Upregulation of Urokinase-type Plasminogen Activator and Inhibitor and Gelatinase Expression via 3 Mitogen-Activated Protein Kinases and PI3K Pathways During the Early Development of Osteoarthritis

YIH-SHOU HSIEH, SHUN-FA YANG, KO-HUANG LUE, SHU-CHEN CHU, TZUNG-JE LI, and KO-HSIU LU

ABSTRACT. ***Objective.*** To examine whether upregulation of urokinase-type plasminogen activator (u-PA), PA inhibitor-1 (PAI-1), and gelatinases [matrix metalloproteinase (MMP)-2 and MMP-9] in early knee osteoarthritis (OA) of humans occurs through 3 major mitogen-activated protein kinases (MAPK): extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 kinase signaling pathways, and the phosphatidylinositol 3-kinase (PI3K) signaling pathway.

Methods. Enzyme linked immunosorbent assay and gelatin zymography were used to investigate the effects of ERK 1/2 inhibitor U0126, JNK and p38 inhibitor SB203580, and PI3K inhibitor LY294002 on the secretion of u-PA, PAI-1, MMP-2, and MMP-9 in early osteoarthritic tissue cultures, with or without interleukin 1α (IL-1α) and lipopolysaccharide (LPS) induction.

Results. Our findings were: (1) latent and active forms of MMP-9 secretion in synovial and some meniscal cultures were inhibited significantly by U0126, SB203580, and LY294002; (2) latent and active forms of MMP-2 secretion were also inhibited significantly by U0126 and LY294002, but not by SB203580, except for active MMP-2 in synovial cultures; (3) a similar observation was seen in IL-1α- and LPS-treated cultures; and (4) U0126, SB203580, and LY294002 significantly decreased u-PA and PAI-1 levels in all cultures in the presence or absence of IL-1α and LPS.

Conclusion. MAPK ERK, JNK, and p38 signaling pathways and the PI3K signaling pathway are involved in upregulation of u-PA, PAI-1, and gelatinase expression during early development of knee OA. Thus, blocking PA/plasmin and gelatinase expression by novel physiologic and pharmacological inhibitors could be an important therapeutic or preventive approach for early OA. (First Release Feb 1 2007; J Rheumatol 2007;34:785–93)

Key Indexing Terms:
PLASMINOGEN ACTIVATOR INHIBITOR
OSTEOARTHRITIS
MATRIX METALLOPROTEINASE
SIGNALING PATHWAY

In osteoarthritis (OA), alterations of chondrocyte metabolism by inappropriate mechanical loading and/or with increased levels of soluble mediators may result in extracellular matrix (ECM) degradation and remodeling and contribute to the progression of degenerative changes. These changes in cartilage are mediated by the ECM-degrading enzymes including serine proteases, matrix metalloproteinases (MMP), and cathepsins (cysteine proteinases)[1]. MMP are probably created in response to the stimulation of cytokines such as interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α)[2-4]. Although collagenase 3 (MMP-13) is thought to be mainly responsible for cartilage collagen degradation[5-7], MMP-9 is also likely to be involved in degradation of joint collagen[8,9].

Strong circumstantial evidence shows that MMP-2 participates in the turnover of normal cartilage matrix, whereas MMP-9 and some MMP-2 facilitate the progressive destruction of the cartilage matrix in OA[10]. In addition, increased MMP-9 levels have been seen in effusion samples from patients with inflammatory arthritis, such as rheumatoid arthritis (RA)[11,12] and gouty arthritis[13]. Also, more gelatinases appear in effusions of septic arthritis than in aseptic arthritis[14]. In an acute attack of gouty arthritis, there is a correlation between the plasminogen activator (PA)/plasmin system and MMP-9[15]. Higher PA/plasmin activity appears in effusions of septic arthritis than in aseptic arthritis[16].

Tissue cultures more closely approximate *in vivo* develop-

From the Institute of Biochemistry and Biotechnology, Institute of Medicine, Chung Shan Medical University; Department of Orthopaedic Surgery, Chung Shan Medical University Hospital; and Department of Food Science, Central Taiwan University of Science and Technology, Taichung, Taiwan.

Supported by a grant from the Research Section of Chung Shan Medical University (CSMU94-OM-B-019) and National Science Council, Taiwan (NSC93-2314-B-040-003).

Y-S. Hsieh, PhD, Institute of Biochemistry and Biotechnology, Chung Shan Medical University; S-F. Yang, PhD; K-H. Lue, MD, Institute of Medicine, Chung Shan Medical University; S-C. Chu, PhD, Department of Food Science, Central Taiwan University of Science and Technology; T-J. Li, MD, Department of Orthopaedic Surgery, Chung Shan Medical University Hospital; K-H. Lu, MD, PhD, Department of Orthopaedic Surgery, Chung Shan Medical University Hospital, Institute of Medicine, Chung Shan Medical University.

Address reprint requests to Dr. K-H. Lu, 110, Section 1, Chien-Kuo N. Road, Taichung 402, Taiwan. E-mail: cshy307@csh.org.tw

Accepted for publication November 1, 2006.

ment. We have previously demonstrated that proinflammatory cytokines (IL-1α and TNF-α) and endotoxin lipopolysaccharide (LPS) upregulate MMP-2 and MMP-9 expression, and the regulation of MMP-2 and MMP-9 is mediated via a protein kinase C signaling pathway in human osteoarthritic knees[2]. However, the involvement of other pathways such as 3 major mitogen-activated protein kinases (MAPK) signaling pathways remains unclear, and the mechanisms through which IL-1α and LPS increase MMP-2 and MMP-9 expression have not been fully deciphered. To obtain further information on signal transduction pathways of urokinase-type PA (u-PA), PA inhibitor-1 (PAI-1), MMP-2, and MMP-9, we tested the hypothesis that upregulation of u-PA, PAI-1, MMP-2, and MMP-9 during early development of the human osteoarthritic knee occurs through 3 major MAPK extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), p38 pathways, and the phosphatidylinositol 3-kinase (PI3K) pathway.

MATERIALS AND METHODS

Chemicals and reagents. IL-1α and LPS were purchased from Sigma Chemical (St. Louis, MO, USA). U0126 (ERK 1/2 inhibitor), SB203580 (JNK and p38 inhibitor, which at 10 to 25 μM concentrations can inhibit both p38 and JNK pathways[17,18]), and LY294002 (PI3K inhibitor) were purchased from Promega Corp. (Madison, WI, USA). All culture materials were obtained from Gibco (Grand Island, NY, USA). According to *in vitro* studies of other laboratories[17-20], the final concentrations used in this study were U0126, 10 μM; SB203580, 25 μM; LY294002, 10 μM; IL-1α, 10 ng/ml; and LPS, 1 μg/ml.

Sampling and chondral, meniscal, and synovial cultures. Diseased cartilage, torn menisci, and hypertrophic synovia were obtained from patients with primary knee OA undergoing arthroscopic debridement at our hospital by the same author[10]. All patients fulfilled the American College of Rheumatology criteria for knee OA[21] and gave informed consent for their surgical specimens to be studied. This study was conducted in accord with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the Chung Shan Medical University Hospital of Taichung, Taiwan. The knees all showed grade II or III OA in anteroposterior weight-bearing and lateral radiographs, according to the Kellgren and Lawrence grading scale for the medial, lateral, and patellofemoral compartments[22]. The specimens were divided and weighted equally 50 mg, transferred into 24-well plates, respectively, and then incubated[23]. The remainders of the specimen were subjected to pathological examination to confirm the diagnosis.

Cytokine, endotoxin, and pharmacological agent treatment. The chondral, meniscal, and synovial tissues were cultured for 3 h, and then the medium was changed to a medium containing pharmacological agents (U0126, SB203580, and LY294002) for 30 min pretreatment. Without changing the medium, the cultured media were collected at 24 and 48 h after treatment with or without appropriate concentrations of IL-1α and LPS, and then subjected to gelatin zymography and ELISA for the measurement of u-PA and PAI-1 antigens[2].

Gelatin zymography. Gelatinolytic activity was assayed by gelatin zymography[24,25]. Each conditioned medium containing 10 μg of total protein was loaded onto a precast sodium dodecyl sulfate-polyacrylamide gel containing 0.1% gelatin. After electrophoresis, gels were processed[10,12]. Nonstaining bands representing the activities of MMP-2 and MMP-9 were quantitatively measured by spot density measurement using a digital imaging analysis system (Alpha Innotech, Mt. Prospect, IL, USA). Then the levels of MMP-2 and MMP-9 from treated groups were expressed as optical density (percentage of control) in comparison with the corresponding control group.

Measurement of u-PA and PAI-1 levels. u-PA and PAI-1 levels in conditioned media were measured by u-PA and PAI-1 ELISA kits from Biopool, Umea, Sweden. From each conditioned sample, 200 μl of the sample were directly transferred to the microtest strip wells of the ELISA plate. All further procedures were performed following the manufacturer's instructions. The absorbance at 495 nm was measured in a microtest plate spectrophotometer and u-PA and PAI-1 levels were quantitated with a calibration curve using human u-PA and PAI-1 as a standard[15,16].

Measurement of lactate dehydrogenase activity. As an indicator of cell viability, cytoplasmic enzyme lactate dehydrogenase (LDH) was measured in the culture medium. For the cell viability experiment, an optimized LDH test (Promega) was used to quantify LDH activity in the conditioned medium of chondral, meniscal, and synovial cultures after treatment with or without various inhibitors (U0126, SB203580, and LY294002) and higher concentrations of SB203580 (50 μM) for 3, 24, 48, and 96 h. Like extraction of RNA from the tissue block in our previous study[10], each tissue of cartilage, menisci, and synovia was homogenized in 1 ml of phosphate-buffered saline to determine total LDH activity as positive control.

Statistical analysis. Statistical calculations of the levels of MMP-2 and MMP-9 and the activity of LDH between control and various inhibitor-treated groups were performed using analysis of variance (ANOVA). Student's t test was used for the analysis of data concerning MMP-2 and MMP-9 in IL-1α- and LPS-induced groups as well as u-PA and PAI-1 between control and treated groups. Statistical significance was set at $p < 0.05$.

RESULTS

In zymograms, the main gelatinase secreted in all chondral, meniscal, and synovial cultures migrated at 72 kDa and represented the latent form of MMP-2 (proMMP-2; Figure 1). In all synovial and some meniscal cultures, minor gelatinolytic bands were also observed at 92 kDa regions that correspond to proMMP-9. The activated forms of MMP-2 and MMP-9 showed a loss of the propeptide of roughly 10 kDa. As expected[2], the levels of MMP-2 and MMP-9 increased in IL-1α- and LPS-treated cultures.

Effect of various inhibitors on MMP-2 and MMP-9 levels. Generally, both ERK 1/2 inhibitor U0126 and PI3K inhibitor LY294002 significantly suppressed the levels of latent and activated forms of MMP-2 in all tissue cultures at 24 and 48 h ($p < 0.05$; Tables 1-3). However, these suppressive effects could not be found in JNK and p38 inhibitor SB203580-treated cultures ($p = 0.068$), except for the activated form of MMP-2 levels in synovial cultures at 48 h ($p < 0.05$). In all groups treated with ERK 1/2 inhibitor U0126, JNK and p38 inhibitor SB203580, and PI3K inhibitor LY294002, proMMP-9 levels significantly decreased at 24 and 48 h ($p < 0.05$). The activated form of MMP-9 only appeared in synovial cultures and also significantly repressed its levels in all U0126-, SB203580-, and LY294002-treated groups at 24 and 48 h ($p < 0.05$).

Effect of various inhibitors on IL-1α- and LPS-induced MMP-2 and MMP-9 levels. The increases in IL-1α-induced latent and activated forms of MMP-2 levels were abrogated significantly in ERK 1/2 inhibitor U0126- and PI3K inhibitor LY294002-treated cultures ($p < 0.05$), but these suppressive effects were not observed in JNK and p38 inhibitor SB203580-treated cultures except for the levels of proMMP-2 at 48 h ($p < 0.01$) and activated MMP-2 at 24 ($p < 0.05$) and

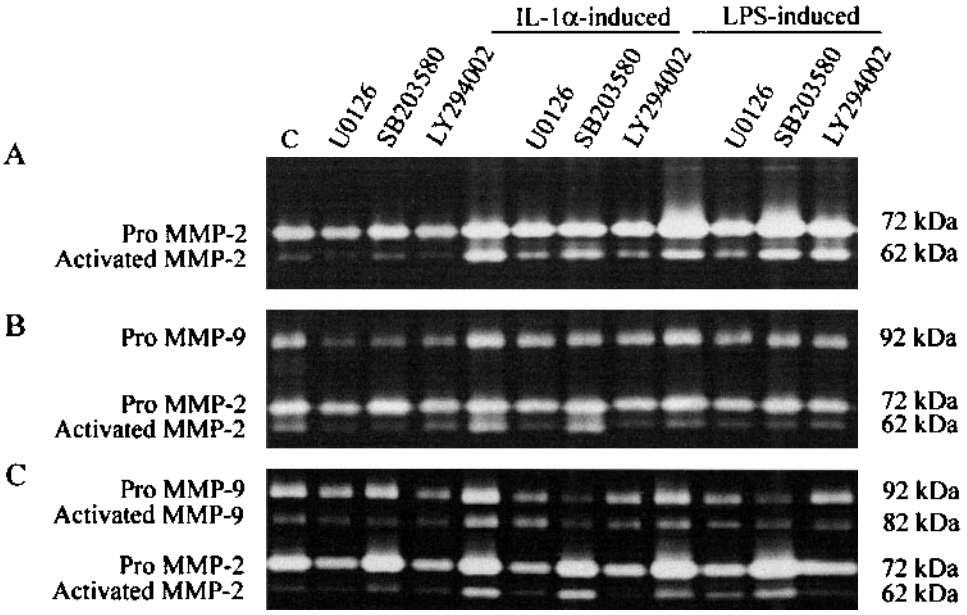

Figure 1. Gelatinolytic activity in osteoarthritic (A) chondral, (B) meniscal, and (C) synovial cultures co-treated with or without various inhibitors for 24 h were assessed by gelatin zymography.

48 h ($p < 0.05$) in synovial cultures (Figure 2). However, all inhibitors U0126, SB203580, and LY294002 significantly cancelled out the increases in IL-1α-induced MMP-9 levels ($p < 0.05$). Similarly, the responses to various inhibitors U0126, SB203580, and LY294002 were observed in cultures with LPS induction ($p < 0.05$), except that SB203580 was found to reduce activated MMP-2 levels at 48 h ($p < 0.01$), but not the levels of proMMP-2 at 24 and 48 h and activated MMP-2 at 24 h in synovial cultures (Figure 3).

Effect of IL-1α, LPS, and various inhibitors on u-PA and PAI-1 levels. As illustrated in Figure 4, u-PA levels varied among individual conditioned media at 48 h and were significantly induced by IL-1α and LPS ($p < 0.05$), while the levels were significantly reduced by ERK 1/2 inhibitor U0126, JNK and p38 inhibitor SB203580, and PI3K inhibitor LY294002 ($p < 0.05$). All inhibitors U0126, SB203580, and LY294002 significantly abrogated the increases in u-PA levels in response to IL-1α and LPS at 48 h ($p < 0.05$). As u-PA did in the presence or absence of IL-1α and LPS at 48 h, PAI-1 levels showed similar changes, which increased significantly in IL-1α- and LPS-treated cultures and decreased significantly in U0126-, SB203580- and LY294002-treated cultures ($p < 0.05$; Figure 5).

Effect of various inhibitors on the cell viability of chondral, meniscal, and synovial cultures. We examined whether the 3 inhibitors affected cell viability in chondral, meniscal, and synovial cultures. We were unable to detect any significant increase of LDH activity in the conditioned medium of tissue cultures treated with various inhibitors, including higher concentrations of SB203580 (50 μM). Therefore, this indicates that neither the 3 inhibitors in this study nor higher concentrations of SB203580 (50 μM) have cytotoxic effects on chondral, meniscal, and synovial tissues during 3, 24, 48, or 96 h of culture (data not shown).

DISCUSSION

OA is classically defined as a progressively degenerative disease rather than an inflammatory disease, but the key role of inflammation in OA has been pointed out recently[26-28]. Synovial fibroblasts have been implicated in tissue destruction of inflammatory synovitis and modulating the local cellular and cytokine microenvironment then conditioning the inflammatory infiltrate[29,30]. Moreover, proinflammatory cytokines mediate autocrine, paracrine, and endocrine effects on cells of the synovial membrane, then activate different signal transduction pathways at different sites of the synovial membrane[31]. Although, somewhat unexpectedly, JNK and p38 inhibitor SB203580 could not decrease the levels of latent and activated forms of MMP-2 in chondral and meniscal cultures and proMMP-2 in synovial cultures, SB203580 did suppress activated MMP-2 levels in synovial cultures at 48 h (Figure 6). Intriguingly, IL-1α- and LPS-induced groups also showed similar changes implicating JNK and p38 pathways in MMP-2 secretion in synovial cultures. However, the differences of signaling pathways in MMP-2 production and activation between chondral, meniscal, and synovial tissues deserves to be pursued further.

Previous research demonstrated that both gelatinases exhibit a broad substrate specificity towards denatured collagens and other ECM macromolecules, and thereby contribute to the degradation of collagen fibrils, basement membranes, and other suprastructures of the ECM[8,32]. Indeed, articular chondrocytes that fail to produce MMP-9[10,33] are not innocent

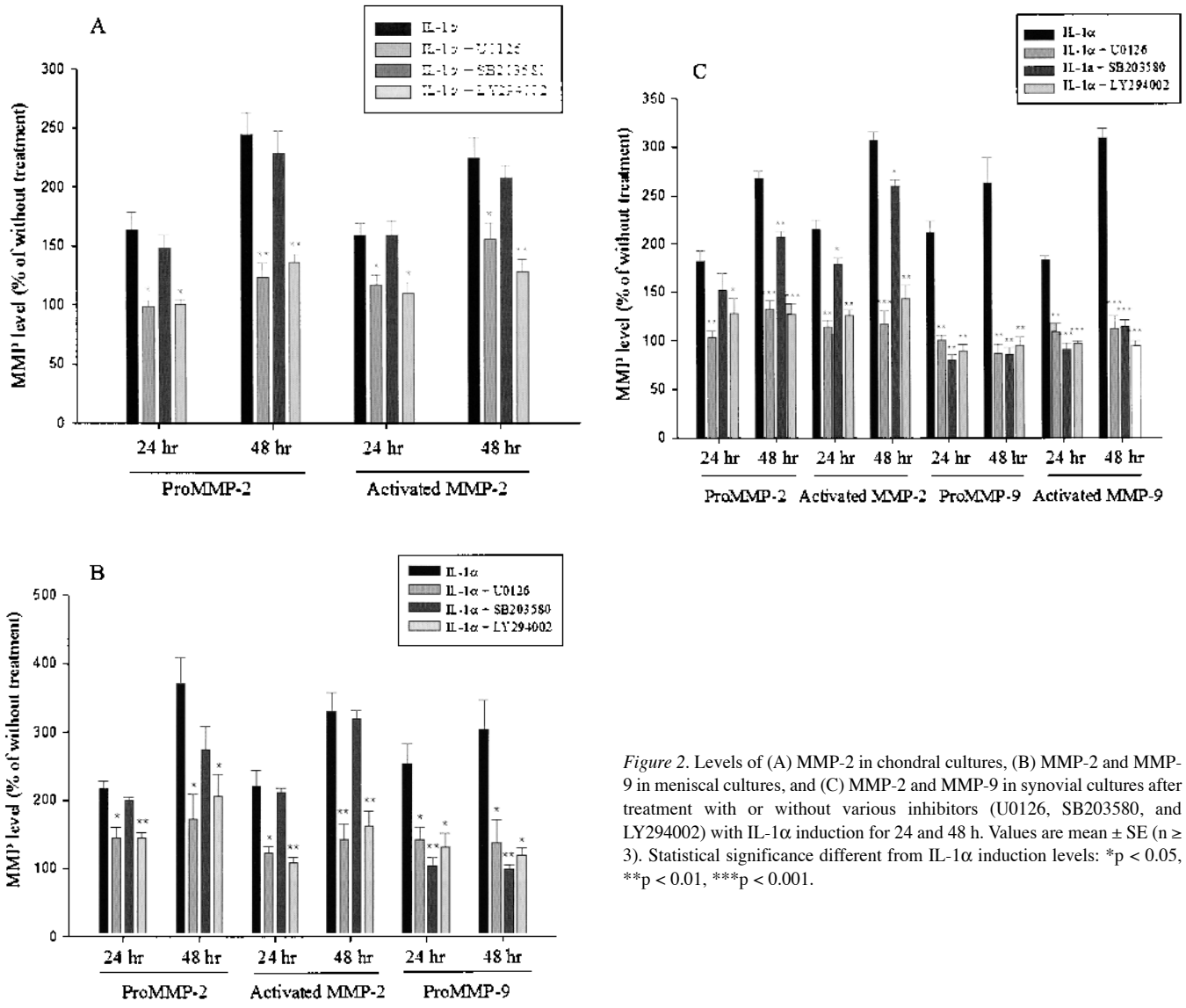

Figure 2. Levels of (A) MMP-2 in chondral cultures, (B) MMP-2 and MMP-9 in meniscal cultures, and (C) MMP-2 and MMP-9 in synovial cultures after treatment with or without various inhibitors (U0126, SB203580, and LY294002) with IL-1α induction for 24 and 48 h. Values are mean ± SE ($n \geq 3$). Statistical significance different from IL-1α induction levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

bystanders in OA[34]. They not only produce destructive enzymes guided by environmental cues but also instruct inflammatory cells or cells from surrounding tissues to trigger 2 alternative activation pathways, which mainly involve MMP-9, MMP-13, and MMP-14 as well as, marginally, serine or cysteine proteinases. Consistent with previous studies[10], we show here that u-PA, PAI-1, MMP-2, and MMP-9 are likely to play a part in the degradation of the collagenous network of early osteoarthritic tissues.

MAPK are a unique family of serine/threonine kinases that are activated via reversible phosphorylation and mediate signal transduction for multiple extracellular stimuli, then regulate a number of transcription factors, with subsequent activation of MMP and cytokine gene expression. The specific IL-1- and TNF-induced MMP expression requires unique combinations of cell type-specific signaling pathways such as ERK, JNK, and p38[31,35-37]. Protein kinases that regulate signal transduction pathways induced by IL-1 and TNF-α have been proposed as therapeutic targets[38]. Indeed, the mechanisms of signal transduction pathways involved in the PA/plasmin system and gelatinase expression in human osteoarthritic tissues and the role of each pathway in IL-1- and LPS-mediated induction of u-PA, PAI-1, MMP-2, and MMP-9 are still not completely understood. However, our observations showed that 3 MAPK pathways are involved in the inhibition of u-PA, PAI-1, MMP-2, and MMP-9 expression in early osteoarthritic chondral, meniscal, and synovial tissues, and this also happens with IL-1α and LPS induction.

Recently, the PI3K signal transduction pathway has emerged as one of the main signal routes that coordinate complex events leading to changes in cell metabolism, cell growth, cell movement, and cell survival[39]. The signaling pathway in gouty arthritis and RA has been well documented[19,20,40,41], whereas no data have been available on how it

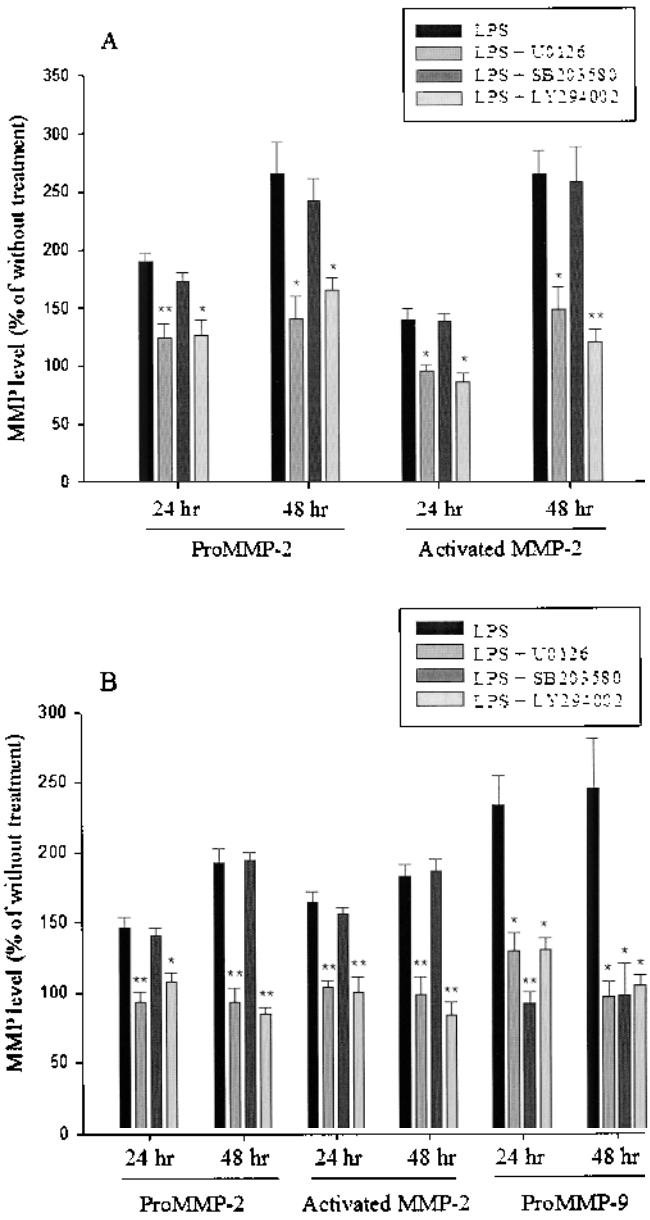

Figure 3. Levels of (A) MMP-2 in chondral cultures, (B) MMP-2 and MMP-9 in meniscal cultures, and (C) MMP-2 and MMP-9 in synovial cultures after treatment with or without various inhibitors (U0126, SB203580, and LY294002) with LPS induction for 24 and 48 hours. Values are mean ± SE ($n \geq 3$). Statistical significance different from LPS induction levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

can regulate MMP expression in OA. Here, the PI3K pathway inhibitor LY294002 not only suppressed u-PA, PAI-1, MMP-2, and MMP-9 secretion, but also decreased IL-1- and LPS-mediated induction of them, indicating that the signal transduction pathway dependent on PI3K is preferentially involved in upregulation of u-PA, PAI-1, MMP-2, and MMP-9 expression in early osteoarthritic tissues.

This short-term *ex vivo* model suggests upregulation of u-PA, PAI-1, and gelatinase expression in the early stage of human knee OA via 3 MAPK and PI3K signaling pathways may occur *in vivo*. Better understanding of IL-1α and LPS signaling and regulatory mechanisms during the early development of OA may lead to novel strategies for inhibiting the catabolic activities in cartilage. However, our study is an indirect method and only indicates that 3 MAPK signaling pathways and the PI3K signaling pathway are involved in upregulation of u-PA, PAI-1, and gelatinase expression during the early development of knee OA. It does not exclude the possibility that other signaling pathways may be involved in this process. Further research into the regulation of IL-1α/LPS — MAPK/PI3K — u-PA/PAI-1—gelatinase signaling pathways will elucidate the mechanisms underlying cartilage destruction in early OA, generating methods to control disease progression at the molecular level by inhibiting the enzymes or their gene expression responsible for cartilage degradation while enhancing tissue repair.

REFERENCES

1. Cunnane G, Hummel KM, Muller-Ladner U, Gay RE, Gay S. Mechanism of joint destruction in rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) 1998;46:1-7.

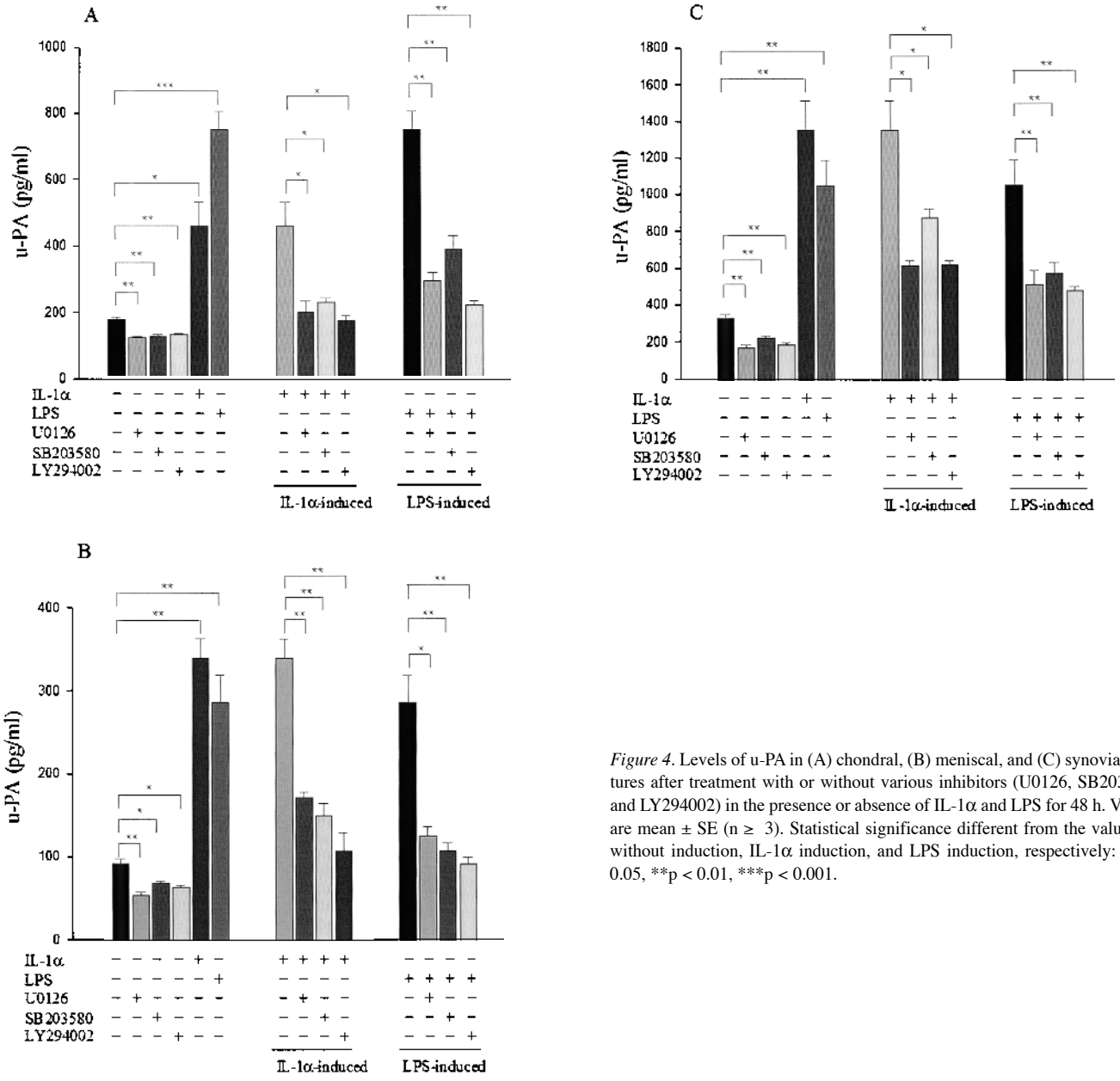

Figure 4. Levels of u-PA in (A) chondral, (B) meniscal, and (C) synovial cultures after treatment with or without various inhibitors (U0126, SB203580, and LY294002) in the presence or absence of IL-1α and LPS for 48 h. Values are mean ± SE (n ≥ 3). Statistical significance different from the values of without induction, IL-1α induction, and LPS induction, respectively: *p < 0.05, **p < 0.01, ***p < 0.001.

2. Chu SC, Yang SF, Lue KH, Hsieh YS, Wu CL, Lu KH. Regulation of gelatinases expression by cytokines, endotoxin, and pharmacological agents in the human osteoarthritic knee. Connect Tissue Res 2004;45:142-50.
3. Goldring MB. The role of the chondrocyte in osteoarthritis. Arthritis Rheum 2000;43:1916-26.
4. Poole AR, Howell DS. Etiopathogenesis of osteoarthritis. In: Moskowitz RW, Howell DS, Goldberg VM, Mankin HJ, editors. Osteoarthritis: diagnosis and management. 3rd ed. Philadelphia: WB Saunders Co.; 2001:29-47.
5. Billinghurst RC, Dahlberg L, Ionescu M, et al. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 1997;99:1534-45.
6. Dahlberg L, Billinghurst RC, Manner P, et al. Selective enhancement of collagenase-mediated cleavage of resident type II collagen in cultured osteoarthritic cartilage and arrest with a synthetic inhibitor that spares collagenase 1 (matrix metalloproteinase 1). Arthritis Rheum 2000;43:673-82.
7. Freemont AJ, Byers RJ, Taiwo YO, Hoyland JA. In situ zymographic localisation of type II collagen degrading activity in osteoarthritic human articular cartilage. Ann Rheum Dis 1999;58:357-65.
8. Murphy G, Knauper V, Atkinson S, et al. Matrix metalloproteinases in arthritic disease. Arthritis Res 2002;4 Suppl 3:S39-49.
9. Tchetverikov I, Ronday HK, Van El B, et al. MMP profile in paired serum and synovial fluid samples of patients with rheumatoid arthritis. Ann Rheum Dis 2004;63:881-3.
10. Hsieh YS, Yang SF, Chu SC, et al. Expression changes of gelatinases in human osteoarthritic knees and arthroscopic debridement. Arthroscopy 2004;20:482-8.
11. Koolwijk P, Miltenburg AM, van Erck MG, et al. Activated gelatinase-B (MMP-9) and urokinase-type plasminogen activator in synovial fluids of patients with arthritis. Correlation with clinical and experimental variables of inflammation. J Rheumatol

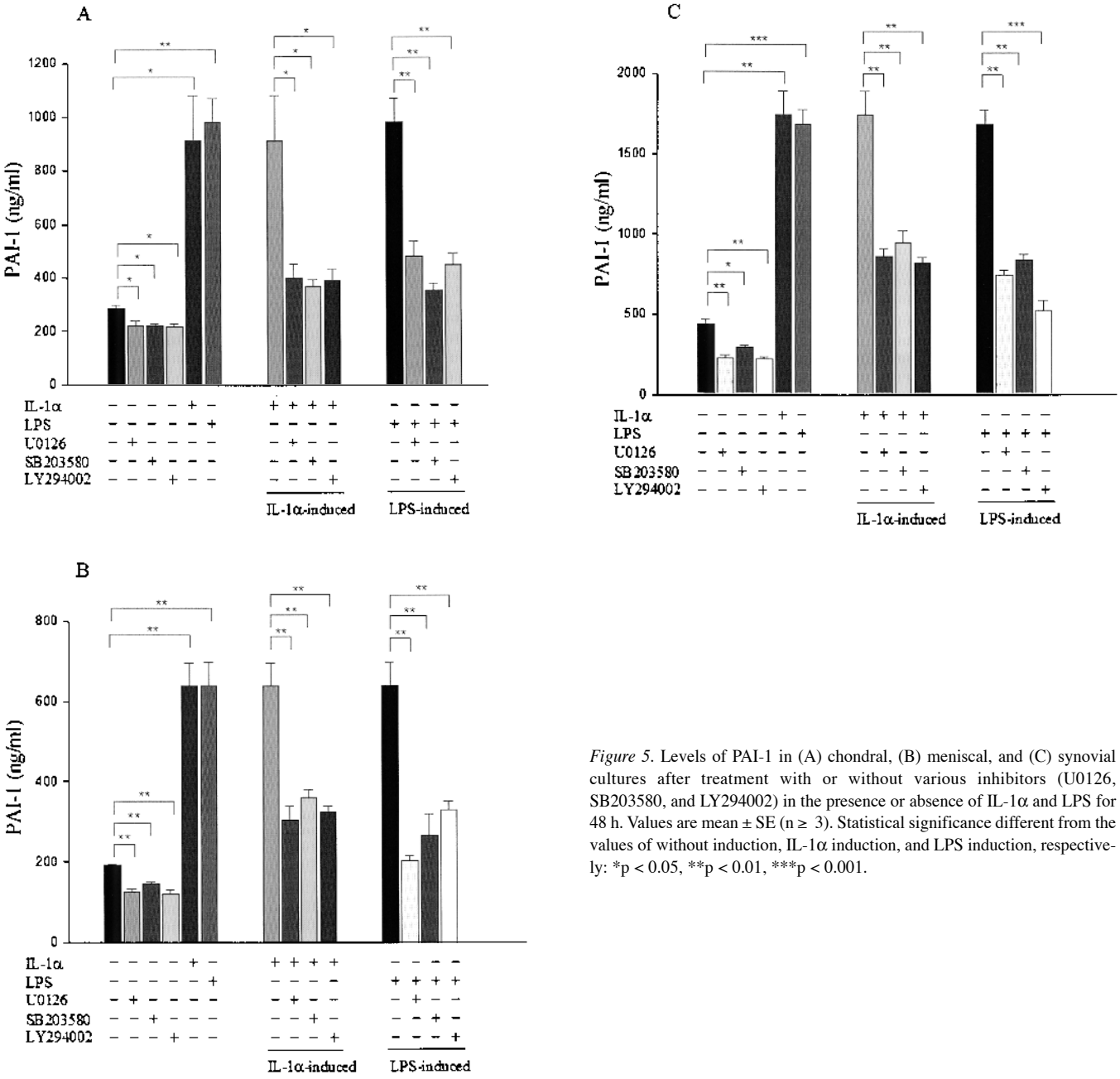

Figure 5. Levels of PAI-1 in (A) chondral, (B) meniscal, and (C) synovial cultures after treatment with or without various inhibitors (U0126, SB203580, and LY294002) in the presence or absence of IL-1α and LPS for 48 h. Values are mean ± SE ($n \geq 3$). Statistical significance different from the values of without induction, IL-1α induction, and LPS induction, respectively: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

1995;22:385-93.
12. Lu KH, Yang SF, Chu SC, et al. The significance of altered gelatinase expression in the synovium of patient with arthritic effusions. Clin Rheumatol 2004;23:21-6.
13. Chu SC, Yang SF, Lue KH, Hsieh YS, Hsiao TY, Lu KH. The clinical significance of gelatinase B in gouty arthritis of the knee. Clin Chim Acta 2004;339:77-83.
14. Chu SC, Yang SF, Lue KH, Hsieh YS, Lin ZI, Lu KH. Clinical significance of gelatinases in septic arthritis of native and replaced knees. Clin Orthop Relat Res 2004;427:179-83.
15. Chu SC, Yang SF, Lue KH, Hsieh YS, Hsiao TY, Lu KH. Urokinase-type plasminogen activator, receptor, and inhibitor correlating with gelatinase-B (MMP-9) contribute to inflammation in gouty arthritis of the knee. J Rheumatol 2006;33:311-7.
16. Hsieh YS, Yang SF, Lue KH, Lu KH. Clinical correlation with the PA/plasmin system in septic arthritis of the knee. Clin Orthop Relat Res 2006;447:172-8.
17. Han Z, Boyle DL, Aupperle KR, Bennett B, Manning AM, Firestein GS. Jun N-terminal kinase in rheumatoid arthritis. J Pharmacol Exp Ther 1999;291:124-30.
18. Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. Matrix Biol 2002;21:251-62.
19. Chen L, Hsieh MS, Ho HC, Liu YH, Chou DT, Tsai SH. Stimulation of inducible nitric oxide synthase by monosodium urate crystals in macrophages and expression of iNOS in gouty arthritis. Nitric Oxide 2004;11:228-36.
20. Kim KW, Cho ML, Park MK, et al. Increased interleukin-17

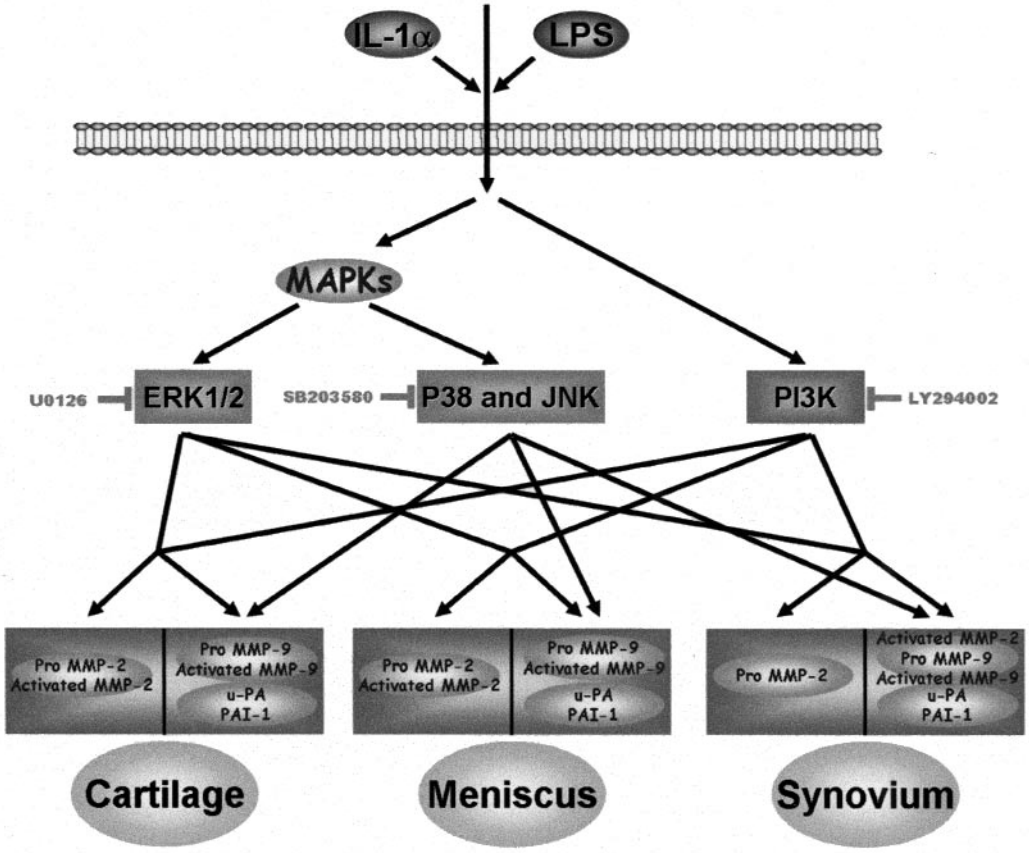

Figure 6. Three MAPK and PI3K signaling pathways involved in u-PA, PAI-1, MMP-2, and MMP-9 expression during the early development of OA. JNK and p38 inhibitor SB203580 could not decrease the levels of latent and activated forms of MMP-2 in chondral and meniscal cultures and proMMP-2 in synovial cultures, whereas SB203580 did suppress the level of activated MMP-2 in synovial cultures. IL-1α- and LPS-induced groups showed similar changes.

Table 1. MMP-2 levels in chondral cultures after treatment with or without various inhibitors for 24 and 48 hours. Values are mean ± SD; n ≥ 3.

	ProMMP-2 (% of control)	Activated MMP-2 (% of control)
U0126		
24 h	74.59 ± 5.02[a]	83.96 ± 5.51[a]
48 h	64.35 ± 8.51[a]	68.97 ± 5.04[a,b]
F value	31.057**	38.876***
SB203580		
24 h	94.09 ± 3.94	105.42 ± 11.05
48 h	99.89 ± 6.48	103.31 ± 10.49
F value	1.790	0.290
LY294002		
24 h	69.65 ± 3.85[a]	80.09 ± 4.57[a]
48 h	58.80 ± 4.26[a,b]	70.27 ± 4.89[a]
F value	124.522***	46.087***

ANOVA with Scheffe posteriori comparison was used. ** $p < 0.01$, *** $p < 0.001$. [a] Significantly different, $p < 0.05$, compared to control (control = 100%). [b] Significantly different, $p < 0.05$, compared to 24 hours.

Table 2. MMP-2 and MMP-9 levels in meniscal cultures after treatment with or without various inhibitors for 24 and 48 hours. Values are mean ± SD; n ≥ 3.

	ProMMP-2 (% of control)	Activated MMP-2 (% of control)	ProMMP-9 (% of control)
U0126			
24 h	84.74 ± 12.38	71.55 ± 13.13[a]	65.55 ± 10.41[a]
48 h	69.52 ± 10.06[a]	61.90 ± 7.55[a]	54.86 ± 18.10[a]
F value	8.218*	15.405**	11.489**
SB203580			
24 h	95.26 ± 6.05	104.84 ± 9.07	57.27 ± 6.69[a]
48 h	95.63 ± 21.04	97.99 ± 18.65	52.64 ± 9.26[a]
F value	0.131	0.259	46.975***
LY294002			
24 h	73.72 ± 11.76[a]	61.92 ± 10.53[a]	63.46 ± 16.26[a]
48 h	65.79 ± 6.95[a]	56.67 ± 4.30[a]	54.37 ± 17.63[a]
F value	15.469**	38.915***	9.127*

ANOVA with Scheffe posteriori comparison was used. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. [a] Significantly different, $p < 0.05$, compared to control (control = 100%).

Table 3. MMP-2 and MMP-9 levels in synovial cultures after treatment with or without various inhibitors for 24 and 48 hours. Values are mean ± SD; n ≥ 3.

	ProMMP-2 (% of control)	Activated MMP-2 (% of control)	ProMMP-9 (% of control)	Activated MMP-9 (% of control)
U0126				
24 h	84.77 ± 5.01[a]	81.77 ± 4.04[a]	61.02 ± 8.04[a]	83.97 ± 4.28[a]
48 h	77.90 ± 4.81[a]	74.17 ± 5.16[a]	50.92 ± 1.51[a]	76.64 ± 6.54[a]
F value	23.882**	36.936***	90.380***	21.017**
SB203580				
24 h	90.88 ± 5.05	93.89 ± 3.80	60.63 ± 1.83[a]	77.79 ± 4.66[a]
48 h	87.98 ± 7.47	88.69 ± 2.59[a]	66.92 ± 9.09[a]	76.25 ± 6.44[a]
F value	4.351	13.609**	46.860***	25.177**
LY294002				
24 h	81.48 ± 6.82[a]	71.66 ± 1.76[a]	68.14 ± 8.94[a]	79.64 ± 3.85[a]
48 h	81.96 ± 2.58[a]	68.75 ± 3.69[a]	63.54 ± 8.02[a]	74.27 ± 5.41[a]
F value	18.881**	160.822***	24.619**	37.564***

ANOVA with Scheffe posteriori comparison was used. ** $p < 0.01$, *** $p < 0.001$. [a] Significantly different, $p < 0.05$, compared to control (control = 100%).

production via a phosphoinositide 3-kinase/Akt and nuclear factor kappa B-dependent pathway in patients with rheumatoid arthritis. Arthritis Res Ther 2005;7:R139-48.

21. Altman RD. Criteria for classification of clinical osteoarthritis. J Rheumatol 1991;18 Suppl 27:10-2.
22. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494-502.
23. Chu SC, Yang SF, Lue KH, et al. Glucosamine sulphate suppresses the expressions of urokinase plasminogen activator and inhibitor and gelatinases during the early stage of osteoarthritis. Clin Chim Acta 2006;372:167-72.
24. Kleiner DE, Stetler-Stevenson WG. Quantitative zymography: detection of picogram quantities of gelatinases. Anal Biochem 1994;218:325-9.
25. Makowski GS, Ramsby ML. Calibrating gelatin zymograms with human gelatinase standards. Anal Biochem 1996;236:353-6.
26. Abramson SB, Attur M, Amin AR, Clancy R. Nitric oxide and inflammatory mediators in the perpetuation of osteoarthritis. Curr Rheumatol Rep 2001;3:535-41.
27. Moskowitz RW. The role of anti-inflammatory drugs in the treatment of osteoarthritis: a United States viewpoint. Clin Exp Rheumatol 2001;19 Suppl 25:S3-8.
28. Pincus T. Clinical evidence for osteoarthritis as an inflammatory disease. Curr Rheumatol Rep 2001;3:524-34.
29. Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. Trends Immunol 2001;22:199-204.
30. Pap T, Muller-Ladner U, Gay RE, Gay S. Fibroblast biology. Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis. Arthritis Res 2000;2:361-7.
31. Schett G, Tohidast-Akrad M, Smolen JS, et al. Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-JUN N-terminal kinase, and p38 mitogen-activated protein kinase, in synovial tissue and cells in rheumatoid arthritis. Arthritis Rheum 2000;43:2501-12.
32. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001;17:463-516.
33. Dreier R, Wallace S, Fuchs S, Bruckner P, Grassel S. Paracrine interactions of chondrocytes and macrophages in cartilage degradation: articular chondrocytes provide factors that activate macrophage-derived pro-gelatinase B (pro-MMP-9). J Cell Sci 2001;114:3813-22.
34. Dreier R, Grassel S, Fuchs S, Schaumburger J, Bruckner P. Pro-MMP-9 is a specific macrophage product and is activated by osteoarthritic chondrocytes via MMP-3 or a MT1-MMP/MMP-13 cascade. Exp Cell Res 2004;297:303-12.
35. Geng Y, Valbracht J, Lotz M. Selective activation of the mitogen-activated protein kinase subgroups c-Jun NH2 terminal kinase and p38 by IL-1 and TNF in human articular chondrocytes. J Clin Invest 1996;98:2425-30.
36. Mengshol JA, Vincenti MP, Coon CI, Barchowsky A, Brinckerhoff CE. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappa B: differential regulation of collagenase 1 and collagenase 3. Arthritis Rheum 2000; 43:801-11.
37. Thomas B, Thirion S, Humbert L, et al. Differentiation regulates interleukin-1 beta-induced cyclo-oxygenase-2 in human articular chondrocytes: role of p38 mitogen-activated protein kinase. Biochem J 2002;362:367-73.
38. Lewis AJ, Manning AM. New targets for anti-inflammatory drugs. Curr Opin Chem Biol 1999;3:489-94.
39. Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002;296:1655-7.
40. Liu-Bryan R, Pritzker K, Firestein GS, Terkeltaub R. TLR2 signaling in chondrocytes drives calcium pyrophosphate dihydrate and monosodium urate crystal-induced nitric oxide generation. J Immunol 2005;174:5016-23.
41. Morel J, Audo R, Hahne M, Combe B. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces rheumatoid arthritis synovial fibroblast proliferation through mitogen-activated protein kinases and phosphatidylinositol 3-kinase/Akt. J Biol Chem 2005;280:15709-18.