

Original Paper

Interleukin-1 Induction of Aggrecanase Gene Expression in Human Articular Chondrocytes is Mediated by Mitogen-Activated Protein Kinases

Judith Sylvester Mohammed El Mabrouk Rasheed Ahmad Ataf Chaudry
Muhammad Zafarullah

Département de Médecine, Université de Montréal and Centre de recherche du CHUM (CRCHUM)-
Hôpital Notre-Dame, 1560 Sherbrooke E, Montreal Québec

Key Words

Arthritis • Chondrocytes • Interleukin-1 • Aggrecanases • Signal transduction

Abstract

Background/Aims: We investigated the unknown molecular mechanisms of Interleukin-1 (IL-1 β)-induced cartilage aggrecan degeneration by aggrecanase (ADAMTS-A Disintegrin And Metalloproteinase with ThromboSpondin motifs) in human articular chondrocytes, a model mimicking human arthritis. **Methods:** Chondrocytes were pretreated with various pharmacological inhibitors and then stimulated with IL-1 β for 24 h. ADAMTS-4 expression or activity was studied by RT-PCR or ELISA and other proteins measured by Western blotting. **Results:** MAP kinase kinase-specific inhibitor, U0126 inhibited IL-1-induced phosphorylation of ERK1/2 and down-regulated ADAMTS-4 expression and activity. Protein 38 inhibitor, SB203580 down-regulated the phosphorylation of p38 and its target, activating transcription factor-2 (ATF-2), ADAMTS-4 mRNA and activity. C-Jun N-terminal kinase (JNK) inhibitor, SP600125 diminished IL-1-stimulated JNK phosphorylation, ADAMTS-4 mRNA expression and enzyme activity. A c-fos/lipoxygenase pathway inhibitor and antioxidant, nordihydroguaiaretic acid (NDGA) significantly suppressed ADAMTS-4 mRNA induction and activity. Activating protein (AP-1) and nuclear factor kappa B (NF- κ B) transcription factor inhibitors, curcumin and pyrrolidine dithiocarbamate (PDTc) partially inhibited ADAMTS-4 induction and activity. **Conclusion:** These results suggest partial involvement of ERK-, p38- and JNK-MAPKs as well as AP-1, ATF-2 and NF- κ B transcription factors in IL-1-induced ADAMTS-4 in chondrocytes. Inhibition of these targets by the specific pharmacological agents could be useful for reducing aggrecanase-driven cartilage resorption in arthritis.

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Introduction

Interleukin-1 (IL-1) is a major proinflammatory cytokine implicated in cartilage and bone loss during the pathogenesis of arthritis. Its levels are increased in the synovial fluid and cartilage of patients with rheumatoid arthritis (RA) and osteoarthritis (OA) [1, 2]. Its expression is also induced by cartilage injuries [3]. IL-1 receptor levels are increased in OA chondrocytes rendering them more sensitive to this cytokine [4]. IL-1 inhibits the cartilage-specific, type II collagen but enhances matrix metalloproteinases (MMP) mRNA expression in human chondrocytes [5, 6]. Blocking IL-1 with specific antibodies and its signal transduction by IL-1 receptor antagonist reduce the signs of arthritis such as cartilage and bone loss and synovial invasion of cartilage in animal models and patients [7-9]. Blockade of IL-1 and TNF- α in human OA cartilage explants resulted in reduced aggrecan and collagen loss [10]. Proinflammatory cytokines involved in rheumatic diseases activate multiple signaling cascades including ERK, p38 and JNK pathways [11].

Aggrecan is the principal cartilage extracellular matrix proteoglycan that gives cartilage its characteristic compressibility. Aggrecanases or ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin motifs) are a family of proteases [12, 13] implicated in degradation of aggrecan at Glu 373-Ala 374 bond [14]. Indeed, fragments of aggrecan generated by aggrecanases and MMPs are found in the OA synovial fluid [15]. Principal aggrecanases, ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) also cleave biglycan in OA cartilage [16]. ADAMTS-5 gene knockout protects mice from arthritis-induced aggrecan degradation and is the major aggrecanase in mice [17-19]. In the earlier phases of focal cartilage degradation, ADAMTS-5 and not ADAMTS-4 is increased [20]. *In vitro* knockdown experiments revealed that both ADAMTS-4 and -5 are implicated in human cartilage aggrecan cleavage prior to collagen degradation by MMP-13 [21]. Aggrecanases are currently major targets for developing cartilage-protective physiologic and pharmacological inhibitors for treating arthritis [22].

The mechanisms through which IL-1 increases the expression of ADAMTS-4 have not been fully elucidated. A better understanding of these signaling and regulatory mechanisms in chondrocytes may lead to novel strategies for suppressing the catabolic actions of IL-1. Here we show that blocking ERK, p38 and JNK-MAP kinase signal transduction-pathways as well as AP-1 and NF- κ B transcription factors in human articular chondrocytes leads to down-regulation of the IL-1-induced ADAMTS-4 gene expression.

Materials and Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from Invitrogen (Burlington, ON). Recombinant human IL-1 β was from R&D Systems (Minneapolis, MN). Curcumin and PDTC were obtained from Sigma while NDGA was from Calbiochem (La Jolla, CA).

Chondrocyte cell culture and treatments

The normal human knee articular chondrocytes between passages 2-4 (from Cambrex, Walkersville, MD) were grown in 6-well plates as high-density monolayer cultures to confluence in DMEM supplemented with 10% fetal calf serum (FCS) and growth factors (IGF-I, TGF- β , insulin) that maintain chondrocyte differentiated phenotype. These cells express type II collagen mRNA and protein at the passages used. Cells were washed with phosphate buffered saline (PBS) and kept in serum-free DMEM for 48 hours. Chondrocytes were pretreated for 1 h either with potential inhibitors dissolved in ethanol or dimethyl sulfoxide (DMSO) at different doses and treated further for 24 h with IL-1 β (10 ng/ml) and inhibitors. U0126 (1,4-Diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene; from Calbiochem) that selectively inhibits both the inactive and active forms of MEK-1 and MEK-2 [23], was dissolved in DMSO at 10 mM stock and used at the doses of 2.5 and 5 μ M final. SB203580 or 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole (Calbiochem) which inhibits p38 kinase [24] was dissolved in DMSO at 2.5 mM and used at 2.5 and

5 μ M final concentrations. SP600125, a JNK-specific inhibitor [25], was used at 10-20 μ M concentrations. Nordihydroguaiaretic acid (NDGA) a 5-, 12, 15-lipoxygenase inhibitor that also blocks AP-1 binding activity [26] was used at 5 and 10 μ M doses. Curcumin or diferuloylmethane (from Sigma-Aldrich), an inhibitor of JNK, AP-1 and NF- κ B [27-29], was dissolved at 1 mM in ethanol and used at the 2.5- and 5- μ M doses. Pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF- κ B [30] was dissolved in water as a 100 mM solution and used at the final concentrations of 25 and 50 μ M. The doses used are optimal for numerous in vitro systems and are not toxic to chondrocytes. The control cells were exposed to vehicles (DMSO, ethanol and water), which had no effect on ADAMTS-4 gene expression.

RNA extraction and RT-PCR

Total RNA was extracted as previously described [31] and aliquots of 3-5 μ g analyzed by electrophoretic fractionation in 1.2% formaldehyde-agarose gels. The integrity and quantity of applied RNA were verified by ethidium bromide staining of the gels and photography of the 28S and 18S ribosomal RNA bands. For RT-PCR, 2 μ g of RNA was heated for 5 min at 80°C and reverse transcribed in the mixture consisting of oligo d (T) 12-18mer, dNTPs, RNase inhibitor (Pharmacia), acetylated BSA (Promega) with Moloney murine leukemia-virus reverse transcriptase (MMLV-RT) (Invitrogen) according to the protocols of Clontech Laboratories Inc. (Paolo Alto, CA). Conditions of RT-PCR were same as described before for ADAMTS-4-specific primers [32]. The amplification profile was one cycle at 94°C for one min, 35 cycles of 94°C for one min, hybridization at 60°C for 2 min and extension at 72°C for 3 min, followed by one extension cycle of 7 min at 72°C. The PCR was performed in a DNA cycler (Techne, Princeton, NJ) in a 50 μ l reaction with 1.25 mM dNTPs, Taq DNA polymerase (Pharmacia) and respective primers. Aliquots of 10 μ l from the 50 μ l PCR reaction were analyzed on 1.4 % agarose gels to detect ADAMTS-4 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA amplification products of 692 and 226 bp respectively. Negative controls included either all the RT-PCR reagents except cDNA or additionally, RT was omitted before PCR. None of these controls gave any bands. The ADAMTS-4 cDNA product was cloned in pGEM-4Z and its identity confirmed by DNA sequence analysis that matched with the published sequences.

Aggrecanase activity by ELISA

For measuring the biological activity of ADAMTS-4 enzyme, supernatants were collected from the cells cultured in the 6-well plates and were kept at -20°C until analysis. For quantitative determination of aggrecanase activity, Sensitive Aggrecanase Activity ELISA (Cat #SEN-AGG-96, MD Biosciences Inc., St. Paul, MN) was used according to their protocol. The aggrecanase in supernatant cleaves the recombinant interglobular domain of aggrecan releasing a peptide with the N-terminal sequence of ARGSVIL (peptides S) which is quantified by two anti-peptide monoclonal antibodies [33]. The absorbance values were read at 450 nm and a mean $\pm$ SD of three independent experiments is shown as bar graphs using the GraphPad Prism software.

Western blotting

For Western blot analysis of phosphorylated ERK1/2, pATF-2 and JNK, human chondrocytes were pretreated for each time point with the respective inhibitors and then stimulated with IL-1 (20 min). These cells were washed three times with cold PBS and lysed in a buffer which contained 20 mM HEPES (pH 7.5), 20 mM NaCl, 1 mM orthovanadate, 1 mM sodium fluoride, 5 mM EDTA, 1% Nonidet P-40, 1 μ g/ml aprotinin, 0.7 μ g/ml pepstatin, 1 μ g/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. Cell lysates were centrifuged at 14,000 rpm for 20 min. Proteins (15 μ g) were separated by 10% SDS-PAGE. Blots were treated with polyclonal phospho ERK1/2, p38, p-ATF-2 and p-JNK-specific antibodies (1/1,000 dilution) in blocking buffer at 4°C overnight. The same nitrocellulose membranes were subsequently used to probe the total forms of ERK, p38 and JNK kinases.

All the experiments reported in this study were performed at least three times and the results were reproducible.

Results

Inhibition of ERK-MAPK pathway decreases IL-1 induction ADAMTS-4 gene expression

We have previously shown that IL-1 time-dependently (within 15 min) stimulates the phosphorylation of ERK, p38 and JNK MAPKs in articular chondrocytes [6]. To explore if the

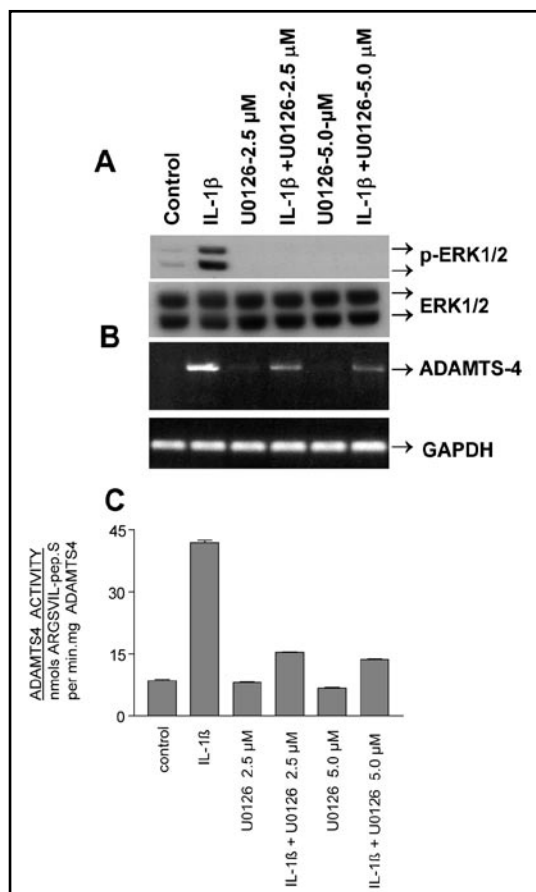


Fig. 1. Down-regulation of IL-1-induced ERK1/2 phosphorylation and ADAMTS-4 mRNA expression and activity by the ERK pathway inhibitor, U0126. Human chondrocytes were made quiescent by maintaining them in low-serum medium and pretreated with the indicated doses of the U0126 or inhibitor vehicles (DMSO, PBS-0.1% BSA, Control) for 30 min, followed by additional incubation with recombinant human IL-1 (10 ng/ml) for 20 min (A) or 24 h (B and C). Panel A shows result of Western blots with anti-phospho ERK1/2 and total ERK1/2 levels demonstrating activation with IL-1 and complete inhibition of phosphorylation with U0126. B shows representative RT-PCR and agarose gel analysis of ADAMTS-4 and GAPDH mRNAs demonstrating induction with IL-1 and partial inhibition with U0126. The panel C depicts the aggrecanase activity in cell culture medium as determined by specific ELISA and depicted as mean $\pm$ SD of three independent experiments using the GraphPad Prism software. P value = $P < 0.0001$.

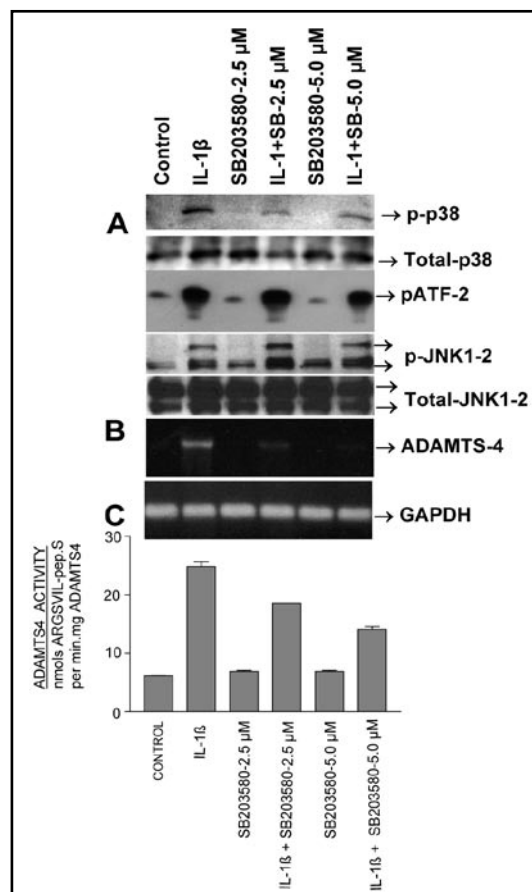


Fig. 2. Decrease of IL-1 β -induced p38 and ATF-2 activation and ADAMTS-4 mRNA expression and activity by the p38 inhibitor, SB203580. Human chondrocytes kept in low-serum medium were exposed to DMSO (vehicle, Control) or pretreated with SB203580 for 30 min followed by stimulation with IL-1 β for 24 h. A is a Western blot with the anti-phosphorylated p38, JNK, ATF-2 (p38 target) and total p38 and JNK antibodies. B is representative RT-PCR and agarose gel analysis of ADAMTS-4 and GAPDH mRNAs while panel C shows aggrecanase-specific ELISA depicted as mean $\pm$ SD of three independent experiments using the GraphPad Prism software. P value = $P < 0.0011$.

MAPKs are involved in the IL-1-stimulated ADAMTS-4 gene expression, human chondrocytes were pretreated with a specific pharmacological inhibitor of ERK pathway, U0126 [23], and then simultaneously exposed to IL-1 β . This inhibitor completely suppressed ERK1/2 phosphorylation

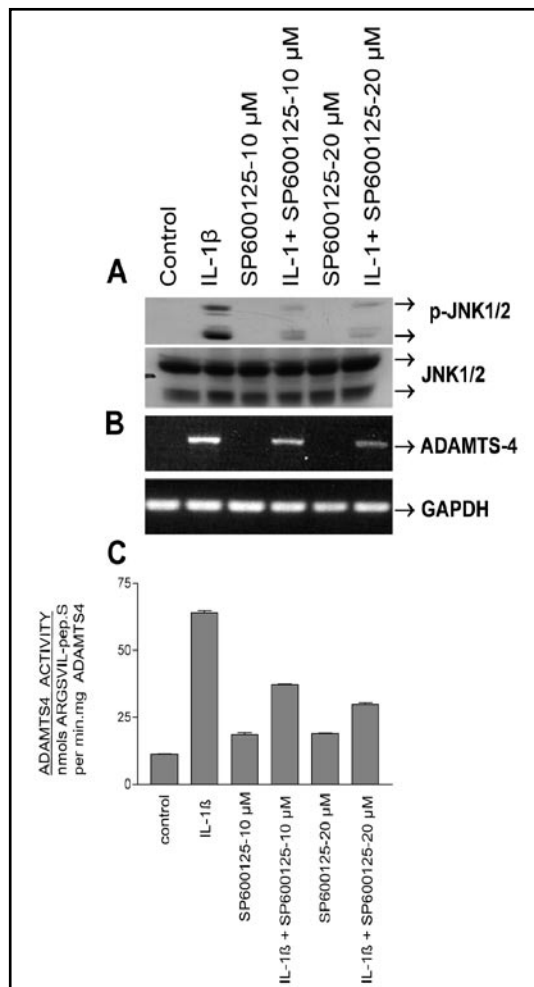


Fig. 3. Inhibition of IL-1 β -stimulated JNK phosphorylation and ADAMTS-4 mRNA expression and enzyme activity by SP600125. Human chondrocytes kept in serum-deficient medium were exposed to DMSO (vehicle, Control) or pretreated with the indicated doses of SP600125 for 30 min followed by stimulation with IL-1 β for 20 min (A) or 24 h (B and C). Panel A shows result of Western blots with anti-phosphorylated JNK and total JNK levels demonstrating activation with IL-1 and inhibition of phosphorylation with SP600125. B is representative RT-PCR analysis of ADAMTS-4 and GAPDH mRNAs demonstrating inhibition of ADAMTS-4 induction by SP600125. The panel C shows aggrecanase-specific ELISA depicted as mean $\pm$ SD of three independent experiments using the GraphPad Prism software. P value = P<0.0001.

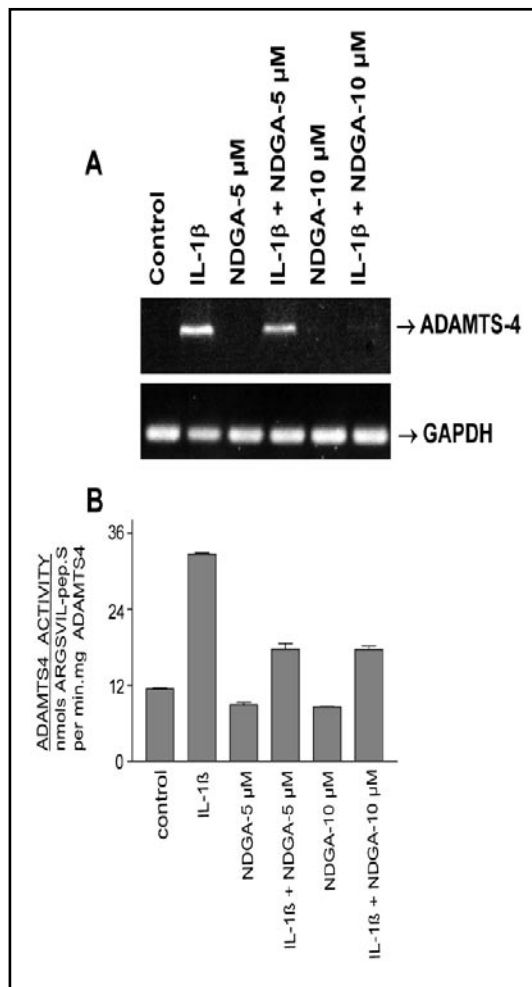


Fig. 4. Diminution of IL-1 β -stimulated ADAMTS-4 mRNA expression and activity by nordihydroguaiaretic acid (NDGA). Human chondrocytes maintained in low-serum medium were exposed to ethanol (vehicle, Control) or pretreated with the indicated doses of NDGA for 30 min followed by stimulation with IL-1 β for 24 h. Representative RT-PCR and agarose gel analysis of ADAMTS-4 and GAPDH mRNAs (Panel A) and aggrecanase-specific ELISA of the culture medium (Panel B) depicted as mean $\pm$ SD of three independent experiments using the GraphPad Prism software (Panel B). P value = P<0.0001.

without affecting the total ERK1/2 levels (Fig. 1A). RT-PCR analysis and aggrecanase ELISA revealed a partial inhibition of ADAMTS-4 mRNA (Fig. 1B) and enzyme activity (Fig. 1C) with the two doses. These results demonstrate critical but partial role of ERK-MAPKs in the IL-1-induced ADAMTS-4 gene expression.

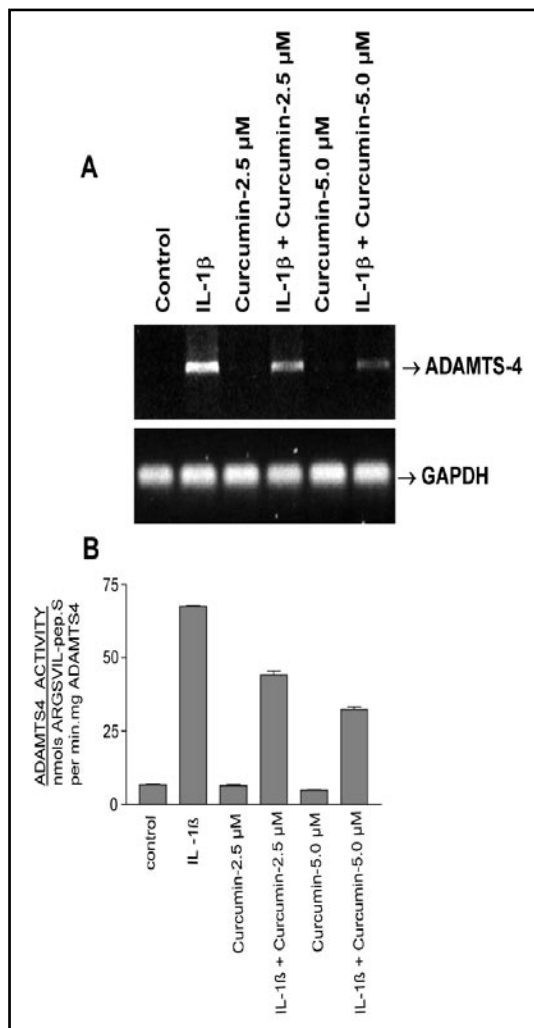


Fig. 5. Suppression of IL-1 β -stimulated ADAMTS-4 gene expression and aggrecanase activity by curcumin. Confluent cultures of human chondrocytes were pre-exposed for 30 min to vehicle (ethanol) or 2.5–5 μ M of curcumin for 30 min and then treated with IL-1 β for an additional 24 h. Representative RT-PCR and agarose gel analysis of ADAMTS-4 and GAPDH mRNAs (Panel A) and aggrecanase-specific ELISA depicted as mean $\pm$ SD of three independent experiments using the GraphPad Prism software (Panel B). P value = P<0.0001.

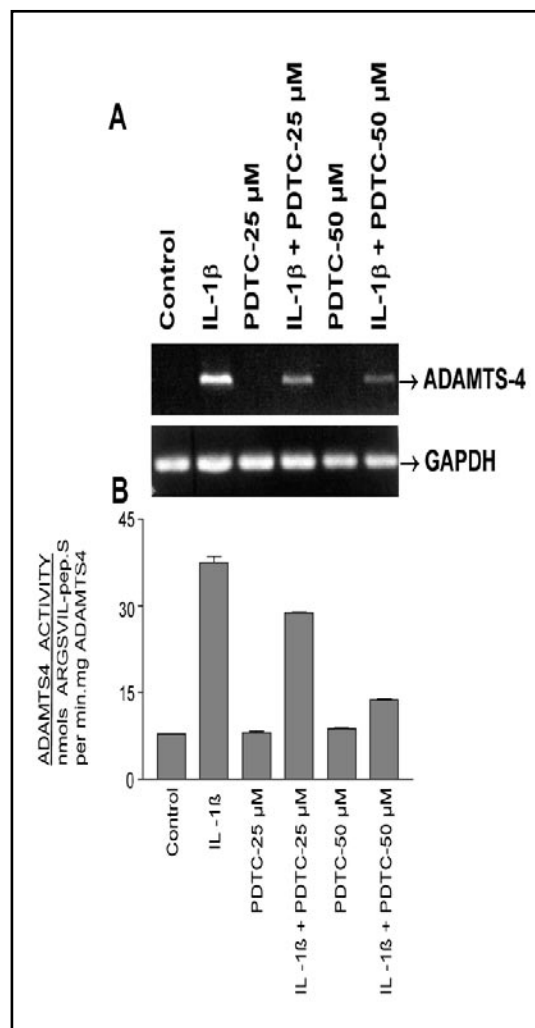


Fig. 6. Inhibition of IL-1 β -inducible ADAMTS-4 mRNA expression and enzyme activity by pyrrolidine dithiocarbamate (PDTC). Quiescent and confluent human chondrocytes were pretreated with the indicated concentrations of PDTC for 30 min followed by additional treatment with IL-1 β for 24 h. Representative RT-PCR and agarose gel analysis of ADAMTS-4 and constitutive GAPDH mRNAs (panel A) as well as aggrecanase-specific ELISA (panel B) depicted as mean $\pm$ SD of three independent experiments using the GraphPad Prism software. P value = P<0.0001.

Inhibitors of Protein 38 and JNK suppress IL-1 induced-ADAMTS-4 gene expression

To investigate the implication of p38 pathway in the induction of ADAMTS-4, human chondrocytes were treated with a p38 inhibitor, SB203580, which at different concentrations can inhibit both p38 and JNK pathways [24]. While phosphorylation of JNK was not affected by the inhibitor, activation of p38 and the major target transcription factor of p38, ATF-2, by IL-1 was partially inhibited by SB203580 (Fig. 2A). Total p38 and JNK levels remained constant. SB203580 repressed the IL-1 induction of ADAMTS-4 gene expression (Fig. 2B) and aggrecanase enzyme activity (Fig. 2C) with the two concentrations used.

Role of JNK pathway in ADAMTS-4 induction was investigated by pretreatment with the JNK-specific inhibitor, SP600125 [25]. This inhibitor dose-dependently blocked IL-1-stimulated phosphorylation of JNK1/2 without affecting its total levels (Fig 3A). SP600125 down-regulated IL-1-induced expression of ADAMTS-4 mRNA (Fig. 3B) and enzyme activity (Fig. 3C) with the two doses.

Nordihydroguaiaretic acid (NDGA) inhibits induction of ADAMTS-4 by IL-1

NDGA inhibits lipoxygenase (LOX), which in turn blocks arachidonic acid metabolites and thus suppresses expression of the c-Fos component of AP-1 [26]. Since ADAMTS-4 promoter contains AP-1 and AP-2 transcription factor binding sites [34] and activation of MAPK pathways results in downstream activation of c-Fos (ERK) and c-Jun (JNK), we investigated the role of transcription factors AP-1 in the regulation of ADAMTS-4 gene expression. Treatment of chondrocytes with this LOX and AP-1 inhibitor at 5 and 10 μ M, dose-dependently abrogated ADAMTS-4 mRNA induction by IL-1 β (Fig. 4A). The aggrecanase activity was repressed at similar levels with both doses used (Fig. 4B)

Curcumin inhibits IL-1-induced ADAMTS-4 gene expression

Since phosphorylation of MAPK pathways results in downstream activation of c-fos (ERK) and c-Jun (JNK), we investigated the role of JNK/SAPK pathway and AP-1 transcription factors in the regulation of ADAMTS-4 genes expression with the help of an inhibitor of this cascade [27] and an antiinflammatory agent, curcumin, originating from the roots of *Curcuma longa*. This agent also inhibits NF- κ B pathway [28, 29]. Treatment of human chondrocytes with 2.5 and 5 μ M of curcumin and IL-1 stimulation resulted in partial suppression of ADAMTS-4 mRNA induction (Fig. 5A) and enzyme activity (Fig. 5B).

Pyrrolidine dithiocarbamate abrogates IL-1-enhanced ADAMTS-4 gene expression

To further investigate the role of NF- κ B transcription factors, human chondrocytes were pre-exposed to a more specific inhibitor of this factor, PDTC [30], followed by further 24 h combined treatment with PDTC and IL-1. PDTC dose-dependently inhibited the induction of ADAMTS-4 mRNA (Fig. 6A) and enzyme activity (Fig. 6B) by IL-1 without affecting the GAPDH mRNA levels in human chondrocytes.

Discussion

IL-1 is the most prominent proinflammatory cytokine involved in cartilage catabolism in arthritis by ADAMTSs and MMPs. Very little is known about the pathways induced by this cytokine leading to ADAMTSs expression. Here, we have demonstrated that IL-1 clearly induces ADAMTS-4 expression in human articular chondrocytes. Additionally, partial inhibition of ADAMTS-4 gene expression by U0126, SB203580, NDGA, curcumin and PDTC suggests that ERK-, p38- and JNK-MAPK pathways as well as AP-1, ATF-2 and NF- κ B transcription factors mediate ADAMTS-4 induction by IL-1.

Reproducible induction of ADAMTS-4 gene expression and activity by IL-1 in human articular chondrocytes demonstrates that these cells mimic the *in vivo* conditions by responding to IL-1, and that ADAMTS-4 gene is one of the IL-1 targets. Thus these chondrocytes constitute an appropriate model system for studying the IL-1 signal transduction pathways leading to ADAMTS-4 induction. Both ADAMTS-4 and ADAMTS-5 promote degeneration of arthritic hip and knee joints in OA and RA. Induction of ADAMTS-4 gene by IL-1 observed here is in agreement with studies in human primary chondrocytes and SW1353 chondrosarcoma cells [35-37].

MAP kinases have important roles in chondrocyte differentiation during development [38]. Interestingly, these kinases are also found in the active state in rheumatoid synovial tissues and

are phosphorylated in response to IL-1 in RA synovial fibroblasts [39]. IL-1 and physiologically relevant mechanical compression induces ERK MAPKs in chondrocytes under various culture conditions [6, 40, 41]. Complete inhibition of ERKs by U0126 and incomplete suppression of the ADAMTS-4 gene expression by the specific inhibitor of ERK-MAPK pathway suggests that this cascade is partly involved in the ADAMTS-4 induction by IL-1 in human chondrocytes. IL-1 induction of MMP-13 [6] and MMP-1 in chondrocytes also requires ERK pathway, which phosphorylates CCAAT enhancer binding protein beta (C/EBP- β) [42]. Interestingly C/EBP- β sites are found in the ADAMTS-4 promoter [34]. ERK-MAPK pathway is involved in growth and survival of chondrocytes and its inhibition results in chondrocyte apoptosis [43, 44]. Thus therapeutic utility of this target remains to be studied. Interestingly, inhibition of ERKs with PD198306 in an animal model partially reduced symptoms of OA [45]. Ability of ERK inhibitors to suppress ADAMTSs and MMPs may have therapeutic utility in diminishing cartilage degradation by these proteases.

P38 MAPK pathway is an important target in inflammatory diseases such as arthritis that controls the stability of 3'AU-rich sequence containing genes [46]. IL-1 is known to activate p38 pathway in chondrocytes [6]. Suppression of ADAMTS-4 by the specific inhibitor partially implicates p38 pathway in this modulation. Our results are in agreement with a study where another p38 inhibitor, RWJ 67657 inhibited IL-1 β -induced ADAMTS-4 RNA expression in human rheumatoid synovial fibroblasts, a different cell-type important in the pathogenesis of rheumatoid arthritis [47]. ADAMTS-9 induction is also inhibited by the p38 inhibitor [37]. Inhibitors of p38 blocked symptoms of collagen-induced arthritis in mouse and RA-like bone resorption *in vivo* [48, 49]. MKK3 (an upstream regulator of p38)-deficient mice display reduced arthritis [50]. Thus p38 is an important mediator of the catabolic pathways.

IL-1 induces JNK activation among others in chondrocytes [51]. Previously unknown inhibition of IL-1-induced ADAMTS-4 by the specific JNK inhibitor, SP600125 shown here and reported inhibition of MMPs [6] suggests that the JNK pathway can be targeted to diminish cartilage aggrecanolysis and collagenolysis. JNK2 rather than JNK1 appears to be important for cartilage degradation in animal models of arthritis [52, 53].

Decrease in ADAMTS-4 mRNA expression and activity by NDGA may be due to sequential blockade of lipoxygenase activity, inhibition of arachidonic acid metabolite, 5-HPETE, c-fos expression, interruption of fos-jun-DNA complex formation and AP-1 activity [26, 54, 55]. Indeed leukotriene B₄, a metabolite of lipoxygenase pathway has important role in collagen-induced arthritis [56]. Dual inhibitors of 5-LOX and cyclooxygenase (COX) have been suggested to be more effective for treating arthritis [57]. Besides reducing inflammation, such inhibitors may also diminish degradation of cartilage. *Larrea divaricata* extract with elevated content of NDGA has anti-inflammatory and anti-oxidant activities [58].

Partial inhibition of ADAMTS-4 expression by curcumin suggests the implication of JNK, AP-1 and NF- κ B pathways in gene expression as this anti-inflammatory agent from the rhizome of *Curcuma longa* has multiple activities [59] including suppression of JNK phosphorylation and transcription factors AP-1 and NF- κ B binding [27-29]. Indeed, curcumin protects chondrocytes from deleterious effects of IL-1 such as inhibition of type II collagen synthesis and caspase-3-mediated apoptosis [60]. NF- κ B involvement is further supported by down-regulation of ADAMTS-4 mRNA expression by another inhibitor of this transcription factor [30], PDTC. Curcumin and PDTC also have reactive oxygen species-scavenging activities [61]. In support of our results, an I κ B inhibitor, BMS-345541 was shown to block IL-1-stimulated cartilage collagen and aggrecan degradation by aggrecanases and MMPs [62]. Several inflammatory mediators in synovial cells including MMP-1, 3, 9 and 13 are dependent on NF- κ B as shown by its inhibition with I κ B α [63]. Protein kinase C ζ and NF- κ B have been shown to mediate IL-1-induced bovine and human ADAMTS-4 and -5 activities [64, 65].

Human ADAMTS-4 promoter contains Sp1, AP-2 and NF-1 binding sites upstream of transcription initiation site and AP-1, AP-2 and PEA3 consensus sites in the 5' untranslated

region [34]. Runx2 binding sequences have been found in the ADAMTS-5 promoter [66]. By utilizing a transcription factor binding sites recognition software, we have identified several NF- κ B and other sites whose functionality remains to be characterized by additional experimental approaches. Further work is needed to identify the target transcription factors of these signaling pathways.

We have demonstrated that enhancement of ADAMTS-4 expression by IL-1 involves multiple mechanisms including ERK, p38 and JNK MAPK pathways as well as AP-1, ATF-2 and NF- κ B transcription factors whose inhibition down-regulates ADAMTS-4 gene expression. This knowledge may be useful for developing novel therapies for blocking IL-1-stimulated cartilage aggrecan breakdown in arthritis. In view of the partial suppression by individual inhibitors, any therapeutic application in arthritis may require usage of multiple inhibitors to effectively block different pathways and their target MMPs and ADAMTS-4. For multigenic complex diseases such as arthritis, suggested multiple target or polypharmacology approach [67] may be useful.

Abbreviations

ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs); Interleukin-1 (IL-1 β); ERK (extracellular signal-regulated kinase); JNK (C-Jun N-terminal kinase); ATF-2 (activating transcription factor-2); NDGA, nordihydroguaiaretic acid; AP-1 (Activating protein); NF- κ B (nuclear factor kappa B); PDTC (pyrrolidine dithiocarbamate).

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