Glucosamine inhibits IL-1β-induced NFκB activation in human osteoarthritic chondrocytes


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Summary

Objective: Glucosamine sulfate (GS) is a commonly used drug for the treatment of osteoarthritis. The mechanism of the action of this drug does, however, remain to be elucidated. In human osteoarthritic chondrocytes (HOC) stimulated with a proinflammatory cytokine, we studied whether GS could modify the NFκB activity and the expression of COX-2, a NFκB-dependent gene.

Methods: Using HOC in culture stimulated with interleukin-1β (IL-1β), the effects of GS on NFκB activation, nuclear translocation of NFκB/Rel family members, COX-1 and COX-2 expressions and syntheses and prostaglandin E2 (PGE2) concentration were studied.

Results: GS significantly inhibited NFκB activity in a dose-dependent manner, as well as the nuclear translocation of p50 and p65 proteins. Furthermore, GS-preincubated IL-1β-stimulated HOC showed an increase in IκBα in the cell cytoplasm in comparison with HOC incubated with IL-1β alone. GS also inhibited the gene expression and the protein synthesis of COX-2 induced by IL-1β, while no effect on COX-1 synthesis was seen. GS also inhibited the release of PGE2 to conditioned media of HOC stimulated with IL-1β.

Conclusions: GS inhibits the synthesis of proinflammatory mediators in HOC stimulated with IL-1β through a NFκB-dependent mechanism. Our study further supports the role of GS as a symptom- and structure-modifying drug in the treatment of OA.

Key words: Osteoarthritis, Glucosamine sulfate, Nuclear factor kappa B, COX-2, Prostaglandin E2.

Introduction

Osteoarthritis (OA) is the most common joint disorder with an immense socio-economic impact. OA is characterized by quantitative and qualitative changes in the architecture and composition of all the joint structures. An altered imbalance between the biosynthesis and the degradation of matrix components leads to a progressive destruction of the tissue.

The localized nature and lack of signs of general inflammation distinguish OA from systemic inflammatory diseases, such as rheumatoid arthritis, although local inflammatory activity is a well-recognized component of OA. In the past years, some investigators have pointed out the key role of inflammation in OA. Recent studies have shown a superinduction of proinflammatory genes and their corresponding gene products in OA articular cartilage (reviewed in Amin et al.)7. In fact, interleukin-1β (IL-1β), a well-recognized proinflammatory cytokine, is locally increased during the osteoarthritic process. IL-1β induces a large cascade of events that leads to cartilage damage, such as the synthesis of metalloproteases and extracellular matrix proteins that are absent in normal cartilage, the release of other inflammatory mediators, the inhibition of chondrocyte proliferation and induction of cell death.

Nuclear factor kappa B (NFκB) is an ubiquitous protein that specifically binds to DNA consensus sequences, activating its transcription. NFκB exists in the cytoplasm in an inactive form. When an extracellular stimulus induces the phosphorylation of an inhibitory subunit (a member of IκB family) and its subsequent degradation, the active complex becomes capable of migrating to nucleus, where it recognizes the consensus sequences in DNA. NFκB-binding sites are present in the promoter regions of many genes involved in the pathophysiology of joint inflammation and tissue destruction.

Some symptomatic slow-acting drugs, such as glucosamine sulfate (GS), have proved effective in relieving the symptoms of OA. Although GS has been proposed as a scavenger of free oxygen radicals, the mechanism of action of this drug remains to be elucidated. Recent reports suggest that the effects of glucosamine compounds could be due to the control of inflammatory genes expression, but the conclusions seem to be contradictory.

The aim of the present work was to investigate if GS is able to modify the NFκB activation induced by proinflammatory cytokines, such as IL-1β in osteoarthritic chondrocytes. We also looked for subunits participating in NFκB complexes along with the specific activity of GS upon these components. In addition, we studied the effect of GS on the expression and synthesis of a NFκB-dependent gene, such as COX-2. We also assessed whether GS could modify the

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release of prostaglandin E2 (PGE2) to the conditioned media of HOC stimulated by IL-1β.

**Methods**

**CELL CULTURE**

Human osteoarthritic cartilage was obtained after routine total knee replacements from informed donors. Articular cartilages from the femoral condyles and the tibial plateaus were aseptically dissected. Chondrocytes were obtained after sequential digestion with pronase (Roche, 10 g/l) for 30 min and collagenase type IV (Sigma, 1 g/l) for 6 h, both in 0.9% NaCl. Chondrocytes were grown to confluence in DMEM (BioWhittaker) supplemented with 10% fetal calf serum (BioWhittaker), 60 U/ml penicillin, 60 µg/ml streptomycin and 2 mmol/l glutamine (BioWhittaker) at 37°C in the presence of 5% CO₂. Experiments were performed with first or second passage cells. In each experiment, cells were made quiescent for 48 h in DMEM medium without serum and stimulated at different times with IL-1β (Immuno-gex). Where indicated, cells were preincubated with equimolar concentrations of GS (crystalline form, Rottapharm), N-acetylgalactosamine (N-AcG, Sigma) or galactosamine hydrochloride (Gal, Sigma) for 30 min, and these compounds were maintained during the whole period of incubation.

**PREPARATION OF NUCLEAR AND CYTOSOLIC EXTRACTS**

Nuclear and cytosolic extracts were obtained as previously described. After the incubation period, chondrocytes were trypsinized and resuspended in buffer A (10 mmol/l HEPES, pH 7.8, 15 mmol/l KCl, 2 mmol/l MgCl₂, 0.1 mmol/l ethylenediaminetetraacetic acid (EDTA), 1 mmol/l dithiothreitol (DTT), and mmol/l phenylmethylsulfonyl fluoride (PMSF)) and were homogenized. Nuclei and cytosolic fractions were separated by centrifugation at 1000×g for 10 min. The cytosolic fractions (supernatants) were stored at 80°C until Ixbα analysis. The nuclei (pellets) were washed twice in buffer A and resuspended in the same buffer, with a final concentration of 0.39 mol/l KCl. Nuclei were extracted for 1 h at 4°C and centrifuged at 100 000×g for 30 min. Supernatants were dialyzed in buffer C (50 mmol/l HEPES, pH 7.8, 50 mmol/l KCl, 10% glycerol, 1 mmol/l PMSF, 0.1 mmol/l EDTA, and 1 mmol/l DTT) and were then cleared by centrifugation and stored at 80°C. The protein concentration was determined by the bicinchoninic acid (BCA) method.

**ELECTROPHORETIC MOBILITY SHIFT ASSAY**

Transcription factor activity was determined as previously described. Briefly, NFκB or AP-1 consensus oligonucleotides (5′-AGTGGAGGGACTTCCAGGC-3′) and (5′-CGCTTGATGAGTCAGCCGGAA-3′), respectively, were [32P] end-labeled with [γ-32P]ATP and T4 polynucleotide kinase (Promega). Nuclear extracts (5 µg) were equilibrated for 10 min in a binding buffer (4 glycerol, 1 mmol/l MgCl₂, 0.5 mmol/l EDTA, 0.5 mmol/l DTT, 50 mmol/l NaCl, 10 mmol/l Tris–HCl, pH 7.5 and 50 g/ml of poly(dI-dC); Pharmacia LKB), and then the labeled probe (0.35 pmol) was added and incubated for 20 min at room temperature. To establish the specificity of the reaction, negative controls without cell extracts and competition assays with a 100-fold excess of unlabeled oligonucleotide were performed. In competition assays, the corresponding unlabeled probe was added to the reaction mixture 10 min prior to the addition of the labeled probe. Hela cell nuclear extract was used as a positive control of the technique (data not shown). For supershift assays, 1 µg of antibodies was added and incubated with nuclear extracts for 1 h, prior to the addition of labeled probe. The specificity of these antibodies was tested by Western blots (data not shown). The reaction was stopped by adding gel-loading buffer (250 mmol/l Tris–HCl, 0.2% bromophenol blue, 0.2% xylene cyanol, and 40% glycerol) and run on a nondenaturing 4% acrylamide gel in Tris–Borate. The gel was dried and exposed to X-ray film.

**IMMUNOFLUORESCENCE STAINING**

Chondrocytes were grown in eight-well Titer-Tek slides (Costar). To study NFκB proteins, quiescent cells were incubated with 10 U/ml IL-1β with or without GS for 60 min. After incubation, cells were washed, fixed in methanol/acetone (50:50 v/v) for 1 h at −20°C and were then treated with 0.1% Triton X-100 for 1 min on ice to permeabilize nuclear membranes. Cells were incubated with 6% normal goat serum and 4% bovine serum albumin (BSA) in phosphate buffered saline (PBS) (9.1 mmol/l dibasic sodium phosphate, 1.7 mmol/l monobasic sodium phosphate, 150 mmol/l NaCl, pH 7.4) for 1 h at 37°C. Rabbit polyclonal antibodies against p50 and p65 subunits (10 µg/ml) (Santa Cruz Biotech.) were used as primary antibodies, and a 1/200 dilution of FITC-labeled goat antirabbit IgG was used as a secondary antibody (Sigma). Controls were stained with nonimmune serum or with the secondary antibody alone. Preparations were mounted in a 70% glycerol solution and examined under microscope. Images were photographed and printed at equivalent exposures.

**WESTERN-BLOT ANALYSIS**

For IκBα determination, cytosolic fractions were obtained as described in the previous discussion. For COX-1 and COX-2 determinations, cells were homogenized with ice-cold lysis buffer (1% Nonidet P-40 (Sigma); 0.5% SDS, 0.1 mmol/l EDTA, 1 mmol/l DTT and 1 mmol/l PMSF in PBS). The lysates were transferred to eppendorf tubes and centrifuged twice at 12 000×g for 10 min. Protein concentrations were determined by the BCA method. Similar amounts of protein were run on SDS-polyacrylamide gel by electrophoresis and were electrophoretically transferred to polyvinylidene difluoride membranes (Millipore). Membranes were blocked in 0.1 mmol/l Tris, pH 7.4, and 0.1 mmol/l NaCl containing 0.3% Tween-20 and 6% dry skimmed milk for 60 min at room temperature. Then, membranes were incubated overnight with anti-IκBα, anti-COX-1 or anti-COX-2 antibodies (all from Santa Cruz Biotech.) at 4°C. After washing, detections were made by incubation with peroxidase-conjugated secondary antibodies and developed by an enhanced chemiluminescence kit (ECL, Amersham). In order to ensure that equal amounts of total proteins were charged, we also hybridized each membrane with anti-α-tubulin (Sigma).

**TOTAL RNA EXTRACTION AND ANALYSIS OF COX-2 EXPRESSION**

Cells were harvested and total RNA was obtained by the acid guanidinium–phenol–chloroform method. Isolated RNA was reverse transcribed and then amplified with a
commercial kit (Access RT-PCR System, Promega), employing specific primers of human COX-2. PCR analyses for COX-2 and the house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were conducted in the following conditions: 1 min at 94°C to denature the double-stranded DNA, 2 min at 58°C to allow for the annealing of the primers and 2 min at 68°C for primers extension. The primers employed were: (antisense) 5'-TCTTGTCCAGCAGGACTTACG-3' and (sense) 5' CCATTTCAGGATGCTCCTGTT-3' for COX-2 and (anti-sense) 5'-ATACTGTTACTTTACCGATG-3' and (sense) 5'- AATGCTCCTGACACCACCC-3' for GAPDH (515 bp). Amplifications were done for 15, 20, 25, 30 and 35 cycles in order to establish the linearity of the reaction and to decide the appropriate cycle number (28 for COX-2 and 20 for GAPDH). Tubes with mixture reaction were placed on a Thermo Cycler (Perkin–Elmer Cetus). Then, aliquots of each reaction were size-fractionated with 4% acrylamide–bisacrylamide gels. The gels were dried and exposed to X-OMAT AR films (Eastman Kodak, Rochester, NY). Autoradiographies were analyzed using scanning densitometry (Molecular Dynamics). Results were expressed as arbitrary densitometric units.

PROSTAGLANDIN E2 ASSAY

The conditioned media from HOC (cultured at 50 000 cells/well in 96 well-plates in 150 µl media) was collected and stored at −80°C until analysis. PGE2 content was measured by enzyme immunoassay (EIA) (Assay Designs Inc.) according to manufacturer’s instructions.

STATISTICAL ANALYSIS

Densitometric results, expressed as arbitrary units (AU) as n-fold over control, and PGE2 concentration (as µg/ml) are expressed as the mean±S.E.M. Comparisons between groups were made by Student’s test and analysis of variance. P values less than 0.05 were considered significant.

Results

EFFECT OF GS, N-ACG AND GAL ON IL-1β-INDUCED NFκB ACTIVATION

IL-1β caused a time-dependent increase in NFκB activity in human osteoarthritic chondrocytes (HOC). The maximal response was observed at 60 min (Fig. 1). This binding reaction was specific since cold NFκB prevented the formation of the complexes with 32P-labeled NFκB (Fig. 1, lane C). When 60 min was established as optimal period of incubation for NFκB binding, cells were incubated for 30 min with different doses of GS before addition of IL-1β (10–1000 mU/l). At these doses, GS did not affect cell viability (data not shown). As can be seen in Fig. 2, exposure to GS did not modify NFκB binding when incubated alone. However, GS-preincubated IL-1β-stimulated HOC showed a significant inhibition of NFκB binding in a dose-dependent manner in comparison with IL-1β-stimulated HOC. In parallel series of experiments, we also incubated HOC with equimolar concentrations of N-AcG or Gal (0.02–2 mM) before IL-1β stimulation. None of these compounds were able to significantly inhibit NFκB activation induced by IL-1β. Fig. 3 shows only the results corresponding to the higher doses of the sugars, although the effect for Gal and N-AcG was the same with minor doses (data not shown).

In order to study whether this effect on NFκB activation was specific for this nuclear factor, we studied whether the increase in the nuclear translocation of AP-1, that can also be induced by IL-1β, could be modified by GS. Equal amounts of nuclear extracts assayed for NFκB binding were employed to assay AP-1 binding. These studies demonstrated that GS did not modify IL-1β-induced AP-1 binding (Fig. 4).

CHARACTERIZATION OF THE SUBUNITS OF THE NFκB/REL FAMILY INVOLVED IN IL-1β-INDUCED NFκB ACTIVATION

Nuclear extracts from IL-1β-stimulated HOC yielded two specific electrophoretic bands in an electrophoretic mobility shift assay (EMSA). The composition of these NFκB complexes was analyzed with specific antibodies against two different members of the NFκB/Rel proteins. For this purpose, we incubated nuclear extracts from IL-1β-stimulated HOC with 1 µg anti-p50 or/and anti-p65 antibodies. Antibody presence caused the appearance of slower migration.
complexes (shifted complexes) as shown in Fig. 5. According to these results, we concluded that the major band (slower one) of NFκB complexes in our experiment was a heterodimer p50–p65, while the minor band was not related with these two proteins.

Using immunofluorescence assays, we have localized these NFκB subunits in HOC. In control cells, a diffuse cytoplasmic staining was seen, while cells incubated with IL-1β had a clear nuclear staining pattern for p50 and p65, indicating nuclear translocation of these NFκB subunits [Fig. 6(A, B, E, F)]. Preincubation of HOC with 1000 mg/l GS prevented the nuclear translocation of both subunits [Fig. 6(D, H)].

NFκB activation occurs following dissociation of an inhibitory subunit, a member of the IκB family, which is degraded by a proteolytic process. Therefore, we studied the implication of the inhibitory protein IκBα in NFκB activation in our system. In control HOC, IκBα was found as a protein of approximately 37 KDa. After stimulation with IL-1β, this band disappeared, suggesting IκBα degradation (Fig. 7). Preincubation with GS prevented IκBα degradation in HOC stimulated with IL-1β (Fig. 7).

EFFECT OF GS ON COX-1 AND COX-2 SYNTHESIS

In order to study whether the inhibition of NFκB activation correlated with a decrease in the synthesis of COX-2, we studied the effect of 1000 mg/l GS on COX-1 and COX-2 syntheses induced by IL-1β at 24 h of incubation. Results of the experiments demonstrated that GS significantly inhibited the protein synthesis of COX-2 measured by Western blot. However, as shown in Fig. 8, neither IL-1β nor GS modified the synthesis of COX-1.

We also investigated whether the ability of GS to inhibit IL-1β enhanced COX-2 expression in HOC was due to the inhibition of the corresponding gene expression. For this purpose, GS-preincubated HOC were stimulated with IL-1β for 24 h. Figure 9 shows that GS significantly inhibited COX-2 expression.

EFFECT OF GS ON PGE2 PRODUCTION

In order to verify if the GS effect on COX-2 expression and synthesis was related to a diminution of PGE2 concentration in cell supernatants, we measured PGE2 release in GS-preincubated HOC that were stimulated with IL-1β for 6 or 24 h. The sensitivity of PGE2 EIA was 36 pg/ml. The basal release of PGE2 in unstimulated chondrocytes was
nearly invariable for 6 or 24 h (see Table I). IL-1β stimulation caused a time-dependent increase in PGE2 concentration that was inhibited by 1000 mg/l GS both at 6 and 24 h (Table I).

Discussion

In this study, we have demonstrated that GS inhibits NFκB activation induced by IL-1β in HOC. We have shown that GS prevents IκBα degradation in the cell cytoplasm and their migration to the nucleus of the subunits p50 and p65. Furthermore, we have shown that preincubation of HOC with GS inhibits the expression and synthesis of COX-2, a NFκB-controlled protein with a key role in OA pathogenesis.

IL-1β is a proinflammatory cytokine released by synovial cells, chondrocytes and invading macrophages in inflamed joints. IL-1β plays a critical role in the inflammatory process and the connective tissue destruction observed in OA. This cytokine does activate other proinflammatory cytokines, neutral proteases, growth factors and their receptors and the adhesion molecules, all contributors to cartilage injury. Indeed, synovial fluid from patients with OA contains a high IL-1β concentration.

Recently, some interesting studies regarding the effects of GS in the treatment of OA have been published. The data indicate that GS acts as a symptom- and a structure-modifying agent in the treatment of OA. However, the mechanisms associated with these ways of action for GS are still not totally clarified.

Although the origin of pain in OA is under discussion, some explanations have been afforded, relating pain to joint inflammation and local prostaglandin concentration. In experimental animal models of inflammation, GS protected animals from paw edema induced by bradykinin, serotonin and histamine, and also GS impaired serositis induced by carragenan as well as peritonitis induced by acetic acid or formalin. Furthermore, oral administration of GS had an anti-inflammatory effect on adjuvant and kaolin-induced arthritis in rats, although to a lesser extent than that seen for traditional anti-inflammatory agents, such as indometacin or acetylsalicylic acid. Recent in vitro studies have demonstrated that anti-inflammatory actions of GS can be related to the suppression of neutrophil functions and even to the inhibition of immune activity in the synovial tissue. In the present work, we have demonstrated that GS inhibits NFκB activation and PGE2 synthesis induced by IL-1β in human chondrocytes. NFκB is considered a key regulator of tissue inflammation, since it controls the transcription of a number of proinflammatory genes that regulate the synthesis of cytokines, chemokines and adhesion molecules.
We have shown that inhibition of NFκB activation was related to the downregulation of the expression and synthesis of COX-2, the enzyme responsible for PGE2 synthesis in inflammatory situations. There are some NFκB binding regions in the promotor of the COX-2 gene that can account for the inhibition of COX-2 expression observed with GS27. Several in vivo and in vitro studies have approached the hypothesis of COX regulation by glucosamine, although the results seem controversial. Setnikar et al. described that GS had no effect on the total COX activity in inflamed rat paw tissues23. In this study, the dose of GS used to treat rats may not result in critical tissue levels required to achieve the effects described in the present work. Lotz and co-workers found a downregulation of COX-2 expression induced by IL-1 in human chondrocytes17. They demonstrate that this effect can not be observed with different monosaccharides. These data are in agreement with our results regarding the specificity of the action of each sugar. However, they did not find any effect of glucosamine on NFκB activity. In those experiments, N-AcG was used instead of GS. This is an interesting point since it has been suggested that these two drugs could have different mechanisms of action26,28. Lotz et al. described that N-AcG inhibited NO synthesis induced by

Fig. 6. Immunofluorescence assays for p50 (A–D) and p65 (E–H) subunits. In control cells, a slight cytoplasmatic immunostaining was seen with anti-p50 and anti-p65 antibodies (A and E). When cells were treated for 60 min with 10 U/ml IL-1β, an intense nuclear fluorescence was observed with both antibodies (B and F). Incubation with 1000 mg/l GS alone had no significant effect (C and G). GS preincubated IL-1β-stimulated HOC showed a significant diminution in nuclear p50 and p65 immunostaining, showing an inhibition in the translocation of the p50 and p65 subunits into the nuclei (D and H) in comparison with IL-1β-stimulated HOC.
IL-1β, while opposite results have been published for GS. In this sense, it has been hypothesized that the sulfate group could play a key role in the therapeutic action of glucosamine salts.

Our data strongly suggest that the impairment of NFκB activation induced by GS could be responsible for a diminution in COX-2 expression and subsequent PGE2 synthesis. GS could decrease NO concentration and even control neutrophil functions (expression of adhesion molecules, p38 phosphorylation or chemotaxis) through the inhibition of the expression of NFκB-dependent genes. Therefore, NFκB inhibition could account for the beneficial effects observed for this drug in joint inflammation. Other transcription factors have been implicated in the COX-2 human gene stimulation by IL-1β. In particular, C/EBP nuclear factors have been demonstrated to regulate COX-2 expression in IL-1β-stimulated chondrocytes. Additional studies regarding the effect of GS on C/EBP binding to COX-2 promoter would be of interest.

The present study also describes the effect of GS on NFκB subunits nuclear translocation. Preincubation with GS inhibits both p50 and p65 migration to the nucleus. Hence, the heterodimer p50–p65 was the major dimer participating in NFκB activation induced by IL-1β in HOC. These results seem particularly important since p50 subunit has been linked to joint cartilage destruction during inflammation, in contrast with other NFκB/Rel family members.

Besides anti-inflammatory properties, other mechanisms of action can be additionally responsible for the specific action of GS. OA is characterized by severe degradation of matrix proteins, due to the activation of metalloproteinases and increased degradation of proteoglycans. Normalization of articular cartilage metabolism could have structural benefits resulting in an improvement in radiological signs. Recent studies have demonstrated that GS can modify collagenase activation. GS was also able to restore the dysregulated balance of glycosaminoglycan metabolism, both preventing their degradation and stimulating their production. Mechanisms underlying these different actions of GS are still poorly understood. However, recent data have demonstrated that NFκB activity is essential for MMP-1 and MMP-3 upregulation. Hence, GS could have structural benefits on the OA-affected cartilage through the inhibition of NFκB activation.

In these experiments, we have shown that GS is effective in both preventing NFκB activation and COX-2 expression and synthesis in a concentration ranging from 100 to 1000 mg/l (approximately 0.2 to 2 mM). A similar concentration was previously described for collagenase inhibition and proteoglycan production. Pharmacokinetic studies have shown that after oral administration of a therapeutic dose of GS, a blood concentration of 0.1 mM glucosamine can be achieved. This concentration results from both plasma-incorporated and free glucosamine. However, glucosamine was also shown to accumulate in cartilage and synovial cells. Although in vitro data cannot be extrapolated to pathophysiological conditions in humans, our data suggest a novel mechanism of action by which GS could modulate OA cartilage metabolism.

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**Fig. 7.** GS increases IkBα levels in IL-1β-stimulated HOC. Cells were treated with 10 U/ml IL-1β for 60 min after being preincubated with 1000 mg/l GS for 30 min. Cytoplasmic extracts were electrophoresed under reducing, denaturing conditions, stained with affinity-purified anti-IkBα antibody, and visualized by enhanced chemiluminescence. (A) Representative autoradiogram of six different experiments with similar results is shown. (B) Densitometric analysis corresponding to changes in IkBα levels, corrected to those of α-tubulin is also shown (mean±S.E.M.). *P<0.05 vs basal; †P<0.05 vs IL-1β alone.

**Fig. 8.** Effect of GS in HOC stimulated with IL-1β in COX-1 and COX-2 protein syntheses. Cells were preincubated with GS (1000 mg/l) for 30 min and were then stimulated with 10 U/ml IL-1β for 24 h. (A) Representative Western blots corresponding to hybridization with anti-COX-2, anti-COX-1 and anti-α-tubulin antibodies are shown. (B) Densitometric analysis of COX-2 levels in six different experiments, corrected to those of α-tubulin is shown (mean±S.E.M.). *P<0.05 vs basal; †P<0.05 vs IL-1β alone.
In summary, this study shows that GS inhibits NF-κB activation and COX-2 expression induced by IL-1β in OA chondrocytes. The data further support the clinical findings describing GS as a symptom- and structure-modifying drug in the treatment of OA.

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Table I

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<th>Condition</th>
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<tr>
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<td>Basal</td>
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<tr>
<td>IL-1β (10 U/ml)</td>
<td>60±15*</td>
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<td>IL-1β+GS (1000 mg/l)</td>
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HOC were stimulated with the indicated concentrations of IL-1β and/or GS as described in Methods section. Conditioned media was collected after 6 or 24 h for PGE2 EIA. PGE2 concentration is shown as mean±S.E.M. of two independent experiments. In each of them, PGE2 was measured in triplicate. *P<0.01 vs basal; †P<0.01 vs IL-1β alone.

References


