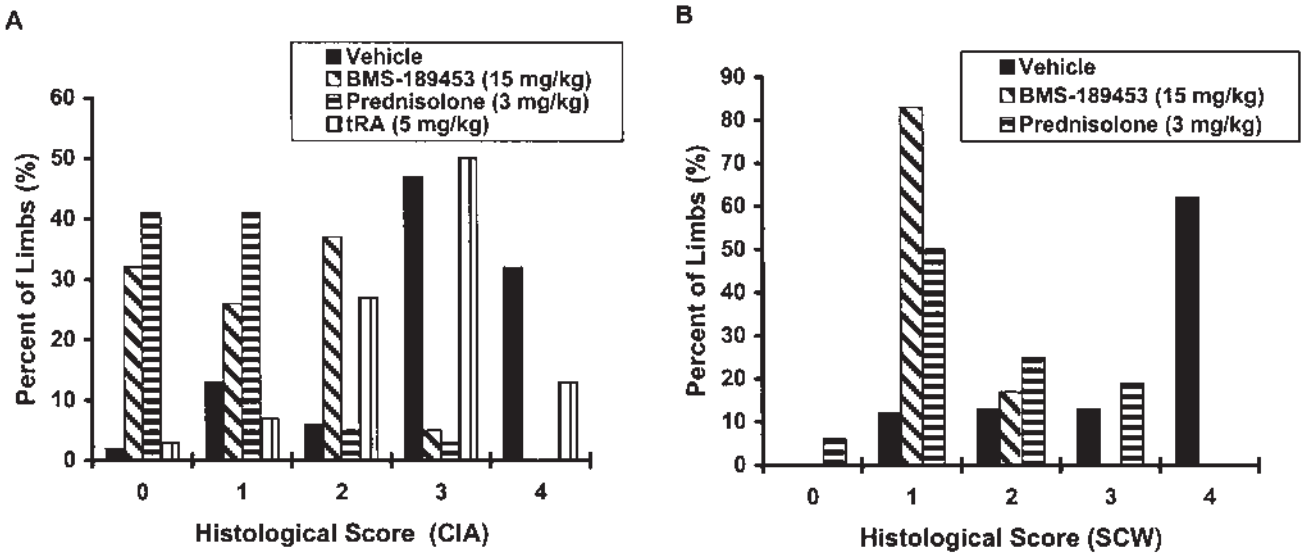

Figure 6. The distribution of histological scores in CIA mice (A) and SCWA rats (B). Histological scores were given to each limb using the following definitions: 0 = normal; 1 = synovial membrane hyperplasia and/or cellular infiltrate into the synovial space; 2 = pannus formation with erosion of the articular cartilage; 3 = pannus erosion into cartilage and bone with > 50% of the joint architecture intact; 4 = loss of joint integrity. The majority of joints in the vehicle treated animals showed histological scores of 3 and 4, while treatment with BMS-189453 shifted the distribution to the lower end, with histological scores of mainly 0–2.

for all the limbs (including additional limbs affected during the treatment period) at the end of the 8 week treatment period showed the protective effects of BMS-189453 similar to that demonstrated in the preventive protocol, with only moderate differences between all treatment groups in histological scoring when comparing either first affected limbs or all limbs (data not shown).

Suppression of mouse collagenase-3 (MMP-13) and stromelysin-1 (MMP-3) expression by BMS-189453 in the limbs of CIA mice. An MMP-1 homolog has not been identified in mice, but many of its functions appear to be served by MMP-13. Studies of the human MMP-13 promoter indicate that it also contains AP-1 sites and is homologous to the mouse gene[25,26]. Therefore, we measured the expression levels of MMP-13 in preventive CIA by Northern blot. As illustrated in Figure 8, mouse MMP-13 and MMP-3 levels were significantly elevated in vehicle and tRA treated CIA mice compared to the naive age matched control animals. In contrast, those mice treated with BMS-189453 had no measurable MMP-13 or MMP-3 expression levels that were similar to those observed for age matched naive control animals. While mRNA for MMP-13 and MMP-3 is not indicative of a corresponding change in the protein levels or in the activation of latent MMP, results from preliminary gel zymography studies using protein prepared from the same limbs used for RNA preparation parallel those from the mRNA (data not shown).

Activity of BMS-189453 in SCWA in rats. BMS-189453 was evaluated in a second model of arthritis in a second species. The SCWA model was chosen due to the severity of symptoms produced and similarity to the human disease. Within 24–72 h of a single IP injection of SCW, vehicle treated rats developed a symmetric erythema and edema of the distal

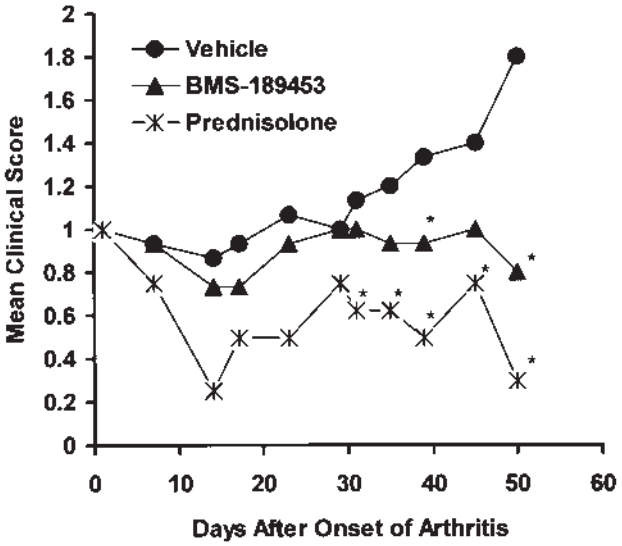

Figure 7. The mean clinical score of first affected limb(s) from mice with established CIA throughout the 8 week treatment period. BMS-189453 effectively blocks the clinical progression of arthritis in CIA mice when dosed therapeutically. Clinical scores were recorded as described in Materials and Methods. *Statistically significant differences ($p < 0.05$) were achieved for BMS-189453 and prednisolone compared to vehicle as determined by ANOVA (Tukey procedure).

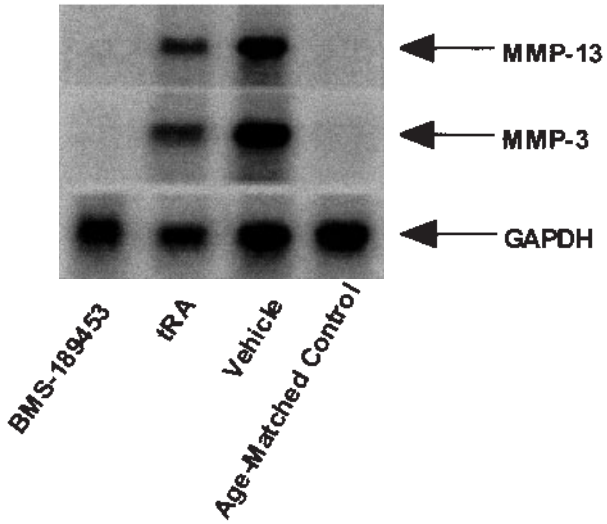

Figure 8. Northern blot analysis of total RNA extracted from limbs of CIA mice treated with the indicated retinoids for 40 days. BMS-189453 significantly reduces MMP-13 and MMP-3 to control levels.

joints primarily involving the wrists and ankles. This acute inflammation subsided by 5 to 8 days. Beginning around 21 days, a chronic remittent arthritis occurred that progressively led to a more severe edema, joint distortion, and joint destruction. Treatment with BMS-189453 (15 mg/kg IP) resulted in significantly reduced swelling compared to the vehicle, with no notable progression to joint distortion/destruction. Figure 9 illustrates the progressive increase in paw thickness for the

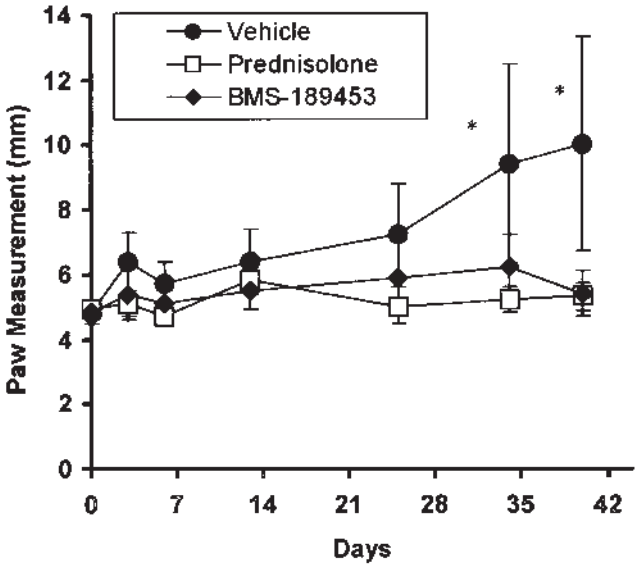

Figure 9. The effects of BMS-189453 on SCWA in rats. The thickness of the hind limb paws as measured throughout the 42 day study. *Statistically significant differences ($p < 0.01$) were achieved for BMS-189453 and prednisolone compared to vehicle on days 35 and 42 of the study as determined by ANOVA.

vehicle treated rats in contrast to the BMS-189453 treated rats, whose hind paws experienced no significant change in paw thickness throughout the 42 day study. On day 42, the average measurement for the hind paws from rats in the vehicle treated group (10.1 mm) was over 2-fold the measured thickness from rats in the normal age matched control group (4.9 mm). Measurements from the BMS-189453 (5.4 mm) and prednisolone (5.3 mm) groups were comparable to the normal age matched controls (4.9 mm). Statistically significant differences ($p < 0.01$) were achieved for both the BMS-189453 and prednisolone groups compared to the vehicle on days 35 and 42 of the study as determined by ANOVA.

At the end of the study, the hind limbs were processed for histological analysis. The slides were read by a blinded investigator using the scoring system described in Materials and Methods. The histological score distribution is illustrated in Figure 6B. Clearly, BMS-189453 protected the integrity of the joint whereas joint destruction was observed in the vehicle treated rats. Greater than 60% of the hind limbs in the vehicle treated group had a histological score of grade 4 (cartilage and bone erosion resulting in a major loss of joint architecture), while 83% of the limbs in the BMS-189453 group recorded a histological score of grade 1 (evidence of soft tissue inflammation with no cartilage or bone erosions). None of the limbs from the BMS-189453 treated groups achieved a histological score of grade 4.

DISCUSSION

RA is a chronic polyarthritis affecting roughly one percent of the population worldwide[27]. Clinical presentation at the time of initial diagnosis includes joint pain, stiffness, and swelling.

Erosive changes in affected joints are detectable by magnetic resonance imaging within 4 months of clinical onset of arthritis, and radiographic data suggest that 50% of the maximum damage to the joints occurs within the first 5 years of the disease[28,29]. The clinical course of RA leads to declines in the functional capacity to perform activities of daily living, frequent work disability, high levels of comorbidities, and earlier mortality[30]. With the exception of the recent introduction of tumor necrosis factor-α (TNF-α) blocking drugs, no single treatment or combination of therapies is associated with a sustained improvement or halt in progression of the disabling course of arthritis[31]. Longterm experience with TNF-α inhibitor therapy is limited and other traditional drug therapies result in side effects that limit their use. The continuing challenge, therefore, is to develop a treatment for early intervention in RA that will block disease progression without causing serious side effects. This report describes the use of a novel RAR antagonist, BMS-189453, in animal models of arthritis and suggests that this compound may be useful in the treatment of the human disease.

Synovial fibroblast cultures provided the first indication that BMS-189453 would be useful in experimental models of arthritis. The ability of this compound to reduce the expression of MMP-1 when induced by physiologically relevant inducers (Figure 2) served as the initial rationale for the study of this compound in animal models. Two *in vivo* models were chosen for further evaluation: the mouse CIA model and the rat SCWA model. In these 2 aggressive, accelerated models of arthritis, BMS-189453 suppresses the pathohistological signs of arthritis, thereby blocking the progression of the disease at the level of joint inflammation (Figures 3–7 and Figure 9). In addition, although toxicological evaluation was not within the design of the studies reported here, it is worthwhile to note that there were no overt signs of the typical toxicities associated with retinoids[32], and the compound did not induce overt signs of hypovitaminosis A (data not shown).

While the mechanism of BMS-189453 action *in vivo* could be very complex, its AP-1 transrepressive activity[12] is consistent with what is known about the role of AP-1 in arthritis. In the CIA model, the finding that arthritic joint destruction could be specifically inhibited by administering oligonucleotides containing the AP-1 consensus sequence to compete for binding of AP-1 at the promoter binding site of target genes strongly suggests that AP-1 activation is involved in disease progression[11]. Further, the role of AP-1 in retinoic acid mediated transrepression of collagenase and stromelysin has been documented[33,34]. Retinoids are known to suppress collagenase transcription by mechanisms that involve downregulation of fos and jun mRNA, sequestration of fos and jun proteins, and the formation of complexes of retinoid receptors and AP-1 proteins on DNA[35]. The downregulation of MMP containing AP-1 in their promoter sites provides a hypothesis for the inhibition of joint destruction by anti-AP-1 compounds such as BMS-189453. Nonetheless, AP-1 is not the only transcription factor involved in the induction of MMP genes. It is known that MMP-1 gene expression is regulated at multiple points, both transcriptionally and post-transcriptionally[35]. The effects of BMS-189453 may also be mediated by other mechanisms such as RAR antagonism, immunosuppression, antiproliferation of the synovial fibroblasts, the induction of tissue inhibitors of metalloproteinases, or the inhibition of inflammatory cytokines important to the pathogenesis of arthritis.

In light of the observations reported here, it is perplexing that the RAR pan-agonist, tRA, was inactive in the CIA model despite its strong anti-AP-1 activity. However, the effects of retinoids in animal models of arthritis have been inconsistent[19-21], and no beneficial clinical effect was observed with the retinoid N-[4-hydroxyphenyl] retinamide in a clinical trial of patients with RA[36]. Despite the inhibition of MMP-1 induction by tRA *in vitro* in a synovial fibroblast cell line described by others[33,37] and reported here (Figure 2), there are also several reports that describe pharmacologic doses of retinoic acid as a stimulus of cartilage degradation[38,39]. While retinoic acid decreases MMP-1 mRNA expression in cultured chondrocytes, it also increases MMP-13 mRNA expression[1]. Interestingly, MMP-13 is much more active against type II collagen compared to MMP-1[40], and overexpression of MMP-13 alone can result in cartilage degradation and joint pathology resembling arthritis[41]. There have also been reports of retinoic acid induced stimulation of urokinase-type plasminogen activator, a serine proteinase with implicated involvement in joint erosion[42]. These findings are consistent with our observations that tRA is a potent AP-1 transrepressor in a synovial fibroblast cell line, yet has no benefit on the clinical progression of arthritis in the mouse CIA model. It is therefore possible that the RAR agonist activities of tRA may contribute to the pathogenic mechanisms of RA *in vivo* dominating over the potential protective effects of anti-AP-1 activity. In such circumstances, an RAR antagonist like BMS-189453 that maintains anti-AP-1 properties could be the preferred profile for effective RA treatment by retinoids. Clearly, more work needs to be done to define the mechanism by which BMS-189453 acts in arthritis models.

In summary, BMS-189453 demonstrated significant effects in 2 animal models of arthritis by delaying the onset of arthritis and limiting the clinical symptoms to inflammation of the soft tissue without progression to joint distortion. The effects of BMS-189453 on joint inflammation are dissimilar to prednisolone, a glucocorticoid, which significantly reduced the soft tissue inflammation as well as the more severe joint damage associated with arthritis. Histological analysis revealed that BMS-189453 was as effective as prednisolone at inhibiting joint destruction. A less striking effect was observed when BMS-189453 was given to mice with established arthritis, suggesting that treatment early in the course of the disease is most beneficial. Studies on synovial fibroblasts in culture and mRNA extracted from the limbs from CIA mice indicate that

the reduction in arthritis symptoms likely involves a suppression of collagenase-3 and stromelysin-1 expression, presumably mediated by an AP-1 transrepression mechanism. Despite the seeming ambiguity regarding the mechanism of action of BMS-189453, the results reported here strongly support the continued investigation of this novel compound as a therapeutic intervention for the treatment of RA.

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