CURCUMIN, NORDIHYDROGUIARETIC ACID, QUERCETIN AND RESVERATROL INHIBIT INTERLEUKIN-1-INDUCED ADAMTS-4 (AGGRECANASE-1) GENE EXPRESSION IN ARTICULAR CHONDROCYTES: NATURAL PRODUCTS AS POTENTIAL ANTI-ARTHRITIC AGENTS

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Summary

Interleukin-1 (IL-1β) is the main proinflammatory cytokine stimulant of cartilagedegeneration in arthritis. Aggrecanases (ADAMTS or A Disintegrin And Metalloproteinase with ThromboSpondin motifs) are enzymes implicated in tissue remodeling and cleavage of aggrecan core protein between Glu^{373}-Ala^{374}, a major structural protein of cartilage extracellular matrix giving its characteristic compressive stiffness. This study screened for natural products with the ability to inhibit IL-1-induced ADAMTS-4 gene expression in human articular chondrocytes. Confluent normal human knee articular chondrocytes were pretreated either with natural products at different doses and stimulated further for 24 h with IL-1β (10 ng/ml). ADAMTS-4 and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) RNA levels were analyzed by RT-PCR. IL-1β induced ADAMTS-4 RNA in high-density human chondrocyte monolayer cultures. Pretreatment with a leukotreine and c-Fos (component of activating protein or AP-1 transcription factor) inhibitor, nordihydroguiaretic acid (NDGA) suppressed the ADAMTS-4 RNA induction. Quercetin and Resveratrol at 50-100 μM partially reduced ADAMTS-4 RNA induction. Further AP-1 and nuclear factor kappa B (NF-κB) transcription factors inhibitor, curcumin partially inhibited aggrecanase-1 induction. The levels of internal control, GAPDH RNA remained consistent. Thus several natural products can interfere with proinflammatory cytokine signal transduction pathways or their target transcription factors and thus inhibit IL-1-induced ADAMTS-4 in chondrocytes. Such inhibition warrants further studies on toxicology and potential for reducing ADAMTS-4-driven cartilage resorption in arthritis.

Key Words: Interleukin-1, Aggrecanases, Natural Products

Introduction

A major pathological manifestation of patients with arthritis is degradation of cartilage in different joints. Interleukin-1 (IL-1) is the main proinflammatory cytokine stimulant of cartilage degeneration in arthritis. It is expressed at high levels in the synovial fluid and cartilage of patients with rheumatoid arthritis (RA) and osteoarthritis (OA) (1, 2). IL-1 drastically alters the physiology of joints and cartilage metabolism by decreasing cartilage-specific, type II collagen and by increasing matrix metalloproteinases (MMP) (3, 4). Blocking IL-1 with specific antibodies and its actions by IL-1 receptor antagonist reduce the symptoms of arthritis in animal models and patients including cartilage and bone loss and invasion of cartilage by synovial membrane (5-8). We have been investigating mechanisms of proinflammatory cytokine signal transduction and have previously shown that IL-1 induces Collagen II-degrading MMP-13 mRNA and protein induction through mitogen-activated protein kinases (MAPKs), activating protein-1 (AP-1) and nuclear factor kappa B (NF-κB) (9).

Besides Collagen II, the other major structural component of cartilage extracellular matrix is aggrecan that gives cartilage its characteristic compressive stiffness. Aggrecanases (or ADAMTS or A Disintegrin And Metalloproteinase with ThromboSpondin motifs) are a family of enzymes implicated in tissue remodeling and cleavage of aggrecan core protein between Glu^{373}-Ala^{374} (10-12). Thus blocking IL-1-induced enzymes or their gene expression by novel physiologic and pharmacological inhibitors (13) is an important therapeutic approach for arthritis (14). Due to cardiovascular and gastrointestinal side effects of current anti-arthritic drugs, there is need for discovering novel non-toxic and natural therapeutic agents. Curcumin (diferuloylmethane) is a natural product from the rhizome (roots) of *Curcuma longa*, which has multiple anti-inflammatory and anti-cancer activities (15). Nordihydroguaiaretic acid (NDGA) is food preservative from *Larrea divaricata* and *Larrea tridentat*a (16), which also has lipoxygenase inhibiting properties. Quercetin from onions and green beans has PI3-kinase inhibiting activity among others (17). Resveratrol is a natural polyphenolic ingredient of red grape skin with anti-oxidant activities (18). The aim of this study was to screen for natural products with the ability to inhibit IL-1-induced ADAMTS-4 gene expression in human articular chondrocytes. We report that curcumin, NDGA, Quercetin and Resveratrol have such capability.

Materials and Methods

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

Chondrocyte cell culture and treatments: The normal human knee articular chondrocytes at passage 2 (from Cambrex) were grown in 6-well plates as high-density primary monolayer cultures to confluence in DMEM supplemented with 10% FCS and growth factors that stimulate chondrocyte differentiated phenotype. 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 natural products dissolved in ethanol or dimethyl sulfoxide (DMSO) at different doses and stimulated further for 24 h with IL-1β (10 ng/ml).

RNA extraction and RT-PCR: Total RNA was extracted as previously described (19) and aliquots of 3-5 μg 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 μg of RNA was heated for 5 min at 65^{o}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 aggrecanase-1-specific primers. The amplification profile was one cycle at 94^{o}C for one min, 35 cycles of 94^{o}C for one min, hybridization at 60^{o}C for 2 min and extension at 72^{o}C for 3 min., followed by one extension cycle of 7 min at 72^{o}C. The PCR was performed in a DNA cycler (Techne, Princeton, NJ) in a 50 μl reaction with 1.25 mM dNTPs, Taq DNA polymerase (Pharmacia) and respective primers (20). Aliquots of 10 μl from the 50 μl PCR reaction were analyzed on 1.4 % agarose gels to detect ADAMTS-4 and glyceraldehyde 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.

Results

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

Since IL-1 activates MAPK pathways such as JNK/SAPK in chondrocytes (9) and cucumin inhibits this signalling cascade (21, 22) as well as NF-κB pathway (23, 24), we investigated the role of JNK/SAPK, AP-1 and NF-κB pathways in the regulation of ADAMTS-4 genes expression. IL-1β clearly induced ADAMTS-4 expression in high-density human chondrocyte monolayer cultures within 24 h of treatment. Pretreament of chondrocytes with 15 μM of curcumin followed by IL-1 stimulation resulted in considerable suppression of ADAMTS-4 RNA induction in human chondrocytes.
The levels of internal control, GAPDH mRNA did not change by these treatments (Fig.1)

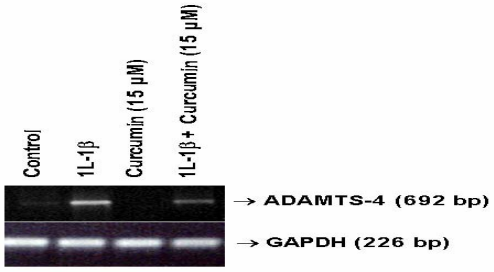

Figure 1. RT-PCR analysis of ADAMTS-4 and GAPDH gene expression by agarose gel showing induction by Interleukin-1 and inhibition by Curcumin.

Nordihydroguaiaretic acid (NDGA) suppresses induction of ADAMTS-4 by IL-1
Since ADAMTS-4 promoter contain AP-1 and AP-2 transcription factor binding sites (25) and activation of MAPK pathways results in downstream activation of c-fos (ERK) and c-jun (JNK), AP-1 components, we investigated the role of transcription factors AP-1 in the regulation of ADAMTS-4 gene expression. Nordihydroguaiaretic acid (NDGA) inhibits lipoxygenase which subsequently blocks arachidonic acid metabolites and thus suppresses expression of the c-fos component of AP-1 (26). Pretreatment of chondrocytes with this AP-1 inhibitor at 20 μM partially abrogated ADAMTS-4 RNA induction by IL-1 without affecting constitutive GAPDH mRNA levels (Fig. 2).

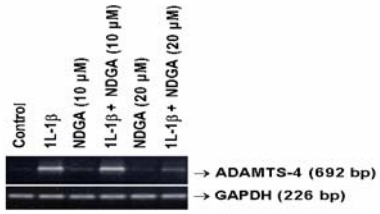

Figure 2. RT-PCR analysis of ADAMTS-4 and GAPDH gene expression by agarose gel showing induction by Interleukin-1 and inhibition by nordihydroguiaretic acid.

Quercetin down-regulates IL-1-induced ADAMTS-4 gene expression
Quercetin has been shown to decrease MMP-2 and MMP-9 expression in prostate cancer cells (27).

To investigate its impact on ADAMTS-4 induction in cartilage cells, chondrocytes were pre-exposed to Qurcetin 50-100 μM and then induced with IL-1. Quercetin dose-dependently decreased IL-1-induced ADAMTS-4 expression. However inhibition at 100 μM dose was partial. The expression of GAPDH internal control remained constant (Fig 3).

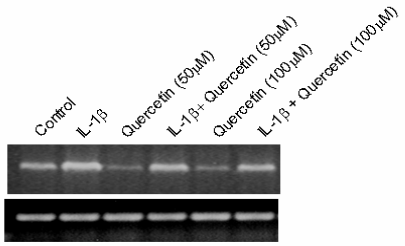

Figure 3. RT-PCR analysis of ADAMTS-4 and GAPDH gene expression by agarose gel showing induction by Interleukin-1 and partial inhibition by quercetin.

Resveratrol partially suppresses IL-1-induced expression of ADAMTS-4 gene
Resveratrol, an active natural ingredient of red grape skin has been recently shown to inhibit tumor necrosis factor-alpha (TNF-α)-induced MMP-9 in human vascular smooth muscle cells (28). We explored its ability to regulate ADAMTS-4 in human chondrocytes. Pretreatment with resveratroal at 100 μM partially reduced ADAMTS-4 RNA induction. The constitutive expression of GAPDH mRNA was not affected (Fig 4).

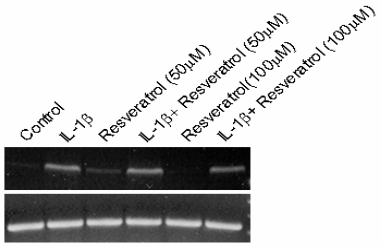

Figure 4. RT-PCR analysis of ADAMTS-4 and GAPDH gene expression by agarose gel showing induction by Interleukin-1 and partial inhibition by resveratrol.

Discussion

We have demonstrated here that proinflammatory cytokine, IL-1-induced cartilage aggrecan-degrading enzyme expression can be inhibited by the active plant-originated natural products such as Curcumin, NDGA, Quercetin and Resveratrol in human articular chondrocytes. While molecular mechanisms of this inhibition are under current investigation, these results suggest potential anti-arthritic activities of these agents.

Inhibition of ADAMTS-4 expression by curcumin suggests the implication of JNK, AP-1 and NF-κB pathways in gene expression as this anti-inflammatory agent from *Curcuma longa* has multiple activities (15) including suppression of JNK phosphorylation and transcription factors AP-1 and NF-κB binding (21-24, 29). Curcumin protects chondrocytes from deleterious effects of IL-1 such as inhibition of type II collagen synthesis and caspase-3 mediated apoptosis (30). Curcumin also has reactive oxygen species-scavenging or antioxidant activities (31). These properties of curcumin make it an interesting prototype for new anti-arthritic agent.

Potent decrease in ADAMTS-4 expression by NDGA may involve blockade of lipoxygenase (LOX) activity and subsequent inhibition of arachidonic acid metabolites such as 5-HPETE that is known to induce c-fos (26). Cyclooxygenase and LOX pathways are important targets of anti-arthritic drugs (32). NDGA can block TNF-α-induced expression of vascular cell adhesion molecules, an important inflammatory mediator in endothelial cells, via inhibition of AP-1 (33).

This agent also prevents the formation of fos-jun-DNA complex formation and AP-1 activity (34). Thus blocking AP-1 activity by inhibition of LOX pathway and c-fos expression may be useful to inhibit degradation of cartilage by ADAMTS and MMPs. Indeed, *Larrea divaricata* extract with elevated content of NDGA has recently been shown to have anti-inflammatory activity (35).

Our results are supported by recent studies where Curcumin and Quercetin were shown to have anti-inflammatory activity by blocking neutrophil activation, synoviocyte proliferation and angiogenesis (36). This bioflavonoid has also been shown to inhibit NF-κB activity in synovial cells (37). By comparing several flavonoids, Quercetin was found to be most effective in carrageenan-induced paw edema (38). Anti-arthritic activity of oral Quercetin may partly due to the observed inhibition of ADAMTS-4 and MMPs.

Our in vitro results with ADAMTS-4 inhibition by resveratrol are supported by in vivo observations with rabbit model of osteoarthritis where this agent significantly reduced cartilage destruction (39). This agent also potently inhibits macrophage migration inhibitory factor, an important factor in the pathogenesis of RA (40). It may work through inhibition of protein kinase C delta and JNK signalling mediators (41).

Plant-based anti-arthritic agents are being used by patients all over the world including the industrialized nations (42, 43). There is increasing interest to decipher the mechanism of their action through latest tools in pharmacology and molecular biology (44). However as for purified drugs, caution is needed for natural products as genotoxic effects have been reported for Quercetin in bacteria (45).

In summary, several natural products can interfere with proinflammatory cytokine signal transduction pathways (such as ERK, p38 and JNK) or their target transcription factors (AP-1 and NF-κB) and thus inhibit IL-1 induction of ADAMTS-4 in chondrocytes. Such inhibition warrants further studies on toxicology, mechanism and potential for reducing ADAMTS-4-driven cartilage resorption in arthritis.

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