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RESEARCH ARTICLES

Short- and long-term exposure of articular cartilage to curcumin or quercetin inhibits aggrecan loss☆

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Abstract

The aim of this study was to determine if curcumin and quercetin inhibit induced aggrecan loss from bovine articular cartilage explants given that these polyphenols have been shown to suppress the expression of matrix-degrading enzymes. The kinetics of loss of ^{35}S-aggrecan and the loss of total aggrecan in cartilage explants maintained in catabolic medium containing either 1 μM retinoic acid or 50 ng/ml interleukin (IL)-1α were studied in the presence of either 1–25 μM curcumin or 10–50 μM quercetin. The reversibility of catabolism of ^{35}S-aggrecan was also studied in catabolically stimulated cultures treated with 25 μM curcumin or 50 μM quercetin for the initial 4–5 days of culture followed by 10–15 days of culture in catabolic medium in the absence of either polyphenol. Curcumin and quercetin suppressed ^{35}S-aggrecan and total aggrecan loss from the explants in a dose-dependent manner. When the exposure of explants to curcumin or quercetin was limited to the first 4–5 days of culture, the suppression of ^{35}S-aggrecan loss was maintained in the extended culture period when the tissue was stimulated with either retinoic acid or IL-1α. Quercetin suppressed IL-1α-stimulated expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4. Curcumin suppressed retinoic acid stimulated expression of ADAMTS-5, and both polyphenols suppressed basal expression of ADAMTS-5. The ability of curcumin and quercetin to protect cartilage from stimulated aggrecan loss and to maintain this protection posttreatment may, at least in part, be due to the suppression of gene expression of ADAMTS-4 and -5.

Keywords: Curcumin; Quercetin; Aggrecan; Cartilage; ADAMTS-4; ADAMTS-5

1. Introduction

The depletion of aggrecan from articular cartilage in osteoarthritis renders the collagen network susceptible to proteolysis, and this then leads to irreversible tissue damage and loss from the surface of articulating bones [1,2]. The aggrecanases a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5 are major aggrecan degrading enzymes in diseased and normal cartilage [3,4]. The regulation of these enzymes in health and disease is not well known, and a treatment that would arrest the stimulated activity of these enzymes in chronic disease is likely to contribute significantly towards providing effective treatments of osteoarthritic cartilage [5].

The current treatments for osteoarthritis reduce pain and inflammation but have no significant effect on disease progression. Effective treatments that would reverse the disease have not been developed, although a limited number of studies have suggested that some antiarthritic agents have the ability to arrest the pathologic process in humans and animals [6,7]; however, further research is required to establish the efficacy of such treatments [8]. In addition to pharmaceuticals such as nonsteroidal anti-inflammatory drugs, nutraceutical therapies are being considered for the treatment of osteoarthritis. The antioxidant, antiproliferative and anti-inflammatory activities of naturally occurring polyphenols such as curcumin and quercetin that are present in vegetables and fruit suggest that these compounds might be useful in the development of new treatments for osteoarthritis [9,10]. Additionally, curcumin has been shown to affect the signaling pathways involved in the expression of matrix-degrading enzymes including matrix metalloproteinases (MMPs) and aggrecanases in chondrocytes and other cells from synovial tissues [9,10]. Similar effects of quercetin on other cell types have also been observed [11]. Curcumin has been reported to ameliorate cytokine stimulated transcription of MMP and ADAMTS genes by suppressing the activation of transcription factors nuclear factor κB (NF-κB) and AP1 [12–15]. These studies have concentrated on the effect of naturally occurring polyphenolic compounds such as quercetin and curcumin on the gene expression of different matrix-degrading enzymes in cell cultures. The studies on the effect of these compounds on the gene expression of matrix-degrading enzymes or catabolism of specific matrix components in tissue explants where

Abbreviations: NF-κB, nuclear factor κB; IL, interleukin; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; MMP, metalloproteinases.

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chondrocytes remain within their native extracellular matrix are lacking. This present study used bovine articular cartilage cultures where chondrocytes are maintained within the native matrix to investigate if curcumin and quercetin can suppress interleukin (IL)-1α or retinoic acid stimulated aggrecan loss from the tissue and if the effect is reversible upon withdrawal of either agent. In order to elucidate a potential mechanism of action of curcumin and quercetin on aggrecan catabolism, their effect on the expression of aggrecanase genes was also determined.

2. Materials and methods

2.1. Materials

Curcumin, quercetin and retinoic acid were from Sigma Chemical Co. (St. Louis, MO, USA). Recombinant human interleukin-1α (rHuIL-1α) was from Peprotech (NJ, USA); Aurum Total RNA Fatty and Fibrous Tissue kit, cDNA synthesis kit and IQ SYBR-Green kit for reverse transcriptase polymerase chain reaction (RT-PCR) were from Bio-Rad (Australia).

2.2. Articular cartilage explants

Articular cartilage from bovine metacarpophalangeal joints was maintained in Dulbecco's modified Eagle's medium (DMEM) containing 20% (vol/vol) NBCS for 1 day at 37°C [16]. The tissue (3–4 g/joint) was then incubated with [^{35}S]sulfate (30 μCi/ml) in DMEM containing 20% (vol/vol) NBCS (10 ml medium/1 g wet weight tissue) for 6 h at 37°C [16]. The tissue was distributed into individual vials and cultured (~100 mg tissue/4 ml medium) for 7 days in DMEM with 1 μM retinoic acid and 0, 1, 10 and 25 μM curcumin or 0, 10, 25 and 50 μM quercetin. The culture medium was changed daily, and the collected medium was stored at −20°C in the presence of proteinase inhibitors [17]. To determine the sulfated glycosaminoglycan content of cartilage, at the end of the culture period, the tissue was extracted with 0.5 M NaOH for 24 h and stored at −22°C. In other experiments, after incubation with [^{35}S]sulfate, the tissue was distributed into individual vials as above and cultured for 6 days in 4 ml DMEM or DMEM with 50 ng/ml IL-1α with or without 25 μM curcumin or 50 μM quercetin. The culture medium was changed every 2 days. Collected medium and tissue extracts from the end of culture period were stored as above.

In other experiments investigating whether the effect of either polyphenol on stimulated aggrecan loss is reversible, after incubation with [^{35}S]sulfate, explants were maintained for 5 days in DMEM or DMEM with 1 μM retinoic acid or DMEM with retinoic acid and 25 μM curcumin, or DMEM with retinoic acid and 50 μM quercetin. On day six of culture, the medium of the explants was switched to DMEM with retinoic acid for an additional 15 days. In other experiments using 50 ng/ml IL-1α, explants were maintained for 4 days in DMEM or DMEM with IL-1α or DMEM with IL-1α and 25 μM curcumin, or DMEM with IL-1α and 50 μM quercetin. On day five of culture, the medium of the explants was switched to DMEM with IL-1α for an additional 10 days.

2.3. Measurement of expression of ADAMTS-4 and -5 by real-time quantitative PCR

Cartilage was cultured in the presence of 20% (NBCS) for 1 day, distributed into separate vials and maintained (100 mg tissue/4 ml medium) in either DMEM or DMEM containing 1 μM retinoic acid with or without 25 μM curcumin or 50 μM quercetin or DMEM containing 50 ng/ml IL-1α with or without 25 μM curcumin or 50 μM quercetin for 3 days. At the end of culture period, tissue samples were frozen in liquid N_2 and stored at −86°C until further use.

Total RNA was isolated from 100 mg tissue and subjected to RT-PCR analysis for ADAMTS-4, ADAMTS-5 and GAPDH as described previously [18]. The RNA levels in cartilage were 25±9 ng RNA/mg tissue, the 260/280 ratio was 2.1±0.05 and the 260/230 ratio was 2.0±0.1 determined by NanoDrop 2000 Spectrophotometer (Thermo Scientific). Listed are primers and gene accession number and base pair length of product in brackets: ADAMTS-4 (AF192770, 102), forward primer 5′-CACTGACTTCC-TAGACAATGG -3′ and reverse primer 5′-TCAGCGTCGTAGTCCTTG -3′; ADAMTS-5 (AF192771, 139), forward primer 5′-TCGGCTCCACGGAAGATAAG -3′ and reverse primer 5′-GGTAGGTCCAGCAAACAGTTAC -3′ and GAPDH (U85042, 123) forward

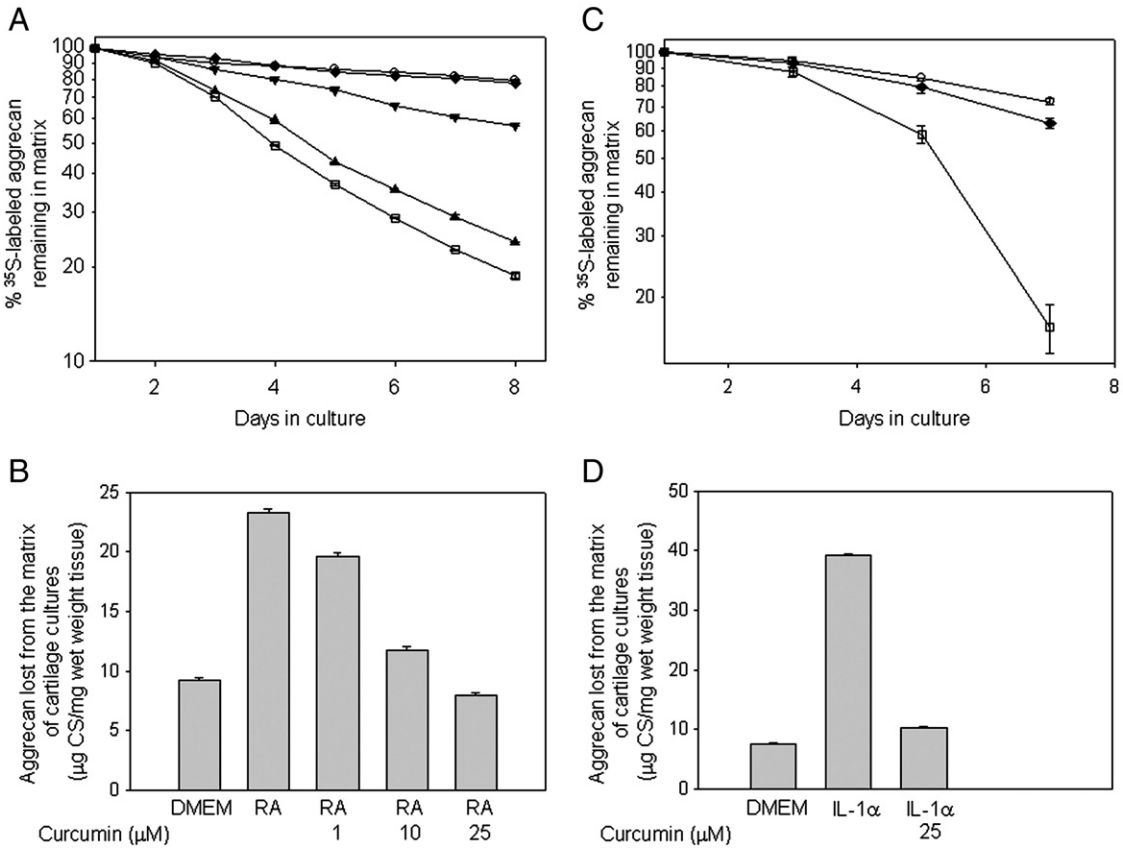

Fig. 1. Curcumin suppresses loss of ^{35}S-labeled proteoglycans and total proteoglycans from the matrix of articular cartilage explant cultures stimulated with retinoic acid or IL-1α. Following the incubation of tissue with [^{35}S]sulfate, cartilage explant cultures were stimulated with retinoic acid for 7 days or IL-1α for 6 days and varying concentrations of curcumin. (A) The percentage of radiolabeled proteoglycans remaining in the matrix with time in cultures maintained in DMEM alone (○), stimulated with retinoic acid (□) and stimulated with retinoic acid and treated with 1 μM (▲), 10 μM (▼) or 25 μM (♦) curcumin. (B) Total levels of proteoglycans lost to the medium of cartilage cultures during the culture period. (C) The percentage of radiolabeled proteoglycans remaining in the matrix with time in culture in cultures maintained in DMEM alone (○), stimulated with IL-1α (□) and stimulated with IL-1α and treated with 25 μM (♦) curcumin. (D) Total levels of proteoglycans lost to the medium of cartilage cultures during the culture period. Values shown represent the mean and the range of duplicate cultures of tissue from same animal. RA: retinoic acid.

primer 5′-CAAGTTCAACGGCACAGTCAAG-3′ and reverse primer 5′-ACATACTCAGCAC-CAGCATCAC-3′. The specificity of primers was determined from the analysis of the melt curve of the PCR products at the end of each run. Each RNA preparation was assayed in triplicate. The efficiencies of the PCRs for each gene were determined from the dilution series of cDNA and were found to be 99.5% for ADAMTS-4 (r=0.97), 105% for ADAMTS-5 (r=0.99) and 110 for GAPDH (r=0.99).

The values obtained for mRNA expression for ADAMTS-4 and ADAMTS-5 were normalized for GAPDH mRNA in the same sample, and the data were expressed as fold change in gene expression compared to control (cultures maintained in DMEM only). This was calculated according to relative quantification method using the formula $2^{-\Delta\Delta CT}$ where CT is the cycle number of the detection threshold, and ΔΔCT shows the difference in threshold cycle (ΔCT) between aggrecanase gene and GAPDH for control culture subtracted from that of the treatment culture [19].

2.4. Measurement of lactate production, protein and proteoglycan synthesis

Explants were maintained in culture in 100 mg wet weight tissue/4 ml medium in DMEM or DMEM containing 1 μM retinoic acid with or without 25 μM curcumin or 50 μM quercetin for 7 days or DMEM or DMEM containing 50 ng/ml IL-1α with or without 25 μM curcumin or 50 μM quercetin for 6 days. The levels of lactate released into the medium of explants on day 6 or 7 were determined by the oxidase/peroxidase method using a commercial kit from Sigma [20].

At the end of culture period, the protein synthesis using [^{3}H]serine and proteoglycan synthesis using [^{35}S]sulfate were determined as reported previously[20].

2.5. Analysis of data

Cultures investigating aggrecan turnover were maintained in duplicate for each experimental condition, whereas cultures investigating metabolic activity of chondrocytes were maintained in triplicate. All experiments were repeated at least three times using tissue from different animals to confirm that the obtained results were valid. Four separate experiments were performed for RT-PCR analysis. Each experiment involved tissue from a single animal, and the tissue from each treatment group was analyzed in triplicate. The results were expressed as fold change relative to control (DMEM). Comparison of differences between treatment groups was done using the Kruskal–Wallis test (analysis of variance by ranks) using SPSS® version 14 (Chicago, IL, USA). Significant differences between the treatment groups were analyzed by the two-tailed Mann–Whitney U test. P values <.05 were taken as significant.

3. Results

3.1. Curcumin and quercetin suppressed the loss of radiolabeled and endogenous aggrecan from articular cartilage explants stimulated with retinoic acid or IL-1α

Fig. 1(A, B) shows that curcumin (1–25 μM) inhibited radiolabeled and endogenous aggrecan loss from the matrix of articular cartilage explants stimulated with 1 μM retinoic acid in a dose-dependent manner. Curcumin (25 μM) also inhibited radiolabeled and endogenous aggrecan loss from the matrix of articular cartilage explants stimulated with 50 ng/ml IL-1α (Fig. 1C, D).

Fig. 2(A, B) shows that quercetin (10–50 μM) inhibited radiolabeled and endogenous aggrecan loss from the matrix of articular cartilage explants stimulated with retinoic acid in a dose-dependent manner. In cultures stimulated with IL-1α, quercetin (50 μM) also inhibited radiolabeled and endogenous aggrecan loss from articular cartilage explants (Fig. 2C, D).

These effects were not due to the toxicity of either polyphenol since, in comparison to the relative controls (see below), the lactate

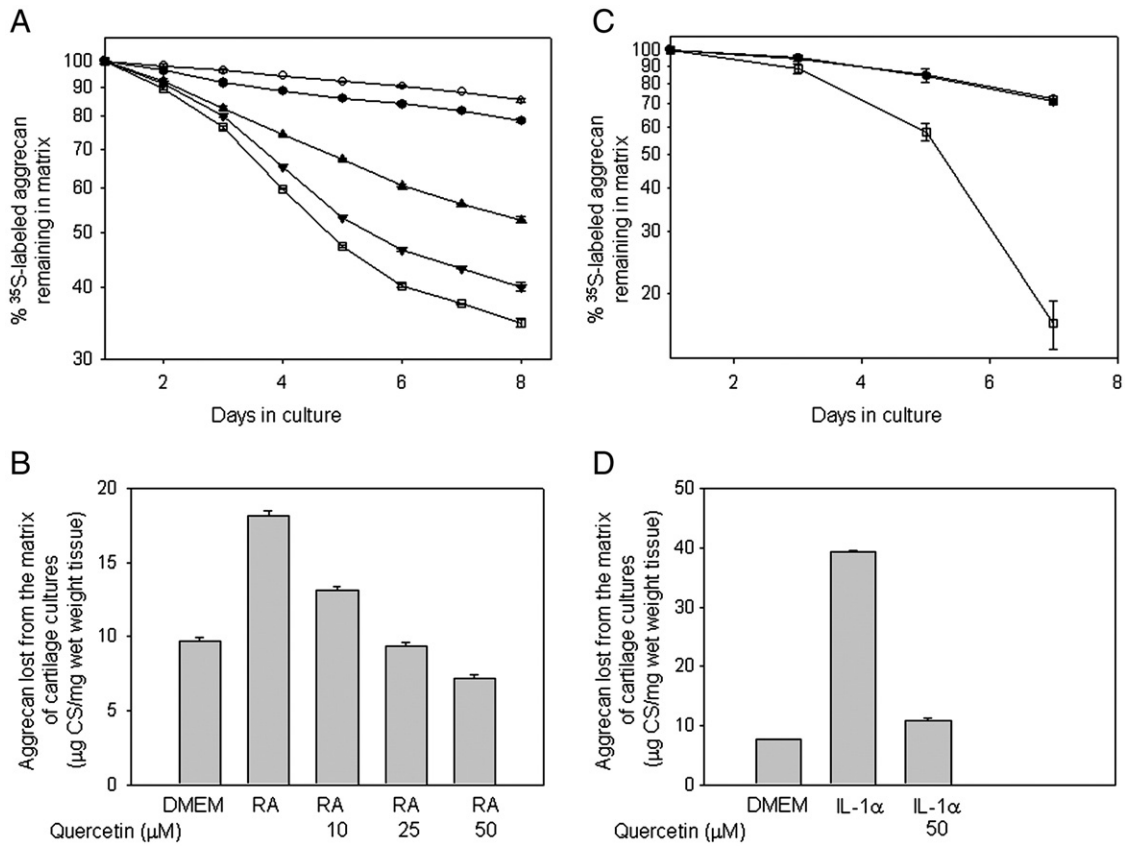

Fig. 2. Quercetin suppresses loss of ^{35}S-labeled proteoglycans and total proteoglycans from the matrix of articular cartilage explant cultures stimulated with retinoic acid or IL-1α. Following the incubation of tissue with [^{35}S]sulfate, cartilage explant cultures were stimulated with retinoic acid for 7 days or IL-1α for 6 days and varying concentrations of quercetin. (A) The percentage of radiolabeled proteoglycans remaining in the matrix with time in cultures maintained in DMEM alone (○), stimulated with retinoic acid (□) and stimulated with retinoic acid and treated with 10 μM (▼), 25 μM (▲) or 50 μM (●) quercetin. (B) Total levels of proteoglycans lost to the medium of cartilage cultures during the culture period. (C) The percentage of radiolabeled proteoglycans remaining in the matrix with time in culture in cultures maintained in DMEM alone (○), stimulated with IL-1α (□) and stimulated with IL-1α and treated with 50 μM (♦) quercetin. (D) Total levels of proteoglycans lost to the medium of cartilage cultures during the culture period. Values shown represent the mean and range of duplicate cultures of tissue from same animal. RA: retinoic acid.

Table 1
Lactate production and incorporation of [^{3}H]leucine and [^{35}S]sulfate into macromolecules by bovine articular cartilage cultured with retinoic acid or IL-1α and quercetin or curcumin

Culture conditions	Days in culture	Lactate concentration in medium (mg lactate/100 mg wet weight tissue/24 h)	[^{3}H]serine incorporation (cpm/100 mg wet weight of tissue/2 h)	[^{35}S]sulfate incorporation (cpm/100 mg wet weight of tissue/2 h)
Medium alone	7	0.746±0.045	2690±84	50444±7611
Medium+50 μM quercetin	7	0.689±0.031 (92)	2612±332 (97)	51759±3301 (103)
Medium+25 μM curcumin	7	0.699±0.089 (94)	2558±144 (95)	51189±5605 (101)
Retinoic acid	7	1.033±0.106 *	2269±134	26607±3146 *
Retinoic acid+50 μM quercetin	7	0.906±0.091 *,** (88)	2486±162 ** (110)	27713±885 *,** (104)
Retinoic acid+25 μM curcumin	7	0.889±0.090 *,** (86)	2712±437 ** (120)	27339±1735 *,** (103)
Medium alone	6	0.897±0.172	2881±294	65856±13690
Medium+50 μM quercetin	6	0.801±0.104 (89)	2645±101 (91)	62032±4644 (94)
Medium+25 μM curcumin	6	0.769±0.043 (86)	2492±122 (87)	66684±8563 (101)
IL-1α	6	0.755±0.218 *	1940±128 *	69326±5853
IL-1α+50 μM quercetin	6	0.758±0.116 *,** (100)	2284±217 *,** (118)	69188±7968 ** (100)
IL-1α+25 μM curcumin	6	0.726±0.101 *,** (96)	2342±116 *,** (121)	70229±8887 ** (101)

Values shown are the mean±S.D. of triplicate determinations. Values in parenthesis are percentage of value observed for cultures maintained in medium alone or medium containing retinoic acid.

* Differences from cultures maintained in DMEM according to Mann–Whitney test ($P \leq .05$).

** Differences from cultures stimulated with retinoic acid or IL-1α according to Mann–Whitney test ($P \geq .05$).

production and protein and proteoglycan synthesis by the explants were not affected in their presence (Table 1). Further, Table 1 shows that retinoic acid had some effect on the cellular metabolism shown by the increase in lactate production and a decrease in the proteoglycan synthesis [20,21]. Additionally, IL-1α down-regulated lactate production by the cartilage explants by 16% and protein synthesis by 23% in comparison to the unstimulated cultures.

3.2. Pretreatment with curcumin or quercetin of retinoic-acid- or IL-1α-stimulated articular cartilage cultures suppressed the loss of radiolabeled aggrecan from the matrix

Curcumin and quercetin at concentrations of 25 μM and 50 μM, respectively, were used to test the reversibility of suppression by these agents of stimulated aggrecan catabolism. This involved a

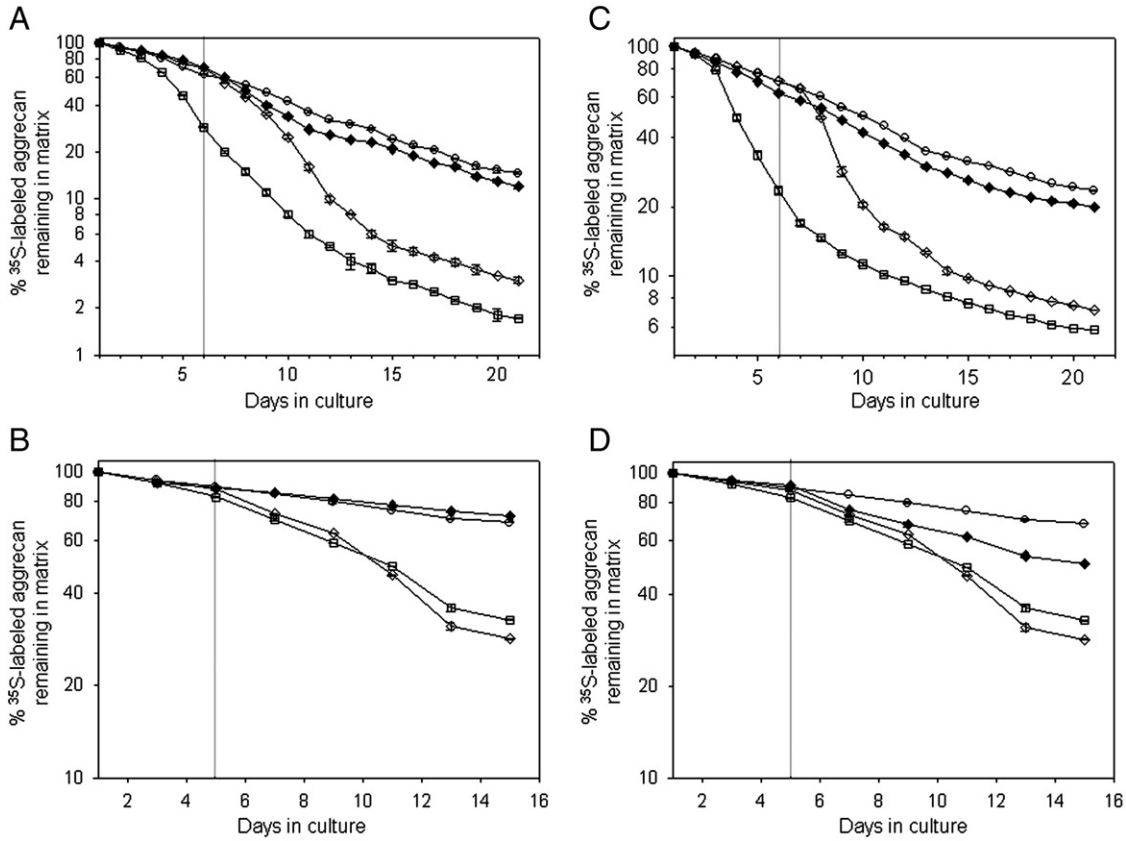

Fig. 3. Pretreatment with curcumin or quercetin suppresses retinoic-acid- or IL-1α-stimulated ^{35}S-labeled aggrecan loss from articular cartilage cultures. (A) Following the incubation of tissue with [^{35}S]sulfate, cartilage explants were maintained in DMEM containing retinoic acid and 25 μM curcumin for 5 days and then continued in DMEM with retinoic acid (♦) for an additional 15 days. Control cultures were maintained in DMEM (○) or DMEM with retinoic acid (□) for 20 days or DMEM for 5 days and then stimulated with retinoic acid for additional 15 days (◇). (B) Following the incubation of tissue with [^{35}S]sulfate, cartilage explants were maintained in DMEM containing IL-1α and 25 μM curcumin for 4 days and then continued in DMEM with IL-1α (♦) for additional 10 days. Control cultures were maintained in DMEM (○) or DMEM with IL-1α (□) for 14 days or DMEM for 4 days and then stimulated with IL-1α for additional 10 days (◇). (C) Same as in "A" except 50 μM quercetin was used instead of curcumin. (D) Same as in "B" except 50 μM quercetin was used instead of curcumin. Values shown represent the mean and range of duplicate cultures of tissue from same animal. The interrupted line indicates the end of treatment with curcumin or quercetin in cultures stimulated with retinoic acid or IL-1α (♦).

period of pretreatment of cartilage cultures with curcumin or quercetin in the presence of the catabolic stimulant followed by an extended culture period in the presence of the catabolic stimulant.

The levels of radiolabeled aggrecan remaining in the matrix at the end of culture period in cultures pretreated with curcumin and stimulated with retinoic acid were similar to those for cultures maintained in DMEM alone: 12% and 14%, respectively. In cultures stimulated with retinoic acid in the absence of curcumin, either for the entire culture period or the last 15 days of culture, 2%–3% of radiolabeled aggrecan remained in the matrix by the end of culture period (Fig. 3A). A similar trend was observed in IL-1α-stimulated cultures where, at the end of culture period, approximately 70% of radiolabeled aggrecan remained in the matrix of cultures pretreated with curcumin. Similar levels of radiolabeled aggrecan were observed in cultures maintained in DMEM alone whereas only 30% radiolabeled aggrecan remained in the matrix of cultures stimulated with IL-1α in the absence of curcumin (Fig. 3B). These results indicate that pretreatment with curcumin suppressed retinoic-acid- and IL-1α-stimulated loss of radiolabeled aggrecan.

A pretreatment with quercetin also suppressed the stimulated loss of radiolabeled aggrecan. In cultures pretreated with quercetin, at the end of culture period, 20% of radiolabeled aggrecan remained in the matrix compared to 24% for cultures maintained in DMEM only or 6%–7% for cultures stimulated with retinoic acid in the absence of quercetin (Fig. 3C). In IL-1α-stimulated cultures, following a pretreatment with quercetin, 50% of radiolabeled aggrecan remained in the matrix by the end of culture period, compared to 68% in unstimulated or 28%–33% in IL-1α-stimulated cultures in the absence of quercetin (Fig. 3D).

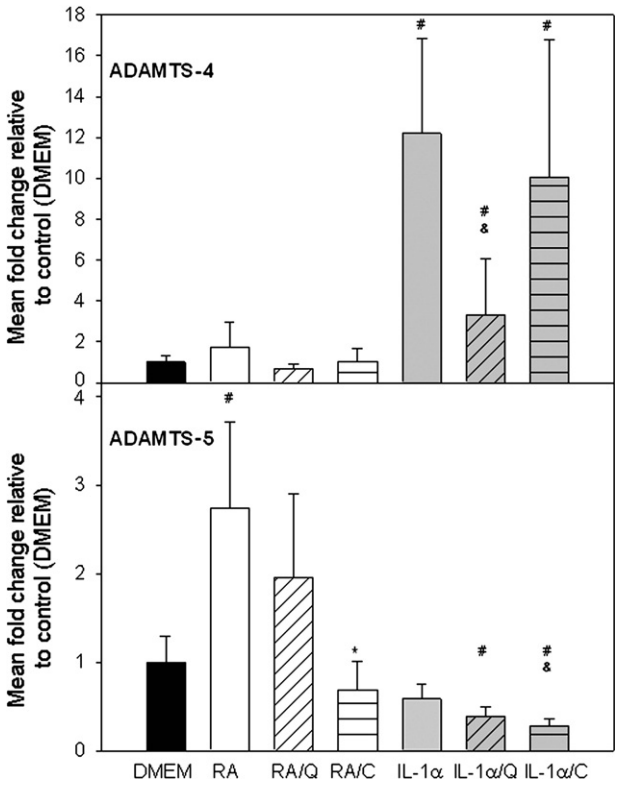

Fig. 4. Effect of quercetin and curcumin on ADAMTS-4 and -5 gene expression in retinoic-acid- or IL-1α-stimulated cultures. Total RNA from four separate experiments was isolated and analyzed in triplicate by RT-PCR. The fold change of ADAMTS-4 and -5 mRNA in comparison to control (DMEM alone) was normalized to GAPDH. Culture incubated with DMEM alone (black bar, DMEM), DMEM with 1 μM retinoic acid (white bar, RA), DMEM with 1 μM retinoic acid and 50 μM quercetin (diagonal hatch, RA/Q) or 25 μM curcumin (horizontal hatch, RA/C) or DMEM with 50 ng/ml IL-1α (shaded bar, IL-1α), DMEM with 50 ng/ml IL-1α and 50 μM quercetin (diagonal hatch, IL-1α/Q) or 25 μM curcumin (horizontal hatch, IL-1α/curcumin). Values shown are the mean±S.D. for four experiments. $P<.05$ #vs. control (DMEM alone), *vs. DMEM with retinoic acid, &vs. DMEM with IL-1α.

3.3. Curcumin and quercetin suppressed the gene expression of ADAMTS-5 and ADAMTS-4

Interleukin-1α stimulated the expression of ADAMTS-4 (Fig. 4), and this was partially suppressed by quercetin but not curcumin. Retinoic acid did not affect the expression of ADAMTS-4.

The ADAMTS-5 expression was stimulated by retinoic acid, and this was suppressed by curcumin. Interleukin-1α did not affect the expression of ADAMTS-5. Curcumin and quercetin suppressed the expression of ADAMTS-5 beyond basal levels in cultures maintained in the presence of IL-1α (Fig. 4).

The high efficiencies for both enzymes made it possible to compare the relative expression levels in different culture conditions. The ADAMTS-5 expression was higher in control cultures where the difference between the CT values for the two genes was five cycles. This difference did not change significantly in the retinoic-acid-stimulated cultures, but the gene expression levels for the two enzymes were similar in the IL-1α-stimulated cultures.

4. Discussion

Both curcumin and quercetin suppressed the loss of radiolabeled and total aggrecan from IL-1α- and retinoic-acid-stimulated cartilage explants in a dose-dependent manner. In addition, a short-term exposure of cartilage explants to curcumin or quercetin in the presence of the catabolic stimulators retinoic acid or IL-1α had the effect of suppressing aggrecan loss in the extended culture period of 10–15 days. As shown in Table 1, these effects were not due to cytotoxicity of curcumin or quercetin. This agrees with a recent report on human articular cartilage showing that up to 20 μM curcumin was not toxic to the explants [22]. There are contradictory reports on the toxicity of a higher concentration of curcumin (50 μM) on cell cultures [23]. At this concentration, curcumin was reported to decrease the viability of immortalized human chondrocyte cell line [23] but did not have an effect on the viability of primary human chondrocytes [24]. Quercetin at concentrations above 50 μM did not appear to affect the viability of human chondrocytes [25].

There are no studies investigating the effect of quercetin on aggrecan loss in cartilage explants; however, curcumin has been reported to inhibit the loss of radiolabeled aggrecan from human articular cartilage explants cultured under basal conditions but not when stimulated with IL-1β [22], a cytokine that has been associated with the progression of osteoarthritis in humans [1]. The difference between this study and the work reported in this paper may be attributed to the potentially different stimulatory pathways between IL-1α and IL-1β [26] and to differences in species given that human IL-1β does not stimulate aggrecan catabolism in bovine articular cartilage explants [27]. Furthermore, the inhibition of aggrecan loss in IL-1β-stimulated equine articular cartilage explants has been reported at 100 μM but not at lower curcumin concentrations [28] such as those used in this present study. In addition, the viability of chondrocytes has not been determined at 100 μM curcumin; and therefore, the authors did not discard the contribution of possible toxic effects of 100 μM curcumin on the inhibition of aggrecan loss in equine cartilage explants [23,28].

It has been established that the loss of aggrecan from cartilage explants stimulated with either retinoic acid or IL-1 is mainly due to the stimulation of aggrecanase activity [29,30]. This activity in normal and diseased cartilage is primarily due to ADAMTS-4 and -5 [3,4,31–34]; however, in murine cartilage, ADAMTS-5 plays a

major part in aggrecan degradation, with a small contribution from ADAMTS-4 [35–37].

The biochemical pathways leading to the stimulation of aggrecanase activity are not well understood. In both cytokine-stimulated normal cartilage and unstimulated osteoarthritic cartilage explants, increased mRNA expression of aggrecanase genes has been shown to contribute to the enhanced aggrecanase activity [33]. However, another study has linked the increased levels of aggrecanase activity with the activation of the endogenous ADAMTS-4 in the IL-1-stimulated explants [38]. Given that quercetin suppressed the expression of ADAMTS-4 in the IL-1-stimulated cultures while curcumin suppressed the expression of ADAMTS-5 in the retinoic-acid-stimulated cultures and both polyphenols suppressed the expression of ADAMTS-5 beyond basal levels in cultures maintained in the presence of IL-1α, this present study suggests that the aggrecanase activity may at least in part be regulated at the transcriptional level by curcumin and quercetin. A recent review pointed out the complexity involved in the regulation of aggrecanase activity at pre- and posttranscriptional level in addition to a current lack of understanding of which of the two recognised aggrecanases, ADAMTS-4 and ADAMTS-5, might prevail at any stage in the pathogenesis of osteoarthritis [39]. The difficulties in distinguishing between these two proteinases are further exemplified by studies that indicated that proteolytic processing of aggrecan core protein does not differ between the two proteinases. Thus, studies on aggrecanase gene expression along with the other steps involved in the regulation of ADAMTS-4 or ADAMTS-5 will contribute to the understanding of the regulation of aggrecanase activity. The mechanism of action of curcumin and quercetin may also contribute to the modulation of the cellular signaling involved in the gene expression of MMPs [12,13,15]. These enzymes have been implicated in the activation of aggrecanases [14,40,41]. Furthermore, it is unlikely that curcumin and quercetin directly inhibited aggrecanase activity since these agents did not inhibit digestion of aggrecan monomer using aggrecanase preparation from synovial capsule explants [30].

Naturally occurring polyphenolic compounds have been shown to have many biological actions, and their suppression of aggrecan catabolism including the long-term sustained suppression of this process in cartilage explants may also result from their antioxidative ability to scavenge for the reactive oxygen species and to suppress nitric oxide production [42]. These molecules are important modulators of intracellular signal transduction in osteoarthritis [43]. Reactive oxygen species may also play a role in depolymerisation of hyaluronan, which is likely to cause the loss of aggrecan complexes from the cartilage matrix [27,44]. Anti-inflammatory activity such as the suppression of NF-κB activity and down-regulation of catabolic factors may also play a role in the suppression of aggrecan loss [9,24,42,45–47]. It would also be of interest to determine in chondrocytes the impact of polyphenols on the production of the endogenous inhibitors of matrix metalloproteinases (TIMPs) particularly TIMP-3, which has the highest activity against aggrecanases [48].

As mentioned above, curcumin and quercetin are present in vegetables and fruit, and it has been shown that these compounds can be tolerated at large doses. Human studies have shown that oral dose of up to 8 g curcumin per day was not toxic, and animal studies using quercetin showed that deleterious effects resulted after 18 months of treatment, at doses above 0.4 g/kg/d [49,50]. The bioavailability of these compounds, however, is low [49,51,52]. This is because they are rapidly metabolized, show poor solubility and stability in aqueous solutions and bind extensively to plasma proteins [53–56]. The maximum concentration of curcumin in plasma detected in humans was 1.8 μM [49], which is below the concentrations of polyphenols employed in this present study. Clearly, there is a need to improve the bioavailability of curcumin and quercetin to realize their potential in the treatment of osteoarthritis. Recent and future studies of natural and synthetic derivatives of polyphenols aim to improve the stability, bioavailability and biological activity of these compounds [54,57–61].

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