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探討非類固醇消炎止痛藥和葡萄糖胺的影響及 IL-1α、TNF-α、LPS 和金黃色葡萄球菌，對於人類骨性關節炎的 MMP-2 和 9 的細胞訊息傳遞

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Research article

Upregulation of urokinase-type plasminogen activator and inhibitor and gelatinases expression via three MAPKs and PI3K pathways in the early human osteoarthritic knee

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Running title: u-PA, PAI-1& Gelatinases in OA via MAPKs & PI3K

Abstract

This study tested the hypothesis that upregulation of urokinase-type plasminogen activators (u-PA), PA inhibitor-1 (PAI-1), gelatinases (matrix metalloproteinase-2 and -9, MMP-2 and -9) in the early human osteoarthritic knee was through three major mitogen-activated protein kinases (MAPKs) 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. 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 chondral, meniscal and synovial tissue cultures with or without interleukin-1α (IL-1α) and lipopolysaccharide (LPS) induction. Together, our results were: (1) inhibition of latent and active forms of MMP-9 secretion in synovial and some meniscal cultures was significantly induced by U0126, SB203580 and LY294002 ($P < 0.05$); (2) latent and active forms of MMP-2 secretion was also inhibited significantly by U0126 and LY294002, but not by SB203580 except for active MMP-2 in synovial cultures ($P < 0.05$); (3) similar observation was seen in IL-1α- and LPS-treated cultures ($P < 0.05$); and (4) U0126, SB203580 and LY294002 significantly decreased u-PA and PAI-1 antigenic values in all cultures in the presence or absence of IL-1α and LPS ($P < 0.05$). These results suggest that upregulation of u-PA, PAI-1 and gelatinases expression in the early human osteoarthritic knee was via the MAPKs ERK, JNK and p38 signaling pathways and the PI3K signaling pathway. Thus blocking PA/plasmin and gelatinases expression by novel physiologic and pharmacological inhibitors is an important therapeutic or preventive approach for early

osteoarthritis.

Keywords: u-PA, PAI-1, MMP-2, MMP-9, osteoarthritis, MAPKs pathways, PI3K pathway

Introduction

In osteoarthritis (OA), alterations of chondrocyte metabolism by inappropriate mechanical loading and/or with increased levels of soluble mediators may culminate in extracellular matrix (ECM) degradation and remoldeling and contribute to the progression of degenerative changes. These changes in cartilage are mediated by the ECM degrading enzymes including serine proteases, matrix metalloproteinases (MMPs), and cathepsins [1]. MMPs are probably 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 likely to be involved in degradation of joint collagen which can be specifically cleaved by collagenases into the characteristic 3/4 and 1/4 fragments [8] and then be further degraded by gelatinases [9,10].

MMPs are a group of more than 25 Zn^{2+} dependent extracellular enzymes and can be divided into at least five subclasses, such as collagenase, gelatinases, stromelysins, matrilysins, and membrane-type MMPs (MT-MMPs). The gelatinases can be divided into gelatinase-A (MMP-2 or 72 kDa type IV collagenase) and gelatinase-B (MMP-9 or 92 kDa type IV collagenase) and they are involved in the normal turnover of ECM, tumor invasion, and inflammatory and degenerative joint diseases [9,11]. These endopeptidases are secreted as latent proenzymes and can be activated by limited proteolysis, which results in the loss of approximately 10 kDa of propeptide. Strong circumstantial evidence exists 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 [12]. In addition, increased MMP-9 levels have been seen in effusion samples from patients with inflammatory arthritis, such as rheumatoid arthritis (RA) [13,14] and gouty arthritis [15]. Also, more gelatinases appear in effusions of septic arthritis than aseptic arthritis [16].

Plasminogen activators (PAs), urokinase- (u-PA) and tissue-type PA (t-PA), are serine proteases that catalyze the conversion of the circulating zymogen, plasminogen, to generate the less specific serine protease, plasmin [17]. Plasmin can degrade cartilage macromolecules and basement membrane and activate both latent collagenases and certain enzyme cascades (complement, clotting and the kinin system), which are important in the development of inflammatory responses [18,19]. PA inhibitor-1 (PAI-1) controls the rate of plasmin generation by forming irreversible inhibitory complexes with u-PA and t-PA and is the major circulating PAI [20]. By single proteolytic cleavage, both u-PA and plasmin produce active forms of MMPs which promote degradation of articular cartilage [21], such as gelatinases [22] and stromelysins [23]. In an acute attack of gouty arthritis, there is a correlation of the PA/plasmin system and MMP-9 [24].

We have previously demonstrated that proinflammatory cytokines (IL-1α and TNF-α) and endotoxin lipopolysaccharide (LPS) upregulate MMP-2 and -9 expressions and the regulation of MMP-2 and -9 is mediated via a protein kinase C signaling pathway in human osteoarthritic knees *ex vivo*. However, the involvement of other pathways such as three major mitogen-activated protein kinases (MAPKs) signaling pathways remains unclear and the expression of different MMPs depends on unique combinations of

different signaling pathways [25-27]. Moreover, the mechanisms through which IL-1α and LPS increase MMP-2 and -9 expressions have not been fully deciphered. In order to obtain further information on signal transduction pathways of u-PA, PAI-1, MMP-2 and MMP-9, this study tested the hypothesis that upregulation of u-PA, PAI-1, MMP-2 and MMP-9 in the early human osteoarthritic knee was through three MAPKs extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 pathways and the phosphatidylinositol 3-kinase (PI3K) pathway.

Materials and methods

Sampling and chondral, meniscal, and synovial cultures

Diseased cartilage, torn menisci, and hypertrophic synovia were obtained from patients with primary early osteoarthritic knees undergoing arthroscopic débridement at our hospital by the same author [12]. The specimens were divided and weighted equally 50 mg, transferred into 24-tissue culture flasks, respectively, and then incubated at 37℃ under a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium supplemented with 2% penicillin (10,000 U/ml) and 10 mg/ml streptomycin. All patients fulfilled the American College of Rheumatology criteria for knee OA [28] and gave informed consent for their surgical specimens to be studied. This study was conducted in accordance with the principles embodied in the Declaration of Helsinki and was approved by the Institutional Review Board of the Chung Shan Medical University Hospital, Taichung, Taiwan. The remainders of the specimen were subjected to pathological examination to confirm the diagnosis. 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 [29].

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 [30,31]) and LY294002 (PI3K inhibitor) were purchased from Promega. All culture materials were obtained from Gibco (Grand Island, NY, USA). All pharmacological agents were first dissolved in dimethyl sulfoxide and then diluted with the culture medium. The final concentration of solvent in the medium did not exceed 0.25% (vol/vol). According to in vitro studies of other laboratories [30-33], the final concentrations used in this study were U0126, 23 μM; SB203580, 25 μM; LY294002, 10 μM; IL-1α, 10 ng/ml; and LPS, 1 μg/ml respectively. These concentrations did not induce cell death, so they should not cause cytotoxicity or apoptosis in human osteoarthritic chondral, meniscal, and synovial cultures.

Cytokine, endotoxin, and pharmacological agents treatment

The chondral, meniscal, and synovial tissues were cultured for 3 hours, and then the medium was changed to a medium in the presence or absence of appropriate concentrations of IL-1α, LPS, and pharmacological agents (ERK 1/2 inhibitor U0126, JNK and p38 inhibitor SB203580 and PI3K inhibitor LY294002). The cultured media were collected at 24 and 48 hours and subjected to gelatin zymography and enzyme-linked immunosorbent assay (ELISA) for the measurement of u-PA and PAI-1 antigens [4].

Gelatin zymography

Gelatinolytic activity was assayed by gelatin zymography. Each conditioned medium containing 10 μg of total protein was loaded onto a precast sodium dodecyl sulfate-polyacrylamide gel electrophoresis containing 0.1% gelatin. After electrophoresis, gels were processed. Nonstaining bands representing the activities of MMP-2 and -9 were quantitatively measured by spot density measurement using a digital imaging analysis system (Alpha Innotech, Mt. Prospect, IL, USA). Results were calculated as integrated density value (IDV) which is the sum of all the pixel values after background correction, i.e., IDV = Σ(each pixel value − background value) as described by Lu et al. [12] and Hsieh et al. [14]. Then the levels of MMP-2 and -9 from treated groups were expressed as optical density (% of control) in comparison with the corresponding control group.

Measurement of u-PA and PAI-1 antigens

u-PA and PAI-1 antigens 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 antigenic values were quantitated with a calibration curve using human u-PA and PAI-1 as a standard [25].

Statistical analysis

All assays were repeated three times to ensure reproducibility. Statistical calculations of MMP-2 and -9 levels between control and various inhibitors treated groups were performed using ANOVA analysis. Student's *t*-test was used for the analysis of data concerning MMP-2 and -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). In all synovial and some meniscal cultures, minor gelatinolytic bands were also observed at 92 kDa regions that correspond to proMMP-9. The activated form of MMP-2 and -9 showed a loss of the propeptide of ~10 kDa, respectively. As expected [4], the levels of MMP-2 and -9 increased in IL-1α- and LPS-treated cultures. In contrast, the level of MMP-9 seemed to decrease in all ERK 1/2 inhibitor U0126-, JNK and p38 inhibitor SB203580-, and PI3K inhibitor LY294002-treated cultures with or without IL-1α and LPS induction. Similarly, the level of MMP-2 seemed to repress in ERK 1/2 inhibitor U0126- and PI3K inhibitor LY294002-treated cultures in the presence or absence of IL-1α and LPS, but not in JNK and p38 inhibitor SB203580-treated ones.

Effect of various inhibitors on MMP-2 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 hours ($P < 0.05$). (Table 1-3) However, these suppressive effects could not be

found in JNK and p38 inhibitor SB203580-treated cultures (P = 0.068) except for the level of activated MMP-2 in synovial cultures at 48 hours ($P < 0.05$).

Effect of various inhibitors on MMP-9 levels

In all groups treated with ERK 1/2 inhibitor U0126, JNK and p38 inhibitor SB203580, and PI3K inhibitor LY294002, the level of proMMP-9 significantly decreased at 24 and 48 hours ($P < 0.05$). Also, the level of activated MMP-9 that only appeared in synovial cultures significantly repressed in all U0126-, SB203580- and LY294002-treated groups at 24 and 48 hours ($P < 0.05$).

Effect of various inhibitors on IL-1α- and LPS-induced MMP-2 and -9 levels

IL-1α-induced latent and activated forms of MMP-2 levels were reduced 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 ones except for the levels of proMMP-2 at 48 hours ($P < 0.05$) and activated MMP-2 at 24 and 48 hours ($P < 0.05$) in synovial cultures. (Fig. 2) Additionally, all inhibitors U0126, SB203580 and LY294002 significantly inhibited the level of IL-1α-induced MMP-9 ($P < 0.05$). Similarly, the responses to various inhibitors U0126, SB203580 and LY294002 were observed in cultures with LPS induction ($P < 0.05$) except that SB203580 could suppress the level of activated MMP-2 at 48 hours ($P < 0.05$), but not the levels of proMMP-2 at 24 and 48 hours and activated MMP-2 at 24 hours in synovial cultures. (Fig. 3)

Effect of IL-1α, LPS and various inhibitors on u-PA and PAI-1 antigenic values

Having observed the increased MMP-2 and -9 levels which were suppressed by various inhibitors, we therefore further examined which signal transduction pathways were involved in PA/plasmin system. As illustrated in Fig. 4, antigenic values of u-PA varied among individual conditioned media at 48 hours and were significantly induced by IL-1α and LPS, while the values were significantly reduced by ERK 1/2 inhibitor U0126, JNK and p38 inhibitor SB203580 and PI3K inhibitor LY294002 ($P < 0.05$). All U0126, SB203580 and LY294002 also significantly abolished the increases in u-PA values in response to IL-1α and LPS at 48 hours ($P < 0.05$). As u-PA did, PAI-1 antigenic values showed the similar changes which significantly increased in IL-1α- and LPS-treated cultures and significantly decreased in U0126-, SB203580- and LY294002-treated ones in the presence or absence of IL-1α and LPS at 48 hours ($P < 0.05$). (Fig. 5)

Discussion

By comparing u-PA, PAI-1, MMP-2 and MMP-9 secreted from chondral, meniscal and synovial cultures of early OA, our results clearly show that early osteoarthritic tissues generally displayed a distinct upregulation of u-PA, PAI-1, MMP-2 and MMP-9 secretion through the MAPKs ERK, JNK and p38 signaling pathways and the PI3K signaling pathway *ex vivo*. Thus, in addition to the well-known fact of further degrading collagenase fragments of collagen [9,34], elucidation of u-PA, PAI-1, MMP-2 and MMP-9 activation mechanism has advanced understanding of cell migration and invasion [23], which is closely related to pericellular rearrangement of ECM. Likewise, the expressions of u-PA, PAI-1, MMP-2 and MMP-9 are regulated by IL-1α and LPS also

via the three MAPKs and PI3K signaling pathways in early osteoarthritic tissues of the knee. The findings—in combination with our previous established results of increased expressions of MMP-2, MMP-9, and MT1-MMP (MMP-14) mRNA in the pathologic lesions of early OA [12]—provide strong evidence to support that u-PA, PAI-1, MMP-2 and MMP-9 play a role in the remodeling of ECM and a variety of physiological and pathological processes in early OA.

OA is classically defined as a progressively degenerative disease rather than an inflammatory disease, while the key role of inflammation in OA has been pointed out recently [35-37]. Synovial fibroblasts have been implicated in tissue destruction of inflammatory synovitis and modulating the local cellular and cytokine microenvironment then conditioning the inflammatory infiltrate [38,39]. 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 [40]. 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 the level of activated MMP-2 in synovial cultures at 48 hours. Intriguingly, IL-1α- and LPS-induced groups also showed the similar changes implicating SB203580 in MMP-2 secretion in synovial cultures. In common with these findings our results implicate the synovium in inflammation and cartilage degradation in OA. However, the differences of signaling pathways in MMP-2 production and activation between chondral, meniscal and synovial tissues deserved to be pursued further.

Previous researches 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 [9,41]. Indeed, articular chondrocytes which fail to produce MMP-9 [12,42] are not innocent bystanders in OA [43]. They not only produce destructive enzymes guided by environmental cues but also instruct inflammatory cells or cells from surrounding tissues to trigger two alternative activation pathways which mainly involved MMP-9, MMP-13 and MMP-14 as well as, marginally, serine or cysteine proteinases. Consistent with previous studies, 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 osteoarthritic tissues.

MAPKs 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. In chondrocytes, IL-1- and TNF-induced different MMPs expression requires combinations of cell type-specific signaling pathways such as ERK, JNK and p38 [25-27]. In fact, the mechanisms of signal transduction pathways involved in PA/plasmin system and gelatinases 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. In response to our current observations of inhibition of u-PA, PAI-1, MMP-2 and MMP-9 expressions via three MAPKs pathways

is evident and also depends on IL-1α and LPS induction in early human osteoarthritic chondral, meniscal synovial tissues.

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 [44]. The signaling pathway in gouty arthritis and RA has been well documented [32,33,45,46], whereas no data have been available on how it can regulate MMPs 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, indicating that signal transduction pathway dependent on PI3K is preferentially involved in upregulation of u-PA, PAI-1, MMP-2 and MMP-9 expressions in early human osteoarthritic tissues.

In this study, we targeted patients with early osteoarthritic knees undergoing arthroscopic débridement and obtained the specimens from early stages of knee OA according to the Kellgren and Lawrence grading scale. This short-term *ex vivo* model, which provides a system representative of the *in vivo* environment, suggests upregulation of u-PA, PAI-1 and gelatinases expression in the early human osteoarthritic knee via three MAPKs and PI3K signaling pathways may occur *in vivo*. Moreover, the better understanding of IL-1α and LPS signaling and regulatory mechanisms in early human osteoarthritic knees may lead to novel strategies for inhibiting the catabolic activities in cartilage. The data also demonstrated that three MAPKs and PI3K inhibitors retain the capacity to reduce u-PA, PAI-1, MMP-2 and MMP-9 expressions. Further research into the regulation of

IL-1α/LPS—MAPKs/PI3K—u-PA/PAI-1—gelatinases signaling pathways should help to unravel the mechanisms underlying cartilage destruction in early OA, thus generating new attempts that could control disease progression at the molecular level by inhibiting the enzymes or their gene expression responsible for cartilage degradation while enhancing tissue repair.

Conclusions

The novel findings of this study are that upregulation of u-PA, PAI-1, MMP-2 and MMP-9 in early OA via three MAPKs and PI3K signaling pathways. In addition, we also demonstrated IL-1α and LPS induced u-PA, PAI-1, MMP-2 and MMP-9 upregulation in early OA via the three MAPKs and PI3K signaling pathways. Thereby, further understanding the precise mechanisms by which upregulation of PA/plasmin system and gelatinases expression in the early human osteoarthritic knee will provide vital insights for development of applications for the treatment and prevention of early osteoarthritic joints.

List of abbreviations used

ECM = extracellular matrix

ELISA = enzyme-linked immunosorbent assay

ERK = extracellular signal-regulated protein kinase

IL = interleukin

JNK = c-Jun N-terminal kinase

LPS = lipopolysaccharide

MAPK = major mitogen-activated protein kinases

MMP = matrix metalloproteinase

OA = osteoarthritis

PA = plasminogen activator

PAI = PA inhibitor

PI3K = phosphatidylinositol 3-kinase

proMMP = latent MMP

RA = rheumatoid arthritis

t-PA = tissue-type PA

TNF = tumor necrotic factor

u-PA = urokinase-type PA

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

YS Hsieh and SF Yang coordinated the study and carried out tissue cultures, gelatin zymography and the measurement of uPA and PAI-1 antigens. KH Lue and TY Hsiao contributed conceived the study and helped to collect the specimens and take part in discussions concerning the manuscript. SC Chu helped to perform gelatin zymography and the measurement of uPA and PAI-1 antigens. KH Lu was responsible for collection of the specimens, participated in the design of the study and statistical analysis, conducted sequence alignment, and drafted the manuscript. All authors read and approved the final manuscript

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Table 1

Levels of MMP-2 in chondral cultures after treatment with or without various inhibitors (U0126, SB203580 and LY294002) for 24 and 48 hours

	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***

Note: Values are mean ± S.D.; n ≧ 3.

ANOVA analysis with Scheffe posteriori comparison was used.

** $P < 0.01$, *** $P < 0.001$.

[a] Significantly different, $P < 0.05$, when compared to control (control = 100%).

[b] Significantly different, $P < 0.05$, when compared to 24 hours.

Table 2

Levels of MMP-2 and MMP-9 in meniscal cultures after treatment with or without various inhibitors (U0126, SB203580 and LY294002) for 24 and 48 hours

	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*

Note: Values are mean ± S.D.; n ≧ 3.

ANOVA analysis with Scheffe posteriori comparison was used.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[a] Significantly different, $P < 0.05$, when compared to control (control = 100%).

[b] Significantly different, $P < 0.05$, when compared to 24 hours.

Table 3

Levels of MMP-2 and MMP-9 in synovial cultures after treatment with or without various inhibitors (U0126, SB203580 and LY294002) for 24 and 48 hours

	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***

Note: Values are mean ± S.D.; n ≧ 3.

ANOVA analysis with Scheffe posteriori comparison was used.

** $P < 0.01$, *** $P < 0.001$.

[a] Significantly different, $P < 0.05$, when compared to control (control = 100%).

[b] Significantly different, $P < 0.05$, when compared to 24 hours

Figure Legends

Figure 1

(A) Levels of MMP-2 in chondral cultures, (B) levels of MMP-2 and MMP-9 in meniscal cultures, and (C) levels of 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 hours. Values are mean ± S.D. ($n \geqq 3$). Statistical significance different from IL-1α induction levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2

(A) Levels of MMP-2 in chondral cultures, (B) levels of MMP-2 and MMP-9 in meniscal cultures, and (C) levels of 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 ± S.D. ($n \geqq 3$). Statistical significance different from LPS induction levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 3

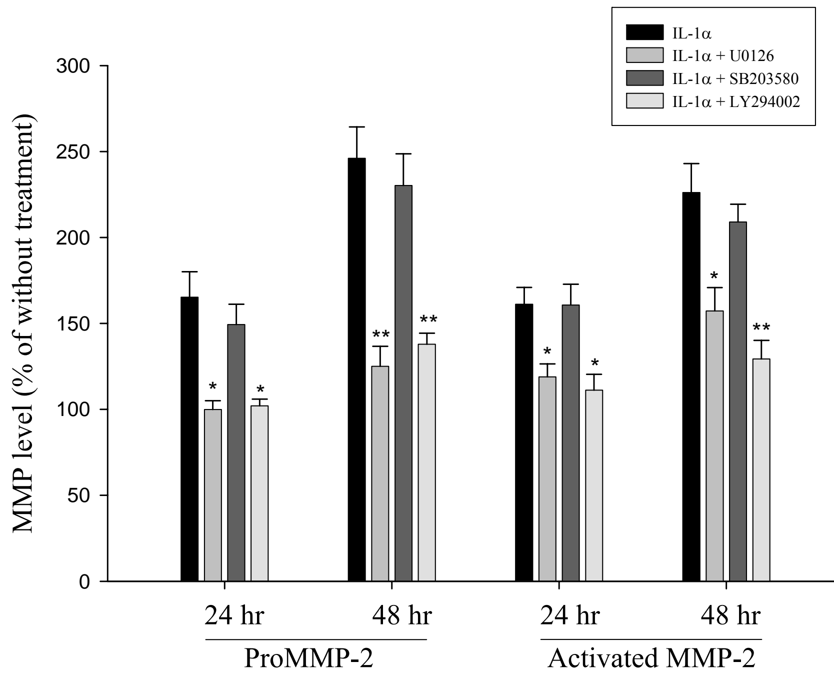
Values of u-PA antigen in (A) chondral, (B) meniscal and (C) synovial cultures after treatment with or without various inhibitors (U0126, SB203580 and LY294002) in the presence or absence of IL-1α and LPS for 48 hours. Values are mean ± S.D. ($n \geqq 3$). Statistical significance different from the values of without induction, IL-1α induction and LPS induction respectively: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4

Values of PAI-1 antigen 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 hours. Values are mean ± S.D. ($n \geqq 3$). Statistical significance different from the values of without induction, IL-1α induction and LPS induction respectively: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 1

(a)



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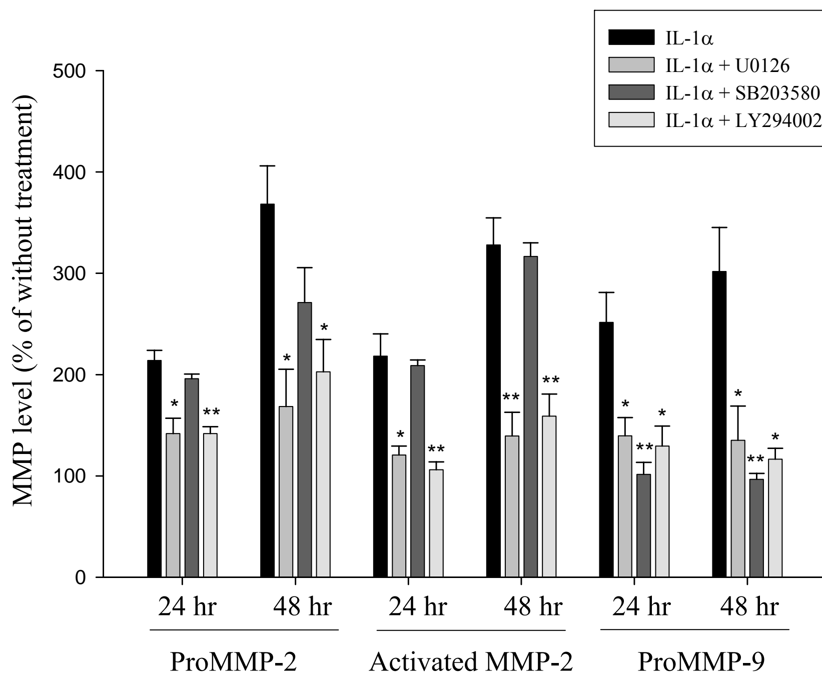

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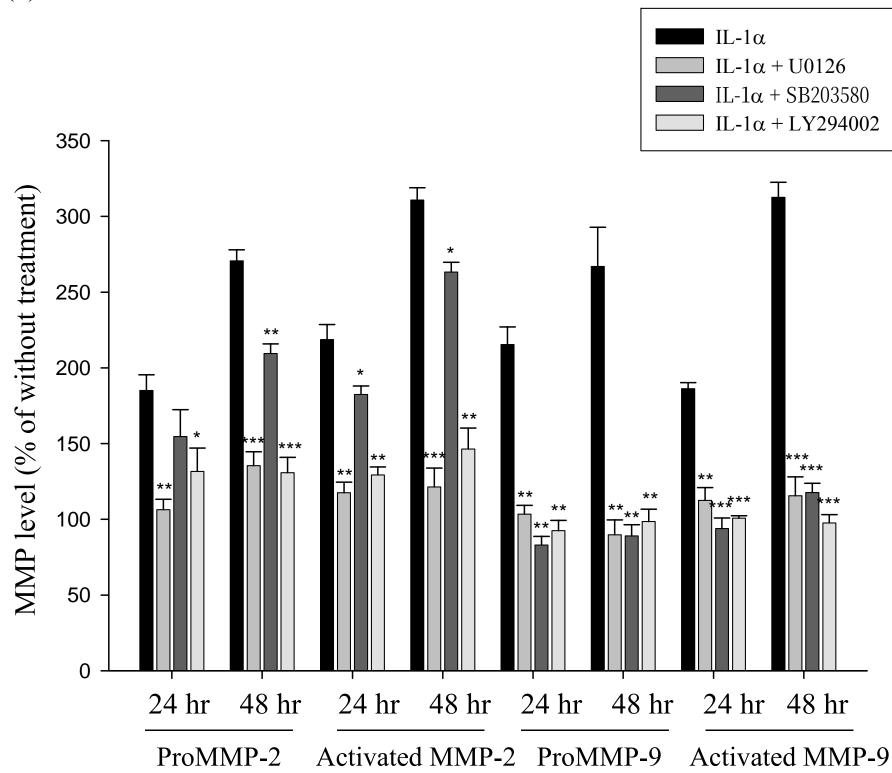

Figure 2
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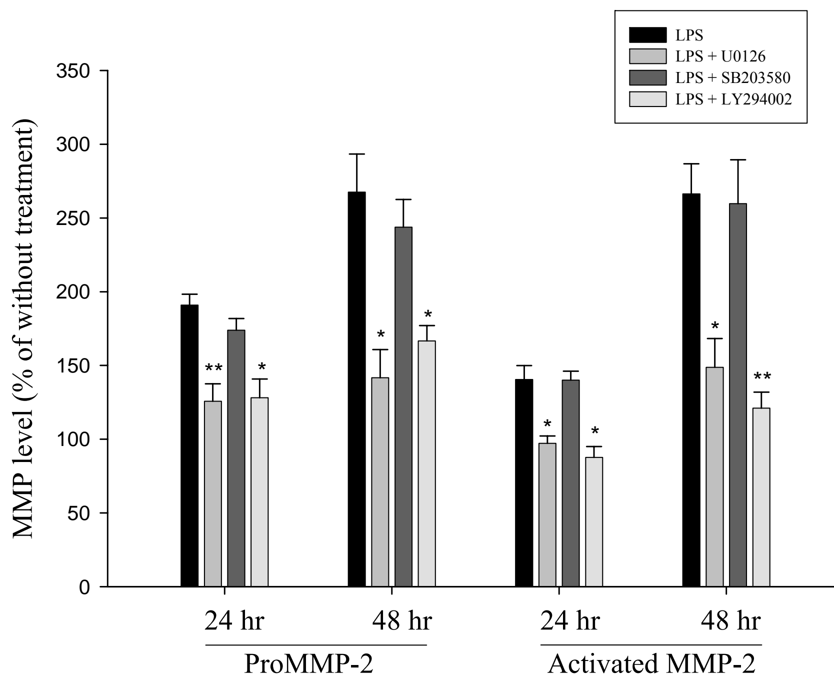

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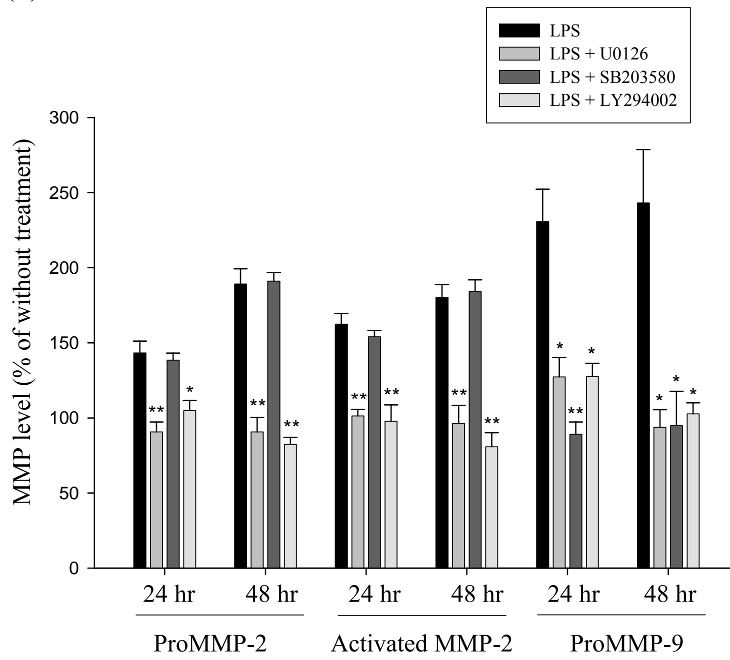

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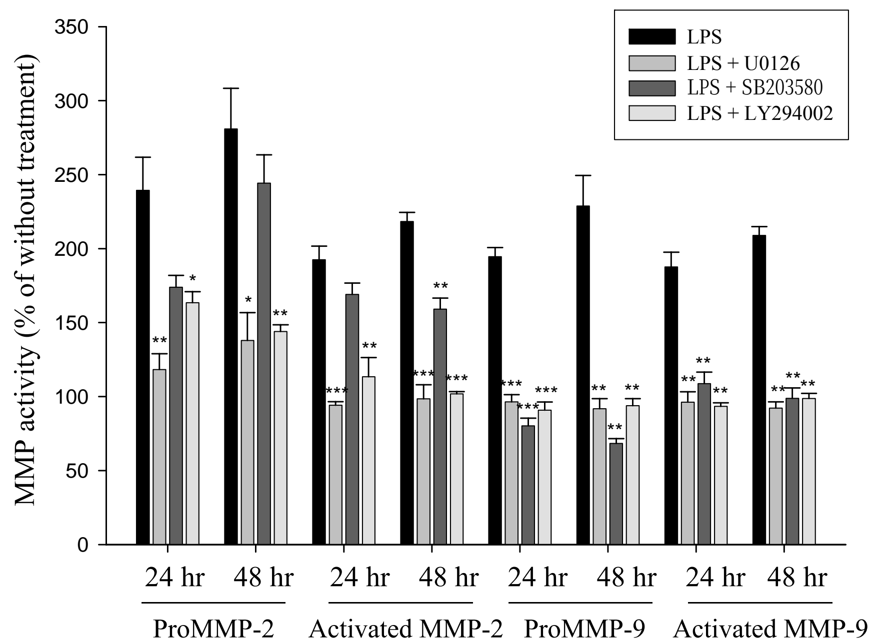
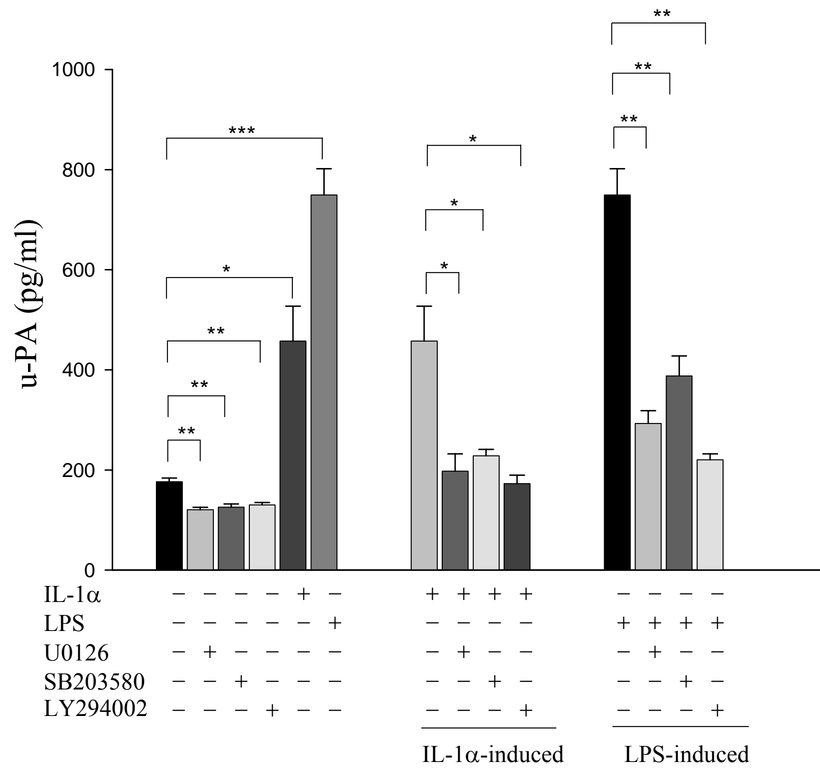


Figure 3

(a)



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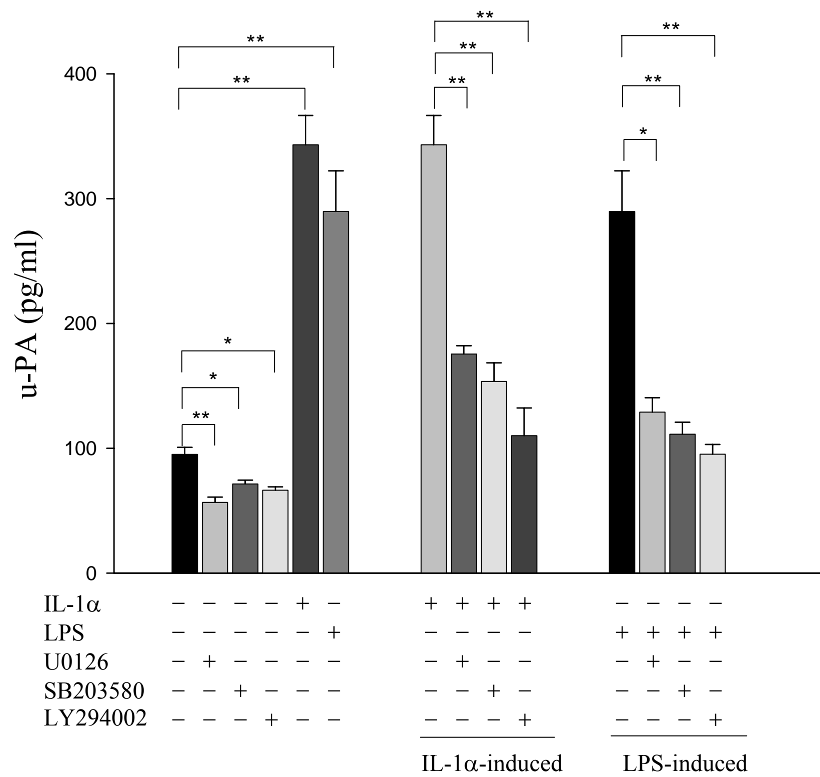


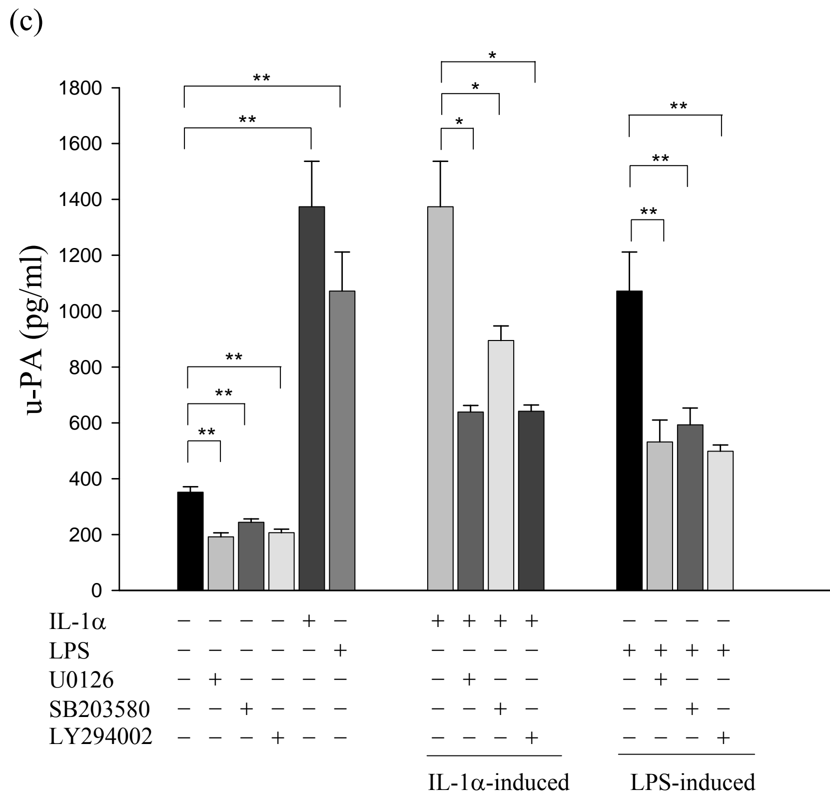
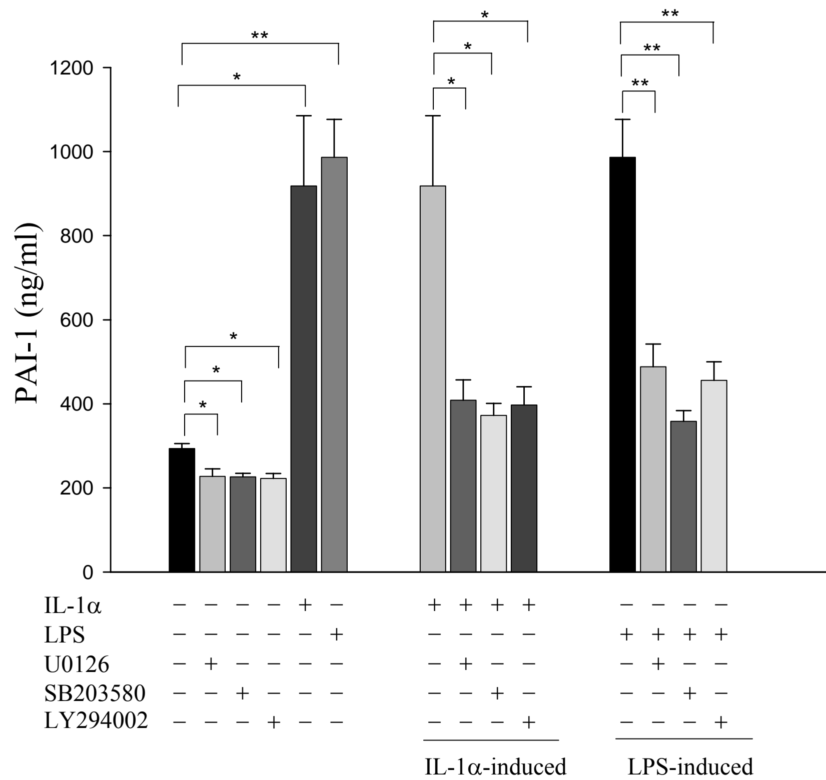
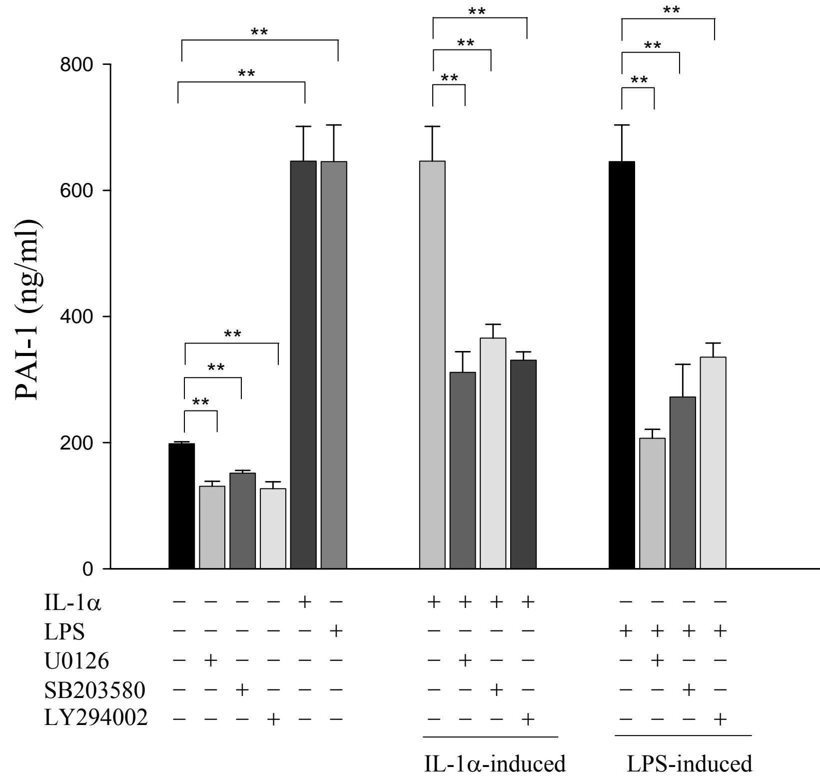
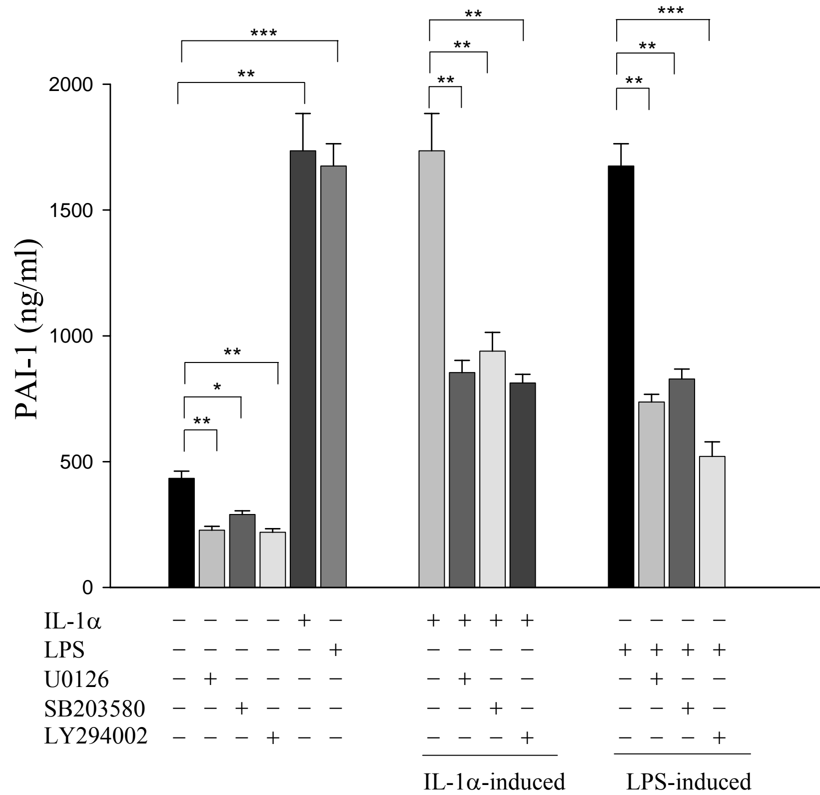


Figure 4
(a)




(b)
PAI-1 (ng/ml)
800
600
400
200
0
**
IL-1α
LPS
U0126
SB203580
LY294002
IL-1α-induced
LPS-induced


(c)
PAI-1 (ng/ml)
2000
1500
1000
500
0
***
**
*
IL-1α
LPS
U0126
SB203580
LY294002
IL-1α-induced
LPS-induced