Collagen and Aggrecan Degradation Is Blocked in Interleukin-1-Treated Cartilage Explants by an Inhibitor of IκB Kinase through Suppression of Metalloproteinase Expression

Mark A. Pattoli, John F. MacMaster, Kurt R. Gregor, and James R. Burke

Immunology, Inflammation, and Pulmonary Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey

Received April 6, 2005; accepted July 7, 2005

ABSTRACT

It has previously been shown that BMS-345541 [4(2’-aminoethyl)-amino-1,8-dimethylimidazo[1,2-a]quinoxaline], a highly-se-lective inhibitor of IκB kinase (IKK), blocks both inflammation and joint destruction in murine collagen-induced arthritis. Although this agent has been shown to inhibit nuclear factor-κB-dependent cytokine expression in mice, we examined whether the inhibitor directly inhibits cytokine-driven metalloproteinase expression and cartilage degradation. In SW-1353 human chondrosarcoma cells, BMS-345541 inhibited interleukin-1 (IL-1)-dependent expression of matrix metalloproteinase (MMP)-1, MMP-3, and MMP-13 in a concentration-dependent manner. IL-1 treatment failed to induce and BMS-345541 did not inhibit the expression of aggrecanases ADAMTS-4 (a disintegrin and metalloproteinase domain with thrombospondin motif) and ADAMTS-5, as well as the tissue inhibitor of metalloproteinase-3. In bovine cartilage explant cultures stimulated with IL-1 to induce aggrecan and collagen degradation over 3 weeks of culture, BMS-345541 was effective in inhibiting the degradation of both aggrecan and collagen. Secreted ADAMTS-4 was not inhibited by BMS-345541 in these explants, whereas ADAMTS-5 secretion was blocked in the same concentration range that inhibited aggrecan degradation. The ability of the IKK inhibitor to block aggrecan and collagen degradation through suppression of metalloproteinase expression, coupled with its ability to block inflammatory cytokine production, shows IKK to be a promising target for the development of novel agents to treat arthritic diseases.

Degenerative joint disorders such as osteoarthritis (OA) and rheumatoid arthritis (RA) are characterized by the de-struction and loss of articular cartilage and an imbalance of proinflammatory cytokines. In both disorders, the chondrocytes appear to play an important role in the pathogenesis of disease. Although chondrocytes are the primary source of type II collagen and aggrecan, two of the most important components of the cartilage matrix, these cells also produce proteolytic enzymes, which degrade collagen and aggrecan/ proteoglycan when stimulated with interleukin-1 (IL-1) or tumor necrosis factor-α (TNF-α). Overproduction of these enzymes results in the erosion of articular cartilage, destruc-tion of tissue and bone, and joint abnormalities. As evidenced by the efficacy of therapies targeting TNF-α (e.g., infliximab and etanercept) or IL-1 (e.g., anakinra), these cytokines play a critical role in both the inflammatory and destructive mechanisms of RA. In OA, IL-1 also appears to play a pivotal role in the degenerative process by driving collagen and aggrecan degradation (for a review, see Chevalier, 1997).

The transcription factor nuclear factor-κB (NF-κB) mediates much of the downstream effects of both TNF-α and IL-1, including the transcription of matrix metalloproteinases (MMP) and other cytokines (Li and Verma, 2002). In unacti-vated cells, NF-κB is inhibited by an interaction with an IκB protein (e.g., IκBα, IκBβ, and IκBε). An essential step in the activation of NF-κB is the phosphorylation of IκB by a mult-isubunit IκB kinase (IKK), which contains two catalytic subunits, termed IKK-1 and IKK-2. IKK-2 appears to play a dominant role over IKK-1 in the activation of NF-κB (Ghosh and Karin, 2002).

Given the importance of NF-κB in regulating immunolog-ical processes and the role IKK plays in NF-κB activation, the

ABBREVIATIONS: OA, osteoarthritis; RA, rheumatoid arthritis; IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-κB; MMP, matrix metalloproteinase(s); IKK, IκB kinase; BMS-345541, 4(2’-aminoethyl)-amino-1,8-dimethylimidazo[1,2-a]quinoxaline; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; PCR, polymerase chain reaction; ADAMTS, a disintegrin and metalloproteinase domain with thrombospondin motif; TIMP, tissue inhibitor of metalloproteinases; INOS, inducible nitric-oxide synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAG, glycosaminoglycan; COX-2, cyclooxygenase-2.
identification of selective IKK inhibitors has received considerable interest (see Burke, 2003; Karin et al., 2004). We have recently reported that BMS-345541 is an inhibitor of the IKK-2 catalytic subunit (IC_{50} = 0.3 μM) with high selectivity over other kinases and some selectivity for IKK-2 over IKK-1 (Burke et al., 2003). The compound appears to bind to an allosteric binding site on the IKK catalytic subunits and inhibits NF-κB-dependent transcription of proinflammatory cytokines both in vitro and in vivo. The compound has been shown to be highly efficacious against both inflammation and joint destruction in collagen-induced arthritis in mice, with inhibition of disease-associated IL-1β mRNA in joints from these mice (McIntyre et al., 2003).

In the present report, we have further investigated the role of IKK in joint matrix destruction by measuring the effect of BMS-345541 on IL-1-stimulated collagenase and aggrecanase transcription in SW-1353 chondrosarcoma cells and on collagen and aggrecan degradation using IL-1-stimulated bovine cartilage explant cultures. The results show an agent that blocks the signal-induced activation of NF-κB by inhibiting IKK while having potent anti-inflammatory activities may also prove to be useful in the treatment of both RA and OA by directly blocking collagen and aggrecan degradation.

Materials and Methods

BMS-345541. The hydrochloride salt of BMS-345541 was prepared as previously reported (Burke et al., 2003).

Chondrosarcoma Cells. Human chondrosarcoma cells (SW-1353 cells) purchased from American Type Culture Collection (Manassas, VA) were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, HEPEs, and penicillin/streptomycin (Invitrogen, Carlsbad, CA). At the beginning of each experiment, cells were detached from culture flasks with 0.05% trypsin/EDTA, placed in 35-mm six-well plates at 1 x 10^6 cells/well and grown to 80 to 90% confluence (typically overnight). Cells were washed twice with phosphate-buffered saline (PBS) (without calcium or magnesium) and replaced with Dulbecco’s modified Eagle’s medium without serum. Compound in dimethyl sulfoxide (DMSO) was added to the desired concentration to give a final DMSO concentration of 1% (v/v). After 1-h incubation with compound, human IL-1β was added to cells to a final concentration of 10 ng/ml and allowed to incubate at 37°C at 5% CO_2 (time course experiments showed that IL-1 stimulation for 8 h was optimal to measure MMP-1, MMP-3, and MMP-13; results not shown). Cells were collected by trypsinization and pelleted. Total RNA was isolated from the cell pellets with RNeasy Mini-columns (Qiagen, Dorking, Surrey, UK) using the manufacturer’s instructions. Before elution of RNA from columns, an on-column digestion of DNA was applied using RNase-Free DNase Set (Qiagen), and the DNase was removed in subsequent wash steps. One microgram of total RNA was reverse-transcribed with 50 ng of random hexamers (Applied Biosystems, Foster City, CA) using SuperScript II reverse transcriptase (Invitrogen) following the specifications outlined by the manufacturer.

For gene expression analysis, real-time polymerase chain reaction (PCR) was performed using the ABI Prism 7700 sequence detection system analytical thermal cycler (Applied Biosystems). Primers were designed using the Primer Express software (Applied Biosystems). The primers used were as follows. MMP-1: 5'-TCATCGGCGAGCTGCTCTGAGA-3' (forward primer), 5'-GAATATTATATATATATGTCTG-3' (reverse primer); MMP-3: 5'-ACATCGGAGGTAGGAGTCT-TTCTACA-3' (forward primer), 5'-CTCATCGCGCGCTCTGCTCTAG-3' (reverse primer); MMP-13: 5'-TGTTAGGCGGCCCTCTGGCCTGC-3' (forward primer), 5'-TCCTGCTCAGAGTTA-3' (reverse primer); TIMP, tissue inhibitor of metalloproteinases-1 (TIMP-1): 5'-AACAGGCTTTAGATGAAATG-3' (forward primer), 5'-CAGATTTCCCTCCCTTACAA-3' (reverse primer); TIMP-3: 5'-ATCATCCAGACAGACTCTCCA-3' (forward primer), 5'-CTGTTGTTACCTCTGGATCACA-3' (forward primer); inducible nitric-oxide synthase (iNOS): 5'-CTCTCAAGCCATTTTTCTCCACAGTCTG-3' (forward primer), 5'-GACGGGCTCCAGGACATCC-3' (reverse primer); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5'-ATCCACCCATCTGCAATCC-3' (forward primer), 5'-CTCTGCAGCTCGGGAATG-3' (reverse primer). PCR reactions were carried out in MicroAmp optical 96-well reaction plates (Applied Biosystems). The PCR master mix for SYBR Green was obtained from Applied Biosystems. A typical 50-μl reaction sample contained cDNA, 1× SYBR Green PCR master mix containing reaction buffer, dNTP mix, 5 mM MgCl_2, 1.25 units of AmpliTaq Gold DNA polymerase, 0.5 unit of AMPerase (uricil N-glycosylase), and 300 nM of each forward and reverse primer. Thermal cycling conditions were 2 min at 50°C and 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Data were collected using the ABI Prism 7700 SDS (Applied Biosystems). Relative gene expression analysis was performed using the standard curve method. Briefly, dilutions of cDNA prepared from human total RNA were used to construct standard curves for the genes of interest and GAPDH amplifications. The normalized data were obtained from standard curves prepared for studied genes and the endogenous reference GAPDH. For each experimental sample, the amount of each gene was determined from the appropriate standard curve and normalized to GAPDH. Normalized gene expression was calculated as the ratio between sample and GAPDH cDNA copy number. Sample aliquots were analyzed by agarose gel electrophoresis to ensure the correct size of each gene fragment.

When measuring IL-1-induced IκBα phosphorylation, cultured SW-1353 cells were washed twice with PBS and incubated for 2 h in serum-free RPMI 1640 medium supplemented with HEPEs. Cells were trypsinized and resuspended in serum-free RPMI 1640 medium/HEPEs and then transferred to 50-ml conical tubes (2 ml at 4 x 10^6 total cells). BMS-345541 in DMSO was added to give a final DMSO concentration of 0.3% (v/v). The proteasome inhibitor PSI (Calbiochem, San Diego, CA) was added to give a final concentration of 1 μM. After 50 min at 37°C, IL-1β was added at 20 ng/ml and incubated for 5 min. Samples were quenched by addition of 15 ml of ice-cold PBS. Cells were centrifuged at 4°C and washed with ice-cold PBS. Pellets were resuspended in 80 μl of lysis buffer containing 50 mM Tris-HCl, 10 mM NaCl, 10 mM 1% NP-40, 1 mM EDTA, 1 mM EGTA, 1 mM β-glycerophosphate, 1 mM NaF, 1 mM Na_3VO_4, 5 mM Na_2P_2O_7, and 1 mM okadaic acid and protease inhibitor mixture set III (Calbiochem) and stored at −80°C. Samples were analyzed for phospho-IκBα by enzyme-linked immunosorbent assay (BioSource International, Camarillo, CA) following the manufacturer’s instructions.

Bovine Cartilage Explant Model. Intact cartil joints from calves (1–3 months old) were dissected, exposing the cartilage. Cartilage disks were obtained by first scoring the surface with a 4-mm biopsy punch and then dissecting the cartilage away from the bone with a scalpel. Four disks weighing a total of ~60 to 70 mg were placed into each well of a 24-well tissue plate with 1 ml of media containing test compound and human recombinant IL-1α (bovine cartilage explants are insensitive to human IL-1β) at 25 ng/ml. Culture supernatants were removed every 3 to 4 days and replaced with media containing fresh test compounds and IL-1α. Supernatants on days 4, 7, and 21 were assayed for cytotoxicity using a lactate dehydrogenase assay as published (Neidel et al., 1998). On day 7, glycosaminoglycan (GAG) content of proteoglycan/aggrecan fragments in culture supernatants was determined using the di-
methylmethylene blue assay according to the procedure of Farndale et al. (1986). On day 21, hydroxyproline content (as a measure of collagen degradation) in the supernatants was quantitated using Ehrlich’s reagent as previously described (Bergman and Losley, 1963; Ellis et al., 1994).

**Immunoblots of ADAMTS-4 and -5 from Explant Cultures.** Culture media supernatants from explant cultures were analyzed by SDS-polyacrylamide gel electrophoresis on 10% polyacrylamide gels under reducing conditions. The proteins were transferred to polyvinylidene difluoride membranes and incubated overnight at 4°C with 1:250 dilution of either anti-ADAMTS-4 or anti-ADAMTS-5 polyclonal antibodies (Triple Point Biologics, Inc., Forest Grove, OR). Subsequently, the membranes were incubated with a 1:10,000 dilution of goat/anti-rabbit IgG alkaline phosphatase conjugate as the secondary antibody. Immunoblots were developed using Western Breeze detection kits (Invitrogen) according to the manufacturer’s instructions.

**Statistical Analysis.** Statistical significance was determined by Dunnett’s one-way analysis of variance using the GraphPad Prism 4 software package from GraphPad Software Inc. (San Diego, CA).

**Results**

**Effect of BMS-345541 on IL-1-Induced IκBα Phosphorylation and Gene Expression in SW-1353 Chondrocytes.** Consistent with the role of IL-1 in inducing IKK activation, stimulation of SW-1353 chondrocytes with IL-1 caused a time-dependent increase in the phosphorylation of IκBα, with the maximal response observed 5 to 10 min after stimulation (results not shown). As shown in Fig. 1, the IKK inhibitor BMS-345541 inhibited the IL-1-stimulated phosphorylation of IκBα in SW-1353 chondrocytes in a concentration-dependent manner. These results with IL-1-stimulated chondrocytes mirror the potency previously reported for the compound against TNF-α-stimulated IκBα phosphorylation in monocyte THP-1 cells (Burke et al., 2003).

In SW-1353 chondrocytes stimulated for 8 h with IL-1, mRNA levels of the NF-κB-dependent genes for cyclooxygenase-2 (COX-2) and iNOS were both stimulated approximately 100-fold (Table 1). As expected, the presence of BMS-345541 inhibited mRNA levels for both COX-2 and iNOS in a concentration-dependent manner, with little to no effect at 0.1 μM inhibitor, 80 to 90% inhibition at 1 μM, and complete inhibition of the inducible mRNA levels at 10 μM. IL-1 also induced large elevations in mRNA levels for the collagenases MMP-1, MMP-3, and MMP-13. As with COX-2 and iNOS, BMS-345541 showed similar concentration-dependent inhibition of the inducible expression of these collagenases.

In contrast, the mRNA levels of aggrecanases ADAMTS-4 and ADAMTS-5 were neither induced by IL-1 stimulation nor affected by treatment with BMS-345541. Expression of the MMP inhibitor TIMP-1 was not significantly elevated on IL-1 stimulation of the cells, but the levels were affected somewhat by BMS-345541, whereas TIMP-3 mRNA levels were not affected by either IL-1 or BMS-345541. To measure only primary effects of BMS-345541 on IL-1β-induced mRNA levels in chondrosarcoma cells, time points longer than 8 h were not evaluated.

**Bovine Cartilage Explant Assay.** Cartilage explants show a progressive degradation of both collagen and aggrecan if cultured in the presence of IL-1. Aggrecan degradation, as measured by the amount of GAG fragments released into the supernatant, was maximal after 7 days of culture in the presence of IL-1 in this ex vivo assay (results not shown; see also Ellis et al., 1994; Kozaci et al., 1997). As shown in Fig. 2, the presence of BMS-345541 during the first 7 days of culture inhibited IL-1-induced GAG release in a concentration-dependent manner, with 10 μM BMS-345541 showing 75% inhibition. The inhibition was not the result of cytotoxicity because the compound failed to elevate lactate dehydrogenase levels in these IL-1-treated cultures (results not shown).

IL-1 treatment also led to collagen degradation as measured by hydroxyproline content in the culture supernatants, although 21 days of culture are needed to observe robust degradation (results not shown; see Ellis et al., 1994; Kozaci et al., 1997). As shown in Fig. 3, BMS-345541 was also effective in inhibiting IL-1-dependent collagen degradation measured after 21 days, with 5 and 10 μM BMS-345541 showing 67 and 95% inhibition, respectively. No measurable hydroxyproline content was evident in control explants not treated with IL-1.

Because the aggrecanases ADAMTS-4 and ADAMTS-5 are thought to play an important role in aggrecan degradation, the levels of these aggrecanases in IL-1-stimulated bovine cartilage explant samples were examined by immunoblotting. As shown in Fig. 4, the p64 form of ADAMTS-4 was detected at all the time points (3, 7, and 10 days), but IL-1 treatment did not appear to elevate these levels, consistent with the lack of induction of ADAMTS-4 mRNA in IL-1-treated SW-1353 chondrocytes. In contrast, the levels of the p73 form of ADAMTS-5 in immunoblots from the cartilage explants were shown to be greatly elevated on IL-1 treatment (Fig. 5). The p73 form represents the full-length ADAMTS-5 (Vankemmelbeke et al., 2001). Interestingly, the levels of
ADAMTS-5 were maximal after 7 days of culture with IL-1, which is coincident with aggrecan degradation. When treated for 7 days with BMS-345541, the compound at 10 μM nearly completely inhibited ADAMTS-5 production in these explants (Fig. 6), which correlates with the effects of the compound on aggrecan degradation. BMS-345541 failed to affect ADAMTS-4 levels in the same samples (results not shown). Although the explants were of essentially identical size, variability in the levels of ADAMTS-5 was observed and likely reflects handling differences between samples of this living tissue. Even with the variability, however, it is clear that no detectable ADAMTS-5 is evident until 7 days of treatment with IL-1 and that only treatment with 10 μM BMS-345541 gives a clear reduction in ADAMTS-5 levels.

**Discussion**

Arthritic diseases such as RA and OA are associated with pronounced degradation of both aggrecan and type II collagen, the major macromolecular components of articular cartilage. In these arthritic diseases, loss of aggrecan precedes collagen degradation and mechanical failure of the cartilage.
Similar processes occur in cartilage explant cultures stimulated to undergo matrix degradation with cytokines such as IL-1, with the aggrecan degraded early (during the first week of culture), whereas the collagen is not rapidly degraded until later in the culture period when essentially all the aggrecan has been lost (Ellis et al., 1994; Kozaci et al., 1997).

Metalloproteinases such as MMP-1 and MMP-13 have major roles in the degradation of collagen (Poole et al., 2003), whereas the aggrecanases ADAMTS-4 and ADAMTS-5 appear to mediate the aggrecan degradation (Arner, 2002). The transcription of MMP-1, MMP-3, and MMP-13 is known to be dependent on NF-κB (Bondonson et al., 1999; Vincenti and Brinckerhoff, 2002; Andreakos et al., 2003), so it is not surprising that the IKK inhibitor BMS-345541 inhibited the transcription of these metalloproteinases in IL-1-stimulated SW-1353 chondrocytes in a concentration-dependent manner.

Consistent with the effects on collagenase transcription in chondrocytes, BMS-345541 inhibited collagen degradation, as measured by hydroxyproline content in the media, in bovine cartilage explants stimulated with IL-1 for 21 days. Interestingly, BMS-345541 also protected against the degradation of aggrecan, as measured by GAG content in the media at 7 days, which precedes the collagen degradation. This was somewhat surprising because the transcription of ADAMTS-4 and ADAMTS-5 was neither induced nor appreciably affected by BMS-345541 in IL-1-stimulated chondrosarcoma cells. However, this may reflect differences in species (SW-1353 cells are human versus bovine cartilage) and IL-1 (IL-1β versus IL-1α) between these two experiments. ADAMTS-5 levels in the media of cartilage explants stimulated with IL-1 were greatly elevated after 7 days of culture, coincident with cartilage degradation, whereas ADAMTS-4 levels in the media were not elevated. This may suggest that ADAMTS-5 plays a more dominant role over ADAMTS-4 in catalyzing the degradation of aggrecan, at least in these cartilage explants. Indeed, treatment with the IKK inhibitor inhibited degradation of both aggrecan and ADAMTS-5 levels in the media after 7 days of culture without affecting ADAMTS-4 levels. Consistent with the idea that ADAMTS-5 plays a more important role than ADAMTS-4 in proteoglycan degradation, a recent report showed cartilage explants from mice deficient in functional ADAMTS-5 were protected from IL-1-induced proteoglycan degradation, whereas explants from ADAMTS-4-deficient mice were not protected (Stanton et al., 2005).

The effect of IKK inhibition on ADAMTS-5 expression and aggrecan degradation may be indirect. IL-1 did not induce and BMS-345541 did not inhibit the baseline expression of ADAMTS-5 in SW-1353 chondrocytes after 8 h of IL-1 treatment, and longer time points were not assessed. BMS-345541 may inhibit the expression of an NF-κB-dependent cytokine or growth factor normally induced during the first few days in IL-1-treated cartilage explants. This unidentified cytokine or growth factor presumably then drives the expression of ADAMTS-5 and the resulting aggrecan degradation after 7 days in culture. This would explain why it takes as long as 7...
days for ADAMTS-5 levels in the media to become elevated in cartilage explants treated with IL-1.

However, differences in species between the chondrocyte and cartilage explant studies, as well as the form of IL-1 used to stimulate, do not make for a perfect comparison. The human chondrosarcoma line was chosen because others have used it to investigate signal transduction mechanisms of IL-1-induced MMP production and NF-κB activation (Cowell et al., 1998; Mengshol et al., 2000; Vincenti and Brinckerhoff, 2001; Liacini et al., 2003; Tardif et al., 2003). When looking at the effect of BMS-345541 on IL-1-stimulated gene expression in chondrosarcoma cells, IL-1β was used as the stimulus because preliminary studies showed IL-1β gave a much more robust (5- to 10-fold) production of MMP-1 and MMP-13 in these cells as compared with IL-1α (results not shown). However, bovine cartilage explants require IL-1α rather than IL-1β to stimulate cartilage degradation. Even with these differences in assay conditions, the effects of BMS-345541 on MMP production in human cells suggest IKK inhibition would be an effective therapy in human arthritic diseases as well. It should be noted that the culture media (including IL-1 and BMS-345541) was replaced every 3 to 4 days in these cartilage explant experiments because in human arthritic disorders, exposure to proinflammatory IL-1 is likely to be of near constant duration rather than of a single, fleeting nature.

We have previously shown that IKK inhibition with BMS-345541 is highly efficacious against the murine collagen-induced arthritis (McIntyre et al., 2003). Although the activity against joint destruction in this collagen-induced arthritis may result largely from the ability of the compound to block cytokine production in vivo (Burke et al., 2003), the present results show the compound to have direct effects on cartilage degradation through suppression of MMP production. That IKK inhibition blocks both aggrecan and collagen degradation is especially important because not only does IKK inhibition block the expression of the major collagenases MMP-1 and MMP-13 in chondrocytes but also blocking the preceding aggrecan production prevents these collagenases from having access to the collagen matrix (Pratta et al., 2003), thereby inhibiting the action of any collagenases whose expression was not blocked by the IKK inhibitor. Because this agent inhibits the IKK-1 subunit nearly as potently as it inhibits IKK-2, the contribution of each subunit to cartilage degradation cannot be determined.

An additional benefit of IKK inhibitors is that whereas MMP-1 and MMP-13 production is NF-κB-dependent, the production of the TIMP-1 (an endogenous inhibitor of MMP) and TIMP-3 (an important inhibitor of ADAMTS-4 and ADAMTS-5; see Kashiwagi et al., 2001; Gendron et al., 2003) in SW-1353 chondrocytes appears to be largely NF-κB-independent. A similar lack of NF-κB-dependent regulation of TIMP expression has been reported in rheumatoid synovial cells (Feldmann et al., 2002). This is especially important because there is evidence to suggest that the imbalance of the MMP/TIMP ratio is a key factor in the progressive cartilage destruction in arthritic patients (Kyeszer et al., 1999; Yoshihara et al., 2000; Cunnane et al., 2001).

In conclusion, pharmacological agents targeting IKK are expected to show efficacy in arthritic diseases through a number of mechanisms. For RA, this includes the inhibition of inflammatory cytokines such as TNF-α and IL-1β as previously shown, but the present results show that inhibition of metalloproteinase expression by an IKK inhibitor will have a direct impact on the cartilage degradation, leading to joint destruction. Moreover, RANK ligand-induced osteoclastogenesis is critical in driving the bone resorption in arthritis, and this process is also IKK-dependent (Dai et al., 2004; Ruocco et al., 2005). The expression of iNOS is also NF-κB/IKK-dependent, and inhibiting iNOS activity has been shown to be beneficial in animal models of inflammatory arthritis (Cuzzocrea et al., 2002).

Although the results presented here using this cartilage explant model are certainly relevant to RA, they are perhaps of even greater relevance to OA, which is less of an inflammation-driven disease, but instead appears to be driven by IL-1-dependent cartilage destruction (Chevalier, 1997). Thus, an IKK inhibitor may be quite efficacious in the treatment of OA, again by inhibiting both IL-1 expression and the resulting production of metalloproteinases.

Because IKK knockouts are embryonically lethal, there are concerns about possible mechanism-based toxicities of a systemic inhibitor of IKK. However, available evidence suggests these effects may be largely developmental (for a review, see Burke, 2003). Because of the broad-based potential applications in inflammatory and autoimmune disorders, the discovery and development of IKK inhibitors continue to receive intense interest at a number of pharmaceutical companies (for a review, see Karin et al., 2004), but only with large-scale clinical trials will the utility of IKK inhibitors in arthritis become fully evident.

Acknowledgments

We thank Dr. Yuping Qiu (Bristol-Myers Squibb) for providing the BMS-345541 used in these studies and Kathleen Gillooly for help in statistical analysis.

References


Pattoli et al.