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Glucosamine modulates IL-1-induced activation of rat chondrocytes at a receptor level, and by inhibiting the NF-κB pathway

J.N. Gouze[a], A. Bianchi[b], P. Bécuwe[b], M. Dauça[b], P. Netter[a], J. Magdalou[a], B. Terlain[a], K. Bordji[a,*]

[a] *UMR 7561 CNRS-Université Henri Poincaré-Nancy I, Physiopathologie et Pharmacologie Articulaires, Faculté de Médecine, P.O. Box 184, 54505 Vandoeuvre-lès-Nancy, France*

[b] *Laboratoire de Biologie Cellulaire du Développement, Unité Propre de Recherche de l'Enseignement Supérieur 2402, Faculté des Sciences, Université Henri Poincaré, 54500 Vandoeuvre-lès-Nancy, France*

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Abstract We recently reported that glucosamine reversed the decrease in proteoglycan synthesis and in UDP-glucuronosyltransferase I mRNA expression induced by interleukin-1β (IL-1β) [Arthritis Rheum. 44 (2001) 351–360]. In the present work, we show that glucosamine does not exert the same effects when chondrocytes were stimulated with reactive oxygen species (ROS). In order to better understand its mechanism of action, we determined if glucosamine could prevent the binding of IL-1β to its cellular receptors or could interfere with its signaling pathway at a post-receptor level. Addition of glucosamine to rat chondrocytes treated with IL-1β or with ROS decreased the activation of the nuclear factor κB, but not the activator protein-1. After treatment with IL-1β, glucosamine increased the expression of mRNA encoding the type II IL-1β receptor. These results emphasize the potential role of two regulating proteins of the IL-1β signaling pathway that could account for the beneficial effect of glucosamine in osteoarthritis.

Key words: Glucosamine; Rat chondrocyte; Osteoarthritis; Nuclear factor κB; Interleukin-1β

1. Introduction

Osteoarthritis (OA) is characterized by cartilage alterations, such as quantitative and qualitative modifications of proteoglycans (PGs) and collagens. An imbalance between the biosynthesis and the degradation of matrix components leads to a progressive destruction of the tissue, resulting in extensive articular damage [1]. Cartilage damage in OA is known to be largely mediated by interleukin 1β (IL-1β), a cytokine that initiates a number of events leading to cartilage destruction, including the inhibition of matrix macromolecule biosynthesis and the increase of catabolic pathways.

IL-1β stimulates the transcription of many genes through the activation of different transcription factors such as nuclear factor κB (NF-κB) or activator protein-1 (AP-1). NF-κB binding sites are present in the promoter regions of many genes involved in the pathophysiology of joint inflammation and tissue destruction [2]. IL-1 binds to the IL-1 receptor type I (IL-1RI) that activates tumor necrosis factor receptor molecule-associated factor-6 (TRAF-6), leading to the activation of NF-κB-inducing kinase (NIK) [3]. In this way, NF-κB can enter the nucleus and activate target genes by binding with high affinity to κB response elements. Alternatively, TRAF-6 can also activate mitogen-activated protein kinase (MAPK). The MAPK pathways promote phosphorylation of other substrates, such as c-Jun N-terminal kinase and the Jun and Fos family, all of which are associated with a transcriptional activity of AP-1 [3].

Some symptomatic slow-acting drugs, such as glucosamine [4], have been shown to be effective in relieving the symptoms of OA. Reports of symptomatic relief afforded by glucosamine on the treatment of OA have spurred new research into its mechanism of action on cartilage [4–6]. In this respect, we have previously shown that glucosamine (4.5 g/l) prevented the IL-1β-mediated decrease in PG synthesis. This process was related, in part, to a decrease in the expression of UDP-glucuronosyltransferase I (GlcAT-I), which is involved in the biosynthesis of glycosaminoglycans. Glucosamine was also suggested in the literature to act through the scavenging of free radicals [7,8]. However, this way of action has never been proved. We only showed in a previous work that glucosamine modulated the expression of inflammatory enzymes (inducible NO synthase) and the production of NO [9].

Since IL-1β treatment is well known to lead to the production of reactive oxygen species (ROS) by cells, we hypothesized that glucosamine could prevent the action of IL-1β by antioxidant effects. Secondly, we wanted to know if the amino sugar could prevent the binding of IL-1β to its cell surface receptors, or could interfere at post-receptor levels. Therefore, the objectives of this study were (i) to look for a potential antioxidant effect of glucosamine by incubating chondrocytes with a ROS-generating system and comparing the results with those obtained in IL-1β-stimulated cells, (ii) to determine if glucosamine could modulate the binding of IL-1 on its receptor via a possible increase in type II IL-1 receptor (IL-1RII) which is a decoy receptor, or an increase in IL-1 receptor antagonist (IL-1ra), and (iii) to examine the possible effects

*Corresponding author. Fax: (33)-3-83 59 26 21.
E-mail address: bordji@facmed.u-nancy.fr (K. Bordji).

Abbreviations: OA, osteoarthritis; PG, proteoglycan; IL-1, interleukin-1; IL-1RII, type II IL-1β receptor; NF-κB, nuclear factor-κB; AP-1, activator protein-1; IL-1ra, IL-1 receptor antagonist; GlcAT-I, UDP-glucuronosyltransferase I; ROS, reactive oxygen species; FCS, fetal calf serum; RT-PCR, reverse transcriptase polymerase chain reaction; EMSA, electrophoretic mobility shift assay

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of glucosamine on the activation of two nuclear factors, NF-κB and AP-1.

2. Materials and methods

2.1. Chemicals

D-(+)-Glucosamine and D-(+)-glucose were supplied by Sigma Chimie (St Quentin Fallavier, France). Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), L-glutamine, gentamicin, reverse transcriptase, and restriction enzymes were obtained from Gibco-BRL (Cergy Pontoise, France). *Taq* polymerase was supplied by Eurobio (Les Ulis, France), and the nucleotides were synthesized by MWG-Biotech (Ebersberg, Germany). Recombinant human IL-1β was purchased from Pepro-Tech (Tebu, Le Perray en Yveline, France).

2.2. Chondrocyte cultures and treatments

Male Wistar rats (130–150 g) (Charles River, Saint-Aubin-lès-Elbœuf, France) were housed under controlled temperature and lighting conditions with food and water ad libitum. Articular cartilage, isolated from femoral head caps, was aseptically dissected, and chondrocytes were obtained after digestion of the cartilage fragments in pronase (2% w/v in 0.15 M NaCl) followed by an overnight digestion in collagenase B (1.5% w/v in DMEM without serum) [10]. The experiments were performed with first-passage cultures, 6 days after collagenase treatment. For this purpose, chondrocytes were grown to confluence for 6 days in 25-cm^2 flasks (about 4×10^6 chondrocytes per flask) in 5 ml of complete medium (DMEM supplemented with 2 mM L-glutamine, 50 μg/ml gentamicin, 10% FCS v/v). Cells were then cultured for 6 h in FCS-free medium containing glucose (4.5 g/l) or glucosamine (4.5 g/l), and finally stimulated with IL-1β (25 or 250 U/ml) for 16 h.

For stimulation with a ROS-generating system, cells were incubated for 30 min in a glucose- or glucosamine-containing medium in the presence of hypoxanthine (4 mM) and xanthine oxidase (10 mU/ml) [11]. Cells were then cultured in glucose- or glucosamine-containing medium for 8 h (mRNA expression determination) or for 16 h (PG synthesis measurement). For the determination of proteoglycan synthesis, chondrocytes were entrapped into alginate beads as follows. The cells were suspended in sterile filtered low viscosity alginate solution (1.2% w/v) at 6×10^6 cells/ml and slowly expressed through a 22-gauge needle into a 100 mM $CaCl_2$ solution. After two washes with 0.15 M NaCl, beads were cultured for 6 days in complete medium containing 4.5 g/l of glucose. Before stimulation with ROS or IL-1β, cells were cultured with complete medium with 2% FCS, for 8 h in the presence of 4.5 g/l of glucose or glucosamine.

2.3. Measurement of PG biosynthesis

The incorporation of radiolabeled sodium sulfate into PGs in rat chondrocytes encapsulated into alginate beads was measured 16 h after ROS or IL-1β stimulation. Alginate beads were incubated in complete medium (4.5 g/l glucose or glucosamine) containing 2% FCS and supplemented with 10 μCi/ml of $Na_2\ ^{35}SO_4$ for 4 h at 37°C. The encapsulated chondrocytes were washed several times with 0.15 M NaCl and solubilized in Soluene 350 (0.5 M quaternary ammonium hydroxide in toluene) overnight. The amount of radiolabeled sulfate incorporated, considered a reliable evaluation of the amount of newly synthesized sulfated glycosaminoglycans [12,13], was quantified by liquid scintillation.

2.4. Analysis of GlcAT-I, and IL-1RII mRNA expression by quantitative reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from cell cultures by a single-step guanidinium thiocyanate–phenol chloroform method using Trizol (Life Technologies) according to the manufacturer's protocol. mRNA expression by rat chondrocytes was analyzed using a quantitative multistandard RT-PCR, a method that takes advantage of sequence conservation of both the gene of interest and β-actin between animals species [14]. The protocol allowed us to normalize the amount of mRNA of the gene of interest with respect to that of β-actin mRNA in each sample. Total RNA samples extracted from rat cells were mixed with a constant amount of RNA prepared from mouse tissues, which brought the sequences of both competitive mouse β-actin and the gene of interest and thus acted as a multistandard source. For amplification of GlcAT-I, the direct primer extended from nucleotide 267 to 290 and the reverse primer from nucleotide 592 to 619, in accordance with the rat sequence (GenBank accession number AB009598). Each amplification product was distinguished by restriction site polymorphism: *Bam*HI digested the mouse GlcAT-I amplification product into two fragments (121 and 304 bp), while the rat product remained uncut.

For amplification of IL-1RII, the direct primer extended from nucleotides 401 to 421, and the reverse primer extended from nucleotides 723 to 744 in accordance with the rat sequence (GenBank accession number Z22812). *Eco*RI digested the mouse IL-1RII product into 2 fragments (228 and 115 bp), while the rat product remained uncut.

Digested RT-PCR products were resolved by agarose gel electrophoresis stained with ethidium bromide. The bands were visualized under UV light and photographed by a computer-assisted camera. Quantitation of each band was performed by densitometry analysis with NIH software. Results are expressed as the ratio (analyzed $\text{gene}_{\text{rat}}/\beta\text{-actin}_{\text{rat}})\times(\beta\text{-actin}_{\text{mouse}}/\text{analyzed gene}_{\text{mouse}})$, in arbitrary units.

2.5. Preparation of nuclear extracts

The nuclear extracts were prepared from rat chondrocytes as described in detail before [15]. Briefly, cell monolayers were washed in ice-cold phosphate-buffered saline and scraped in 1 ml of buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1 mM dithiothreitol) containing a protease inhibitor cocktail (Boehringer Mannheim) and 0.5% Igepal (Sigma). Cell extracts were incubated for 15 min on ice, with intermittent mixing. Nuclei were collected by centrifugation at $1500\times g$ for 5 min at 4°C. The nuclear pellets were washed with 1 ml of buffer A without Igepal and resuspended in 50 μl of buffer B (10 mM HEPES, pH 7.9, 420 mM NaCl, 1 mM dithiothreitol) containing the same protease inhibitor cocktail. The tubes were mixed thoroughly and incubated for 30 min on ice. Nuclear debris were removed by centrifugation at $13\,000\times g$ for 10 min at 4°C. The supernatants were collected and stored at −80°C before use. The protein content was determined according to the method of Bradford [16].

2.6. Electrophoretic mobility shift assay (EMSA)

The DNA sequences of the double-stranded oligonucleotide specific for NF-κB were 5′-GAT CCA GTT GAG GGG ACT TTC CCA GGC G-3′ and 5′-GAT CCG CCT GGG AAA GTC CCC TCA ACT G-3′. Those specific for AP-1 were 5′-GAT CCG CTT GAT GAC TCA GCC GGA AG-3′ and 5′-GAT CCT TCC GGC TGA GTC ATC AAG CG-3′. Complementary strands were annealed, and double-stranded oligonucleotides were labeled with [^{32}P]dCTP using the Klenow fragment of DNA polymerase (Life Technologies). Nuclear extracts (5 μg of protein) were incubated for 10 min at 4°C in a total volume of 20 μl containing 2 μg of poly(dI-dC) and 2 μl of 10× gel shift binding buffer (20 mM Tris–HCl, pH 7.9, 5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, and 20% glycerol). The extracts were then incubated for 30 min at 4°C with 10 000 cpm of ^{32}P-labeled NF-κB or AP-1 probes (MWG-Biotech). The samples were loaded on a 5% native polyacrylamide gel, and run in 0.5× TBE buffer. The gel was dried and subjected to autoradiography. NF-κB- and AP-1-specific bands were confirmed by competition with a 100-fold excess of an unlabeled NF-κB or AP-1 probe, which resulted in no shifted band.

2.7. Statistical analysis

After comparison of data by analysis of variance, the different groups were compared using Fisher's *t*-test. Assays were made in triplicate. *P* values less than 0.05 were considered significant.

3. Results

3.1. Glucosamine prevents the IL-1β-induced changes on both GlcAT-I mRNA expression and PG synthesis, but not after ROS stimulation.

Since IL-1 and tumor necrosis factor have been reported to increase ROS in chondrocytes [17], thus decreasing the biosynthesis of PG, we hypothesized that glucosamine could reverse both the ROS and the IL-1β effects. For this reason, the

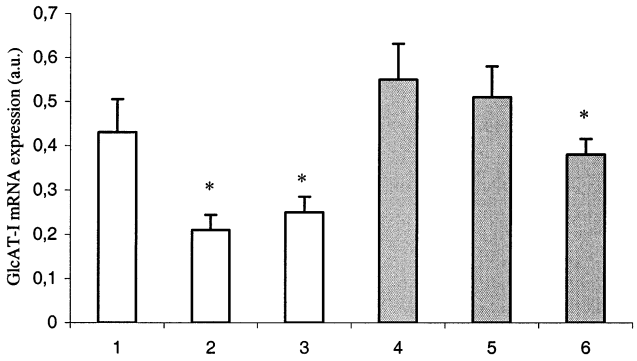

Fig. 1. Effect of ROS or IL-1β on GlcAT-I mRNA expression in rat chondrocytes cultured in the presence of glucose (white bars) or glucosamine (gray bars). Cells were cultured for 6 h in serum-free medium containing glucose or glucosamine before being incubated for 30 min in the same medium in the presence of hypoxanthine (4 mM) and xanthine oxidase (10 mU/ml). mRNA expressions were quantified as described in Section 2. Lane 1: glucose medium; lane 2: glucose+IL-1β (250 U/ml); lane 3: glucose+ROS; lane 4: glucosamine medium; lane 5: glucosamine+IL-1β (250 U/ml); lane 6: glucosamine+ROS. Results are expressed in relative arbitrary units, and are the mean ± S.D. of three different assays ($n = 3$; $^{*}P < 0.05$ versus respective control).

concentration of the hypoxanthine/xanthine oxidase system (4 mM, 10 mU/ml) was chosen to produce approximately similar reductions of PG synthesis and GlcAT-I mRNA expression to those produced by 250 U/ml IL-1β treatment. To investigate the effects of glucosamine on ROS-mediated or IL-1β-mediated PG synthesis decrease, rat chondrocytes were isolated, maintained in culture in the presence of glucose or glucosamine and challenged with ROS or IL-1β. Treatment of chondrocytes with ROS or IL-1β in glucose medium resulted in a significant decrease in both GlcAT-I mRNA expression (−43% for ROS and −51% for IL-1β, $P < 0.05$; Fig. 1) and PG synthesis (−22% for ROS and −24% for IL-1β, $P < 0.05$; Fig. 2). However, in contrast to what we observed with IL-1β

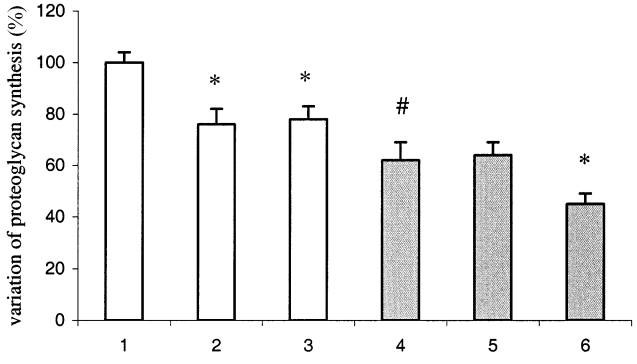

Fig. 2. Effect of ROS or IL-1β on PG synthesis in rat chondrocytes cultured in the presence of glucose (white bars) or glucosamine (gray bars). Cells were cultured in alginate beads in 2% FCS medium containing glucose (4.5 g/l) or glucosamine (4.5 g/l). ROS were produced as reported in the legend of Fig. 1, and 16 h later, radiolabeled sulfate incorporation was analyzed as described in Section 2. Lane 1: glucose medium; lane 2: glucose+IL-1β (250 U/ml); lane 3: glucose+ROS; lane 4: glucosamine medium; lane 5: glucosamine+IL-1β (250 U/ml); lane 6: glucosamine+ROS. Results are expressed as percentage of variations by comparison to the control (value of 100 for glucose medium) and are the mean ± S.D. of three different assays ($n = 3$; $^{*}P < 0.05$ versus respective control, $^{\#}P < 0.05$ versus 4.5 g/l glucose medium).

treatment, addition of glucosamine provided less significant prevention against the ROS-mediated inhibitory effects on GlcAT-I mRNA expression (−31%, $P < 0.05$; Fig. 1) and on PG synthesis (−27.5%, $P < 0.05$; Fig. 2). Moreover, similarly to previous data [9], glucosamine induced a PG synthesis decrease by itself (−38%, $P < 0.05$; Fig. 2), but had no effect on GlcAT-I mRNA expression (Fig. 1). The present results firstly show that glucosamine was able to counteract the inhibition of expression of GlcAT-I mRNA. Secondly, glucosamine was only able to counteract the further inhibition of PG synthesis caused by IL-1β treatment but not after ROS challenge. The decrease in PG synthesis induced by ROS production could be related to different cellular pathways than those involved in the IL-1β challenge.

3.2. *IL-1β induced an increase in the expression of IL-1RII mRNA in the presence of glucosamine*

We therefore investigated the influence of glucosamine on the mRNA expression of the decoy receptor IL-1RII in chondrocytes treated or not with IL-1β. The addition of IL-1β (25 U/ml or 250 U/ml) for 12 h to a culture medium containing glucose did not induce any variation of IL-1RII mRNA expression (Fig. 3). On the other hand, the IL-1β-mediated stimulation of cultured rat chondrocytes in the presence of glucosamine led to a dose-dependent increase in the expression of this mRNA, reaching 51% at the higher dose of the cytokine (Fig. 3). No significant difference was observed between controls containing glucose and glucosamine, in the absence of IL-1β. We also evaluated the effects of IL-1β on IL-1ra mRNA expression. In contrast to IL-1RII, no variation in IL-1ra mRNA expression was measured in the presence of glucosamine (data not shown).

3.3. *Glucosamine inhibits the NF-κB but not the AP-1 signaling pathway*

In order to investigate the influence of glucosamine on IL-1β-mediated effects in rat chondrocytes, we further examined the activation of two transcriptional pathways of the inflammatory response, i.e. NF-κB and AP-1. As expected, treat-

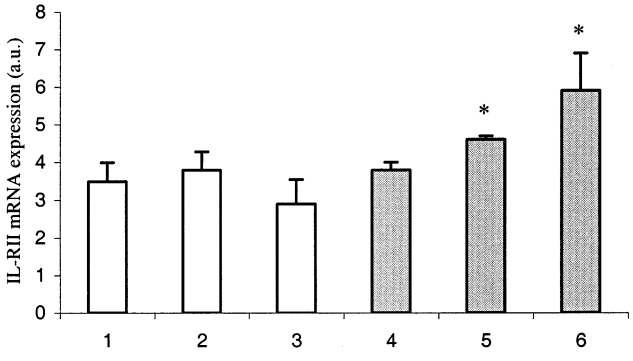

Fig. 3. Effect of IL-1β treatment on IL-1RII mRNA expression in rat chondrocytes in the presence of glucose (white bars) or glucosamine (gray bars). Cells were cultured for 6 h in FCS-free medium containing glucose (4.5 g/l) or glucosamine (4.5 g/l), and finally stimulated with IL-1-β (25 U/ml or 250 U/ml) for 16 h. Lane 1: glucose medium; lane 2: glucose+25 U/ml IL-1β; lane 3: glucose+250 U/ml IL-1β; lane 4: glucosamine medium; lane 5: glucosamine+25 U/ml IL-1β; lane 6: glucosamine+250 U/ml IL-1β. Results are expressed in relative arbitrary units, and are the mean ± S.D. of three different assays ($n = 3$; $^{*}P < 0.05$ versus respective control).

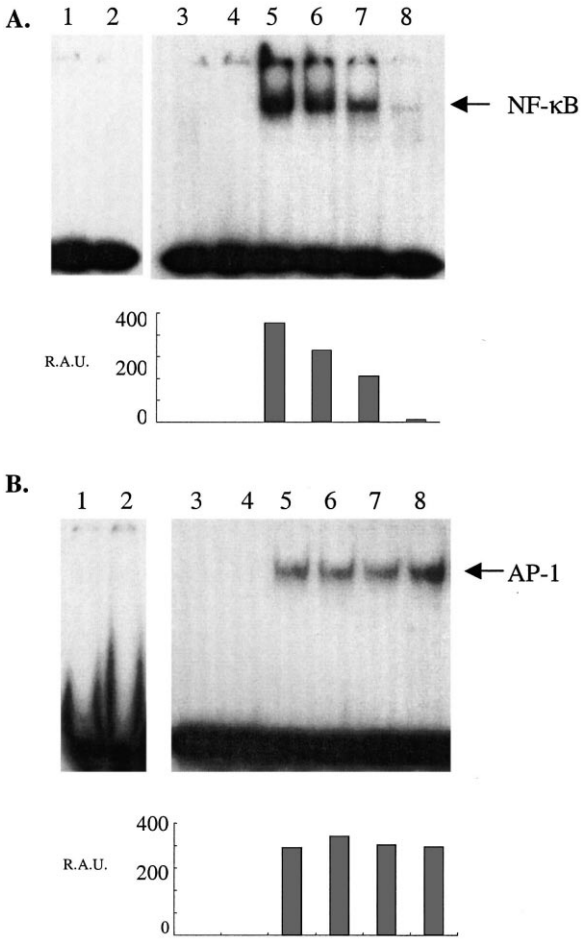

Fig. 4. Effect of glucosamine on NF-κB (A) or AP-1 (B) activation mediated by IL-1β or ROS. Chondrocyte cultures were treated with IL-1β (250 U/ml for 1 h) or ROS (30 min) as described in Section 2. Nuclear proteins were extracted and 5 μg of each sample was subjected to EMSA using NF-κB or AP-1 consensus site radiolabeled probes. Lane 1: radiolabeled probe alone; lane 2: 100× concentrated unlabeled probe; lane 3: glucose control; lane 4: glucosamine control; lane 5: glucose+250 U/ml IL-1β; lane 6: glucosamine+250 U/ml IL-1β; lane 7: glucose+ROS; lane 8: glucosamine+ROS. Band intensities were quantified with densitometry analysis software (NIH image). The experiments were performed three times; representative results are shown.

ments of chondrocytes in glucose-containing medium by IL-1β or ROS induced the activation of both NF-κB (Fig. 4A) and AP-1 (Fig. 4B). However, the NF-κB activation induced by ROS was shown to be lower than that induced by IL-1β. The presence of glucosamine instead of glucose in the culture medium did not modulate the activation of AP-1 induced by IL-1β or ROS treatments. In contrast and interestingly, the presence of glucosamine in the medium significantly reduced NF-κB activation induced by IL-1β and inhibited that induced by ROS (Fig. 4A).

4. Discussion

OA affects approximately 12% of the population, and the incidence increases with age. Current treatments for OA are limited to short-term symptom control with acetaminophen and non-steroidal anti-inflammatory drugs, which do not slow or reverse the degenerative process. Glucosamine has recently received a great deal of attention from the public as a potential treatment for OA [4,5] since this drug has been proposed to slow down and possibly reverse these degenerative processes [18]. However, the mechanisms of the beneficial effect of glucosamine on cartilage disease are still unknown. In a recent study, we have demonstrated that glucosamine at a dose of 4.5 g/l reversed the IL-1β-mediated effects on enzymes such as GlcAT-I and MMP-3 in rat chondrocytes in culture. We also showed that glucosamine not only corrected these effects but also antagonized the production of various pro-inflammatory mediators activated by IL-1β, such as NO and prostaglandin E_2 [9]. Altogether, these results suggested that the pharmacological effects of glucosamine in the treatment of OA would involve a modulating action of this amino sugar at some stages of IL-1 signaling events.

There are many potential ways in which glucosamine could, directly or indirectly, modify IL-1 signaling pathways. The protective effects of glucosamine have been considered at three separate levels: (i) towards the effects induced by radicals, (ii) the variations of mRNA encoding the expression of the decoy receptor IL-1RII and the receptor antagonist IL-1ra, and (iii) the activation of two transcriptional nuclear factors, NF-κB and AP-1, known to be involved in inflammation. IL-1 induces a cellular stress that can generate ROS production, which, in turn, could activate the NF-κB pathway by interacting with NIK [19]. We hypothesized that the presence of ROS could explain the decrease in PG synthesis. If glucosamine reversed the IL-1-mediated decrease in PG synthesis, no effect was observed on ROS-mediated variations in either GlcAT-I mRNA expression or biosynthesis of PG. These data suggest that ROS and IL-1 decreased the PG synthesis via two different pathways.

Two types of IL-1 receptors have been described. The inflammatory effect of IL-1 requires signaling through the cytoplasmic domain of IL-1RI. The decoy IL-1RII can also interact with IL-1. However, as a truncated protein, its interaction with the cytokine is unable to generate signaling. By trapping part of IL-1, the decoy receptor can modulate its concentration and effects. This property makes IL-1RII a potential target in order to block the IL-1β signaling pathways [20]. Many studies suggest that IL-1RII plays an important physiological role in the regulation of IL-1 action in the inflammation sites [21]. Several drugs, such as aspirin or glucocorticosteroids, could induce this decoy receptor, thus decreasing inflammation by preventing the binding of IL-1β to target cells [22,23]. Our study showed that the mRNA expression of IL-1RII was not changed in rat chondrocytes challenged with IL-1β in the presence of glucose. However, the expression of the decoy receptor was significantly increased in chondrocytes cultured in the presence of glucosamine, but only when the cells were treated with IL-1β. By increasing IL-1RII mRNA expression, glucosamine is able to reduce the binding of IL-1β on IL-1RI and therefore modulates its effects. However, at this state of the work, we are unable to explain by which mechanism glucosamine induces the increase in IL-1RII expression in IL-1β-treated chondrocytes.

When IL-1β binds to IL-1R, the signal is transmitted within the cell through a cascade of signaling proteins that can be potential targets of glucosamine. The results presented in this study show that glucosamine significantly decreases the efficiency of the IL-1β signaling pathway by antagonizing NF-κB activation. Several compounds, such as phenylarsine, the anti-

oxidant quercetin or the non-steroidal anti-inflammatory drug tepoxalin, have been described to inhibit the translocation of NF-κB into the nucleus [24–26]. Our results showed that glucosamine had no significant effect on IL-1β-mediated AP-1 activation. Thus, these data would suggest that glucosamine acts downstream of TRAF-6 in the IL-1β signaling pathways [3]. Moreover, we found that glucosamine also blocked the activation of NF-κB induced by ROS, whereas it was inactive on PG synthesis and GlcAT-I mRNA expression. This result shows that the blocking of the NF-κB activation pathway is not sufficient or is not involved in the restoration of proteoglycan synthesis. Induction of cyclooxygenase 2 and inducible NO synthase by inflammatory cytokines is known to be partly mediated by NF-κB [27–29]. Therefore, the anti-inflammatory effects of glucosamine on the production of prostaglandin E_2 and NO [9] could be explained, at least in part, via an inhibition of the binding of NF-κB to its response elements. Moreover, most MMP genes are characterized by the presence of an AP-1 binding site in their proximal promoter that mediates transcriptional activation by growth factor, phorbol ester and oncogenes [30]. For the expression of MMP-3, the transcription factor AP-1 is essential (but not sufficient) for the upregulation of this enzyme under pro-inflammatory cytokines; NF-κB activity is also involved in MMP-3 up-regulation [31]. Hence, the beneficial effects of glucosamine can be explained, at least in part, by the inhibition of NF-κB transcriptional activity.

In conclusion, the present work demonstrates that glucosamine acts, at least, at two different levels in rat chondrocytes (NF-κB activation and IL-1RII expression). Thus, these results contribute to clarify the mechanisms of action of glucosamine on cartilage.

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References

[1] Inerot, S., Heinegard, D., Audell, L. and Olsson, S.E. (1978) Biochem. J. 169, 143–156.
[2] Baeuerle, P. and Baichwal, V. (1997) Adv. Immunol. 65, 211–290.
[3] Ninomiya-Tsuji, J., Kishimoto, K., Hiyama, A., Inoue, J., Cao, Z. and Matsumoto, K. (1999) Nature 398, 252–256.
[4] Bassleer, C., Rovati, L. and Franchimont, P. (1998) Osteoarthritis Cartilage 6, 427–434.
[5] Kelly, G. (1998) Altern. Med. Rev. 3, 27–29.
[6] Reickhelt, A., Forster, K., Fisher, M., Rovati, L. and Settnikar, I. (1994) Arzneim.Forsch. 44, 75–85.
[7] Adams, M.E. (1999) Lancet 354, 353–354.
[8] Müller-Fabbender, H., Bach, G.L., Haase, W., Rovati, L.C. and Setnikar, I. (1994) Osteoarthritis Cartilage 2, 61–69.
[9] Gouze, J.-N., Bordji, K., Gulberti, S., Terlain, B., Netter, P., Magdalou, J., Fournel-Gigleux, S. and Ouzzine, M. (2001) Arthritis Rheum. 44, 351–360.
[10] Kuettner, K.E., Pauli, B.U., Gall, G., Memoli, V.A. and Schenk, R.K. (1982) J. Cell Biol. 93, 743–750.
[11] Kvam, B.J., Fragonas, E., Degrassi, A., Kvam, C., Matulova, M., Pollesello, P., Zanetti, F. and Vittur, F. (1995) Exp. Cell Res. 218, 79–86.
[12] de Vries, B.J., van den Berg, W.B., Vitters, E. and van de Putte, L.B. (1986) Rheumatol. Int. 6, 273–281.
[13] van den Berg, W.B., van de Loo, F.A., Otterness, I., Arntz, O. and Joosten, L.A. (1991) Agents Actions 32 (Suppl.), 159–163.
[14] Khiri, H., Reynier, P., Peyrol, N., Lerique, B., Torresani, J. and Planells, R. (1996) Mol. Cell. Probes 10, 201–211.
[15] Boyault, S., Simonin, M.-A., Bianchi, A., Compe, E., Liagre, B., Mainard, D., Bécuwe, P., Dauça, M., Netter, P., Terlain, B. and Bordji, K. (2001) FEBS Lett. 501, 24–30.
[16] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[17] Oh, M., Fukuda, K., Asada, S., Yasuda, Y. and Tanaka, S. (1998) J. Rheumatol. 25, 2169–2174.
[18] Reginster, J.Y., Deroisy, R., Rovati, L.C., Lee, R.L., Lejeune, E., Bruyere, O., Giacovelli, G., Henrotin, Y., Dacre, J.E. and Gossett, C. (2001) Lancet 375, 251–256.
[19] Bowie, A. and O'Neill, L.A. (2000) Biochem. Pharmacol. 59, 13–23.
[20] Dawson, J., Engelhardt, P., Kastelic, T., Cheneval, D., MacKenzie, A. and Ramage, P. (1999) Rheumatology 38, 401–406.
[21] Colotta, F., Dower, S.K., Sims, J.E. and Mantovani, A. (1993) Science 261, 472–475.
[22] Daun, J., Ball, R., Burger, H. and Cannon, J. (1999) J. Leukocyte Biol. 65, 863–866.
[23] Lukiw, W., Martinez, J. and Pelaez, R. (1999) J. Biol. Chem. 274, 8630–8638.
[24] Estroy, Z., Manna, S. and Harris, D. (1999) Blood 94, 2844–2853.
[25] Ishikawa, Y., Sugiyama, H., Stylianou, E. and Kitamura, M. (1999) J. Am. Soc. Nephrol. 10, 2290–2296.
[26] Fiebich, B., Hofer, T. and Lieb, K. (1999) Neuropharmacology 38, 1325–1333.
[27] Guyton, K.Z., Liu, Y., Gorospe, M., Xu, Q. and Holbrook, N.J. (1996) J. Biol. Chem. 271, 4138–4142.
[28] Plummer, S., Holloway, K. and Manson, M. (1999) Oncogene 18, 6013–6020.
[29] Kaibori, M., Sakitani, K. and Oda, M. (1999) J. Hepatol. 30, 1138–1145.
[30] Borden, P. and Heller, R. (1997) Crit. Rev. Eukaryot. Gene Expr. 7, 159–178.
[31] Bond, M., Baker, A. and Newby, A. (1999) Biochem. Biophys. Res. Commun. 264, 561–567.