Rheumatol Int (2008) 28:1009–1016
DOI 10.1007/s00296-008-0561-4

ORIGINAL ARTICLE

Chondroprotective effects of glucosamine involving the p38 MAPK and Akt signaling pathways

Yi-Cheng Lin · Yu-Chih Liang · Ming-Thau Sheu · Yu-Chen Lin · Ming-Shium Hsieh · Tzeng-Fu Chen · Chien-Ho Chen

Received: 24 November 2007 / Accepted: 1 March 2008 / Published online: 14 March 2008

Abstract The purpose of the present study was to elucidate the possible signal transduction pathway involved in the underlying mechanism of glucosamine (GLN)'s influence on the gene expression of matrix metalloproteinases (MMPs) in chondrocytes stimulated with IL-1β. Using chondrosarcoma cells stimulated with IL-1β, the effects of GLN on the mRNA and protein levels of MMP-3, the activation of JNK, ERK, p38, NF-κB, and AP-1, the nuclear translocation of NF-κB/Rel family members, and PI3-kinase/Akt activation were studied. GLN inhibited the expression and the synthesis of MMP-3 induced by IL-1β, and that inhibition was mediated at the level of transcription involving both the NF-κB and AP-1 transcription factors. Translocation of NF-κB was reduced by GLN as a result of the inhibition of IκB degradation. A slightly synergistic effect on the activation of AP-1 induced by IL-1β was shown in the presence of GLN. Among MAPK pathways involved in the transcriptional regulation of AP-1, phosphorylation of JNK and ERK was found to increase with the presence of GLN under IL-1β treatment, while that for p38 decreased. It was also found that GLN alone, but also synergistically with IL-1β, was able to activate the Akt pathway. The requirements of NF-κB translocation and p38 activity are indispensably involved in the induction of MMP-3 expression in chondrosarcoma cells stimulated by IL-1β. Inhibition of the p38 pathway in the presence of GLN substantially explains the chondroprotective effect of GLN on chondrocytes that regulate COX-2 expression, PGE_2 synthesis, and NO expression and synthesis. The chondroprotective effect of GLN through the decrease in MMP-3 production and stimulation of proteoglycan synthesis may follow another potential signaling pathway of Akt.

Keywords Glucosamine · Matrix metalloproteinases · Chondrocytes

Yi-Cheng Lin and Yu-Chih Liang equally contributed to this work.

Y.-C. Lin
Department of Orthopedics,
Mackay Memorial Hospital, Taipei, Taiwan, ROC

Y.-C. Liang · Y.-C. Lin · C.-H. Chen (✉)
School of Medical Laboratory Science and Biotechnology,
Taipei Medical University, 250 Wu-Hsing Street,
Taipei 110, Taiwan, ROC
e-mail: chenchho@tmu.edu.tw

M.-T. Sheu
Graduate Institute of Pharmaceutical Sciences,
Taipei Medical University, Taipei, Taiwan, ROC

M.-S. Hsieh
Department of Orthopedics and Traumatology,
Taipei Medical University and Hospital, Taipei, Taiwan, ROC

T.-F. Chen
Department of Pharmacology,
Taipei Medical University, Taipei, Taiwan, ROC

Introduction

Cartilage damage in osteoarthritis (OA) is well known for mainly being mediated by interleukin 1β (IL-1β), a cytokine that initiates a number of events leading to cartilage destruction, including inhibition of the biosynthesis of matrix macromolecules and the increase in catabolic pathways. This causes an imbalance between the biosynthesis and degradation of matrix components leading to progressive destruction of tissue and extensive articular damage [1–3]. Some chondroprotective agents, such as glucosamine (GLN), have been shown to be effective in relieving the symptoms of OA. Reports of symptomatic

relief afforded by GLN in the treatment of OA have encouraged research into its mechanisms of action on cartilage. In rat chondrocytes, GLN has been demonstrated that could decrease the IL-1β-induced stromelysin (MMP-3) mRNA expression through downregulation of nuclear factor κB (NF-κB) but not activator protein-1 (AP-1) transcription factor [4]. In human skeletal muscle cells, GLN mimics the stimulatory effects of high glucose on the activation of c-Jun N-terminal kinase (JNK) and ERK1/2 [5]. These results indicated that GLN might play an influential role in the signal transduction pathway involving NF-κB and mitogen-activated protein kinase (MAPK, including ERKs, JNKs, and p38).

IL-1β binds to the IL-1 receptor type I (IL-1RI) that activates tumor necrosis factor receptor molecule-associated factor-6 (TRAF-6), leading to activation of the NF-κB-inducing kinase (NIK) [6] and thereby freeing up NF-κB to interact with the nuclear importation machinery and be translocated to the nucleus, where it binds its target gene to upregulate the expression of many proinflammatory genes, such as COX-2 and iNOS in rheumatic disease [7]. Alternatively, TRAF-6 can also activate MAPK. The MAPK pathways (ERKs, JNKs, and p38) promote phosphorylation of other substrates, such as c-Jun N-terminal kinase and the *Jun* and *Fos* family, all of which are associated with the transcriptional activity of AP-1, which regulates T cell activation, cytokine production, and production of matrix metalloproteinases (MMPs) in rheumatic disease [7]. Therefore, IL-1β is released in OA patients to stimulate the transcription of inflammatory mediators (NO and PGE_2) mainly through activation of NF-κB and that of MMPs mainly through activation of activator protein-1 (AP-1) [8]. However, it was controversially reported in rat chondrocytes that GLN is able to fully prevent the upregulation of stromelysin (MMP-3) mRNA expression induced by IL-1β, but the addition of GLN with IL-1β only decreased the activation of the NF-κB, not AP-1 [4]. On the other hand, in both non-diabetic and diabetic cells, the stimulatory effects of GLN on JNK and ERK1/2 activities were demonstrated [5], both of which are able to activate the translocation of AP-1. Thus, the aim of the present study was to elucidate the possible signal transduction pathway involved in the underlying mechanism of GLN's influence on the gene expression of MMPs (with particular emphasis on MMP-3) in chondrocytes stimulated with IL-1β.

Materials and methods

Materials

D-(+)-Glucosamine (GLN) was supplied by Sigma (St Louis, MO, USA), and IL-1β was purchased from R&D systems (Minneapolis, MN, USA). Anti-mouse IgG–HRP, anti-rabbit IgG–HRP, the phospho-Akt antibody, phospho-Erk antibody, and total-p38 antibody were provided by Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Chondrocyte culture

Human chondrosarcoma cells (SW-1353, HCCs) were obtained from American Type Culture Collection (ATCC HTB-94; Manassas, VA). HCCs were grown to confluence in L-15 medium supplemented with 10% fetal calf serum, 60 U/mL penicillin, 60 μg/mL streptomycin, and 2 mM glutamine at 37°C. In each experiment, cells were rendered quiescent for 24 h by the addition of L-15 medium without serum and then were stimulated at different times with IL-1β. Where indicated, cells were preincubated with various concentrations of GLN for 1 h, and these compounds were maintained during the entire period of incubation.

Preparation of nuclear and cytosolic extracts

Nuclear and cytosolic extracts were prepared as previously described [9]. After the incubation period, chondrocytes were trypsinized and resuspended in buffer A [10 mM HEPES (pH 7.8), 10.0 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM dithiothreitol (DTT), and 1.0 mM phenylmethylsulfonyl fluoride (PMSF)] and were then homogenized. Nuclei and cytosolic fractions were separated by centrifugation at 14,000 rpm for 5 min. The cytosolic fractions (supernatants) were stored at −20°C. The nuclei (pellets) were washed twice in buffer B (10 mM HEPES, pH 7.8, 10.0 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM DTT, 1.0 mM PMSF, and 0.1% NP-40) and resuspended in buffer C (20 mM HEPES, pH 7.8, 420 mM NaCl, 1.5 mM $MgCl_2$, 25% glycerol, 1.0 mM PMSF, 0.2 mM EDTA, and 0.5 mM DTT) for 30 min on ice. Samples were centrifuged at 10,000 rpm for 30 min, and supernatants (nuclear proteins) were collected and stored at −20°C. The protein concentration was determined by the Bio-Rad Protein assay method.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated as described by Chomczynski and Sacchi [10]. The extracted RNA (2 μg) was reverse transcribed at 37°C for 1.5 h by adding 5 μM of random hexamer oligonucleotides (Gibco BRL), 200 units of reverse transcriptase (Takara), 2.5 mM deoxyribonucleotide triphosphates (dNTP) (Takara) and 10 mM dithiothreitol. PCR primers for amplification of MMP-3 and GAPDH cDNA were synthesized according to the following oligonucleotide sequences: MMP-3, forward 5′-CCTCTGATG GCCCAGAATTGA-3′, reverse 5′-GAAATTGGCCACTC

CCTGGGT-3′; GAPDH, forward 5′-CCACCCCATGGC AAATTCCATGGCA-3′, reverse 5′-TCTAGACGGCAGG TCAGGTCCACC-3′. PCR was carried out with 2 μL of template cDNA and 23 μL of PCR mix buffer containing each primer (0.2 μM), dNTP (2.5 mM) and Taq DNA polymerase (1.25 units) (Takara). After PCR, 15 μL of the reaction mixture was subjected to electrophoresis on a 1.5% agarose gel, and the PCR products were visualized by ethidium bromide staining. The levels of mRNA for MMP-3 and GAPDH were quantified by scanning densitometry.

Western blot analysis

Chondrocytes were stimulated as described in "Results" by lysing cells on the plate with ice-cold lysis buffer (10 mM Tris-HCl pH 7.6, 158 mM NaCl, 1.0 mM EDTA, 0.1% SDS, 1% Triton X-100, 1.0 mg/mL leupeptin, 1.0 mg/mL aprotinin, and 0.5 mM PMSF) which was added immediately before use. The lysates were transferred to Eppendorf tubes and centrifuged at 14,000 rpm for 30 min at 4°C. The supernatants were transferred into fresh tubes, and the protein concentration was determined using the Bio-Rad Protein assay. Similar amounts of protein were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Gelman Laboratory) by electroblotting. The membrane was blocked overnight in a 5% milk powder/TBST solution and then further incubated with one of the following antibodies: MMP-3 antibody (Oncogene, Merck Ltd., Taiwan), anti-phospho-p38 mitogen-activated protein (anti-p-MAP; Cell Signaling Tech. Inc., Beverly, MA), anti-phospho-c-Jun N-terminal kinase (anti-p-JNK), anti-phospho-extracellular signal-regulated kinase (anti-p-ERK) or anti-phospho-Akt (anti-p-Akt) (all three from Santa Cruz Biotech.) for 2 h. Membranes were washed three times with TBST, then further incubated with the appropriate HRP-labeled secondary Ab in 5% milk powder/TBST, and developed using an ECL system (Santa Cruz Biotech.).

Electrophoretic mobility shift assay (EMSA)

Transcription factor activity was determined as previously described [9]. Briefly, NF-κB or AP-1 consensus oligonucleotides (5′-AGTTGAGGGGACTTTCCCAGGC-3′ and 5′-CGCTTGATGAGTCAGCCGGAA-3′, respectively) were end-labeled with [^{32}P] phosphate by T4 polynucleotide kinase (Promega). Nuclear extracts (5 μg) were equilibrated for 10 min in a binding buffer (5% glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 10 mM Tris–HCl (pH 7.5), and 1 μg of poly (dI-dC) (Amersham Biosciences Corp., Piscataway, NJ), 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 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).

Results

Effect of GLN on MMP-3 expression

GLN was reported to inhibit the production and enzymatic activity of MMP-1 and MMP-3 in chondrocytes from OA articular cartilage with MMP-3 being affected to a greater extent than those of MMP-1 [11]. The inhibitory effect of GLN on the synthesis of MMP-3 induced by IL-1β (1 ng/mL) was first validated in chondrosarcoma cells pretreated with various amounts (0.1–1.0 mg/mL) of GLN at 24, 48, and 72 h of incubation. Results, as shown in Fig. 1a, demonstrate that GLN was able to inhibit the protein synthesis of MMP-3 in a dose-dependent manner as measured by Western blotting. Comparisons among the results shown in Fig. 1a indicate that the inhibition of protein synthesis of MMP-3 by GLN at the three different incubation times (24, 48, and 72 h) followed the same dose-dependent manner. Treatment of chondrocytes with IL-1β in the presence of glucosamine resulted in a significant decrease in the expression of MMP-3 mRNA in a dose-dependent manner as shown in Fig. 1b measured by PCR.

Effect of GLN on phosphorylation of the MAPK pathways induced by IL-1β

IL-1β binds to the IL-1 receptor type I (IL-1RI) which activates TRAF-6, through which MAPK is activated to promote phosphorylation of other substrates, such as JNK and the *Jun* and *Fos* family, all of which are associated with the transcriptional activity of AP-1 [7]. Three MAPK signaling cascades, culminating in the activation of the ERK, JNK, and p38 families of MAPK, have been investigated in detail. Therefore, the involvement of each of these in the signaling pathways was examined by detection of the respective phosphorylation products. Figure 2 illustrates the pretreatment effect of GLN (1 mg/mL) for 1 h on the time course of promoting phosphorylation of MAPK (ERK, JNK, and p38) in chondrosarcoma cells stimulated with IL-1β. Results illustrated that IL-1β is able to activate the phosphorylation of ERK starting at 15 min, and maximal induction occurred at 30 min. Pretreatment with GLN for 1 h led to even greater promotion of phosphorylation of ERK stimulated by IL-1β. A similar stimulatory effect as for pretreatment with GLN was found to be true for the

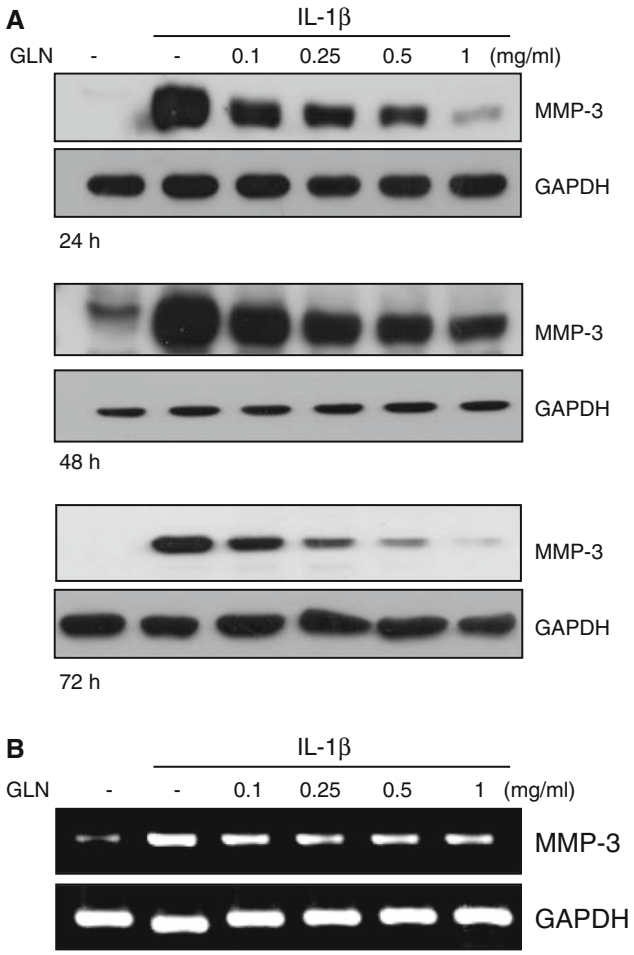

Fig. 1 Time–course effects of glucosamine on the MMP-3 expression in human chondrosarcoma cells (SW1353). **a** Cells were pretreated with different concentrations of GLN (0.1, 0.25, 0.5, and 1 mg/mL) for 1 h and treated with IL-1β (2 ng/mL) for various time periods, and detected MMP-3 protein expression by Western blotting as described in "Materials and Methods". **b** Cells were pretreated with different concentrations of GLN (0.1, 0.25, 0.5, and 1 mg/mL) for 1 h and treated with IL-1β (2 ng/mL) for 6 h, and detected MMP-3 mRNA expression by RT-PCR as described in "Materials and Methods"

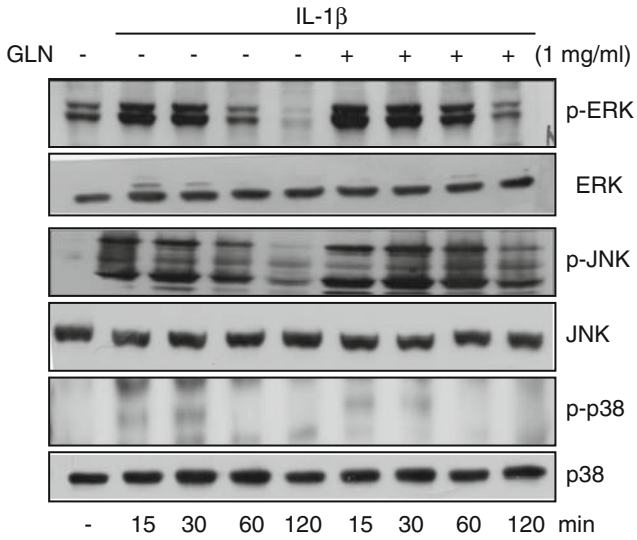

Fig. 2 Time–course effects of glucosamine on the phosphorylation of ERK, JNK, and p38 in human chondrosarcoma cells (SW1353). Cells were incubated with 1 mg/mL GLN for various time periods (15, 30, 60, and 120 min), and phosphorylated-ERK, phosphorylated-JNK, or phosphorylated-p38 was detected by Western blotting as described in "Materials and Methods". Equal loading in each lane was demonstrated by the similar intensities of T-ERK, α-tubulin, and T-p38

phosphorylation of JNK, and this stimulatory effect even lasted for 2 h. However, the inhibitory effect of pretreatment with GLN on the phosphorylation of p38 stimulated by IL-1β was demonstrated starting from as early as 15 min.

Effect of GLN on the phosphorylation of Akt

Phosphatidyl-inositol 3-kinase (PI 3-kinase) was reported to functionally interact with the IL-1 receptor-associated kinase (IRAK), which recruits TRAF6 to activate NF-κB in HepG2 and KB cells [12]. PI 3-kinase seems to be necessary to cooperate with other IL-1-inducible signals to fully activate NF-κB-dependent gene expression, whereas overexpression of PI 3-kinase may be sufficient to induce AP-1 and increase nuclear *c-Fos* protein levels. On the other hand, it is known that Akt serine-theonine kinase is involved in the activation of NF-κB by TNF through TRAF2. A signaling pathway culminating in phosphorylation of IKKα by Akt is necessary for the activation of NF-κB in several types of cells [13]. Akt has been proven to be a critical downstream effector of PI 3-kinase in both non-diabetic and diabetic cells, and GLN impairs the insulin-stimulated tyrosine phosphorylation cascade leading to activation of the PI3-kinase/Akt signaling pathway [5]. Therefore, Akt may be a downstream effector of PI3-kinase in the activation of NF-κB by IL-1β through TRAF6 with Akt being involved in phosphorylation of IKKα which frees up NF-κB for translocation. As demonstrated in Fig. 3a, activation of Akt phosphorylation occurred at 15 min in chondrosarcoma cells after stimulation by IL-1β. Pretreatment of GLN for 1 h led to the promotion of phosphorylation of Akt in chondrosarcoma cells stimulated with IL-1β for all time points examined. Furthermore, treatment with only GLN was also able to induce phosphorylation of Akt with maximal induction occurring at 120 min as illustrated in Fig. 3b.

To test whether GLN activates Akt through PI3 kinase, we used wortmannin to inhibit PI3-kinase activity in IL-1β-treated cells. As shown in Fig. 4, IL-1β alone was able to induce phosphorylation of Akt and the combination with GLN induced it to an even greater extent. In addition, the addition of wortmannin did not inhibit the activation of Akt in the cells with IL-1β and GLN, indicating Akt activation by GLN not mediates by PI3-kinase.

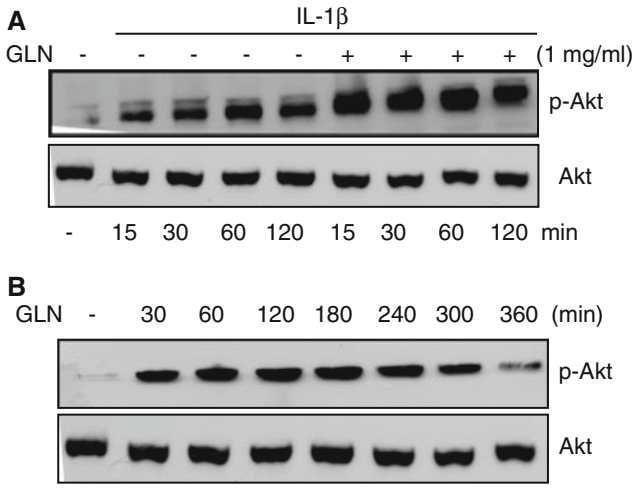

Fig. 3 Effects of glucosamine on IL-1β-induced Akt phosphorylation in human chondrosarcoma cells (SW1353). **a** Cells were stimulated with IL-1β for various time periods (15–120 min) with or without pretreatment with GLN (1 mg/mL) for 30 min, and phosphorylated-Akt was detected by Western blotting as described in "Materials and Methods". **b** Cells were incubated with GLN (1 mg/mL) for various time periods, and phosphorylated-Akt was detected by Western blotting as described in "Materials and Methods". Equal loading in each lane was demonstrated by the similar intensities of T-Akt

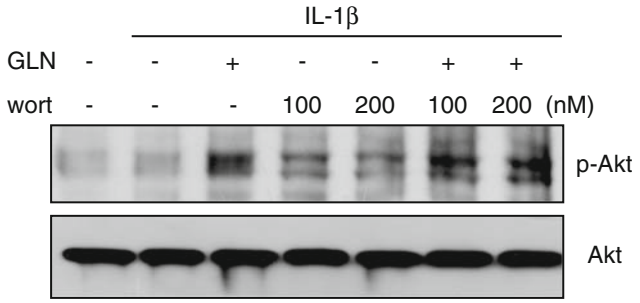

Fig. 4 Effects of wortmannin and glucosamine on the IL-1β-induced production of p-Akt and p-p38 in human chondrosarcoma cells (SW1353). Cells were pretreated with GLN (1 mg/mL) and wortmannin (100 or 200 nM) for 30 min before the addition of IL-1β (2 ng/mL) for 30 min. Phospho-Akt was detected by western blotting as described in "Materials and Methods". Equal loading in each lane was demonstrated by the similar intensities of α-tubulin

Effect of GLN on IL-1β-induced activation of NF-κB and its characterization

It has been demonstrated that GLN preincubated with IL-1β-induced HOCs showed a significant inhibition of NF-κB binding in a dose-dependent manner (10–1,000 mg/L) in comparison with IL-1β-induced HOCs [14]. However, this effect on NF-κB activation was proven to be specific for this nuclear factor since GLN did not modify IL-1β-induced AP-1 binding [14]. As

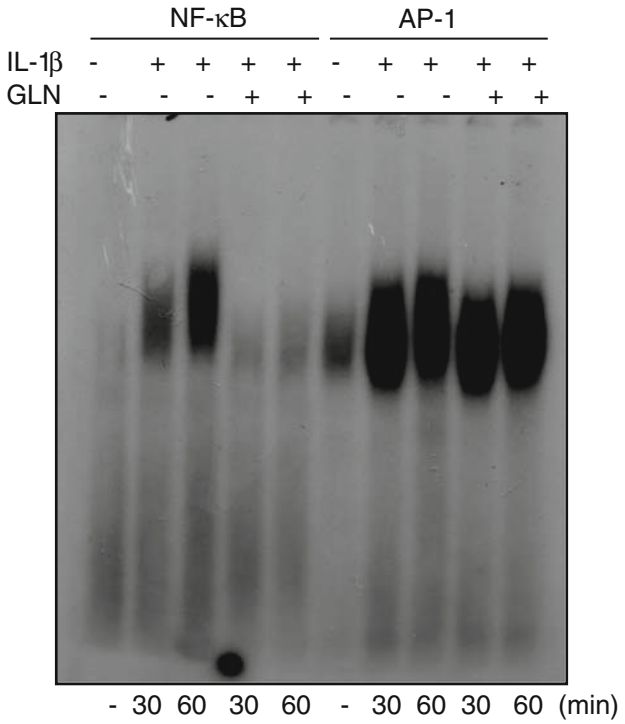

Fig. 5 Effects of glucosamine on IL-1β-induced AP-1 and NF-κB activation in human chondrosarcoma cells (SW-1353). Cells were pretreated with GLN (1 mg/mL) for 30 min and then treated with IL-1β (2 ng/mL) for 30 or 60 min. AP-1 and NF-κB were detected by EMSA

shown by Fig. 5, both NF-κB and AP-1 were activated by treating chondrosarcoma cells with IL-1β, and the NF-κB activation induced by IL-1β was shown to be lower than that of AP-1 activation. However, the presence of GLN at a concentration of 1 mg/mL was able to reduce the activation of NF-κB induced by IL-1β as expected, whereas a slightly synergistic effect of GLN was shown for the activation of AP-1 induced by IL-1β. The translocation of NF-κB subunits into the nucleus was further examined, and the results are demonstrated in Fig. 6a. It clearly indicates that the translocation of one of the NF-κB subunits of p65 into the nucleus was blocked as early as 30 min and for up to 1 h.

NF-κB activation occurs following dissociation of an inhibitory subunit, a member of the IκB family, which is degraded by a proteolytic process. Thus, the effect of GLN on the degradation of IκB leading to NF-κB activation induced by IL-1β was examined. As shown in Fig. 6b, stimulation of chondrosarcoma cells with IL-1β led to the degradation of IκB at 30 min, and then the same lower level was maintained for up to 2 h. However, preincubation with GLN for 1 h reduced the degradation of IκB leading that the level of IκB recovering at 1 h and then returning to the same level as the control at 2 h.

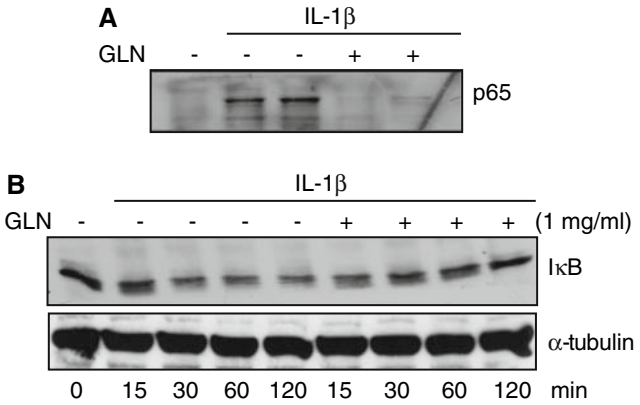

Fig. 6 Effects of glucosamine on the inhibition of IL-1β-induced NFκB activation in human chondrosarcoma cells (SW1353). **a** Cells were pretreated with GLN (1 mg/mL) for 30 min and then treated with IL-1β (2 ng/mL) for 30 or 60 min, and p65 was detected by Western blotting as described in "Materials and Methods". **b** Cells were pretreated with GLN (1 mg/mL) for 30 min and then treated with IL-1β (2 ng/mL) for various time periods, and IκB was detected by Western blotting as described in "Materials and Methods"

Discussion

This study addresses how GLN inhibits the expression and synthesis of MMP-3 induced by IL-1β and how that inhibition is mediated at the level of transcription involving both transcription factors of NF-κB and AP-1. Translocation of NF-κB was reduced by GLN as a result of the inhibition of IκB degradation. A slightly synergistic effect of the presence of GLN on the activation of AP-1 induced by IL-1β was shown. Among the MAPK pathways involved in the transcriptional regulation of AP-1 members of the *Jun* and *Fos* families of transcription factors, phosphorylation of JNK and ERK was found to be increased in the presence of GLN under IL-1β treatment, while that of p38 was decreased. It was also found that the PI′3-kinase/Akt pathway was activated not only by GLN alone but also by synergism with IL-1β.

Regarding to the mechanisms of the beneficial effect of GLN on the treatment of OA, Gouze et al. showed that GLN significantly decreases the efficiency of the IL-1β signaling pathway by antagonizing NF-κB activation, but has no significant effect on IL-1β-medicated AP-1 activation. However, 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 [15]. For the expression of MMP-3, the transcription factor AP-1 is essential (but not sufficient) for the upregulation of this enzyme under proinflammatory cytokines [16]; NF-κB activity is also involved in MMP-3 upregulation. Therefore, the beneficial effects of GLN can be explained, at least in part, by the inhibition of NF-κB transcriptional activity [4]. This was confirmed in the present study by the presence of GLN being able to reduce the translocation of NF-κB through inhibition of the degradation of IκB. However, the activity of AP-1 was slightly enhanced by IL-1β synergistically in the presence of GLN. This indicates that both NF-κB and AP-1 activities are required for the induction of MMP-3 by IL-1β in chondrosarcoma cells. Therefore, the inhibition of one of two possible transcriptional factors, NF-κB and AP-1, in the presence of GLN is able to reduce the expression of MMP-3.

It has been shown that IL-1β induces MMP-3, MMP-13, and MAPKs in chondrocytes. The inhibition of MMP gene expression by using corresponding inhibitors suggests that the ERK-, p38- and JNK-MAPK pathways as well as the AP-1 and NF-κB transcription factors are mediators of MMP induction by IL-1β [17]. However, it has been demonstrated that IL-1β induction of MMP-13 in articular chondrocytes and chondrosarcoma cells requires p38 activity, JNK activity, and NF-κB translocation, whereas MMP-1 induction in chondrosarcoma cells depends on p38 and MEK (an ERK pathway) but does not require JNK or NF-κB; in articular chondrocytes, inhibition of MEK had no effect, while inhibition of p38 gave variable results [18]. These results further suggest that induction of MMPs by IL-1β in chondrocytic cells depends on unique combinations of signaling pathways that are cell type specific. In this study, it was demonstrated that the presence of GLN inhibited the induction of MMP-3 in chondrosarcoma cells stimulated by IL-1β in concert with the inhibition of NF-κB translocation and p38 activation. This indicates that NF-κB translocation and p38 activity in chondrosarcoma cells are required to induce the expression of MMP-3 stimulated by IL-1β.

The important role of p38 MAPK in mediating the response induced by IL-1β to regulate the expressions of NO, COX-2, and mPGES-1 (microsomal prostaglandin E synthase 1) has been disclosed. Thomas et al. [19] reported that overexpression of p38 MAPK induces the COX-2 reporter, whereas overexpression of the dominant negative p38 MAPK represses IL-1β-induced promoter expression in articular chondrocytes. This result suggests that differentiated articular chondrocytes are highly responsive to IL-1β and that p38 MAPK exclusively mediates this response by inducing COX-2 gene expression. It was concluded by Masuko-Hongo et al. [20] that the mPGES-1 gene is regulated by IL-1β via ERK-1/2 and putative β-isoform signaling of the p38 MAPK pathway in human chondrocytes. Targeting p38β MAPK may inhibit the proinflammatory mediator, PGE_2, without interfering with the prostacyclin pathway. Furthermore, monosodium urate (MSU) crystals were found to activate MMP-3 and iNOS expression and NO release in chondrocytes in a p38-dependent manner that

did not require IL-1β [21]. It was confirmed that the p38 pathway was the principal MAPK cascade involved in the induction of both NO and MMP-3 expression. Thus, inhibition of the p38 pathway induced by IL-1β in the presence of GLN demonstrated in this study, substantially but not fully, explains the chondroprotective effect of GLN on chondrocytes that has been reported by regulating COX-2 expression [14], PGE_2 synthesis [14, 22], and NO expression and synthesis [22].

Several reports have demonstrated that PI 3-kinase was associated with the downregulation of MMPs expression. HC-gp39 (human cartilage glycoprotein 39) binding to a putative receptor leads to PI 3-kinase-mediated phosphorylation of Akt, which downregulates ASK1 (apoptosis signal-regulating kinase 1) activity by catalyzing the phosphorylation of Ser^{83}. This in turn leads to decreased activation of JNK and p38, and finally decreased production of MMPs (including MMP-1, MMP-3, and MMP-13) [23]. It was also demonstrated in a study conducted by Starkman et al. [24] that IGF-1 (insulin-like growth factor 1) stimulation of proteoglycan synthesis by articular chondrocytes requires activation of the PI 3-kinase/Akt/mTOR/p70S6 kinase signaling pathways but not the Ras/Raf/MEK/ERK pathways. Thus, the phosphorylating activation of Akt in the presence of GLN downregulates ASK1 activity, which in turn decreases activation of JNK and p38 which may be induced followed by binding of IL-1β to its membrane receptor. However, our results showed that inhibition of PI 3-kinase had no effect on the activation of Akt induced by IL-1β and GLN (Fig. 4), indicating that Akt activation by GLN is not mediated by PI 3-kinase. This reveals that the chondroprotective effect of GLN on chondrosarcoma cells by at least decreasing MMP-3 production and stimulating proteoglycan synthesis may follow another potential signaling pathway of Akt. However, it remains to be elucidated that which isoform of GLUT transporter interacted with IL-1β to regulate the influx of GLN into chondrocytes and what is the binding receptor inside chondrocytes for GLN to initiate signaling cascades that regulate its chondroprotective effect.

Acknowledgments Financial support by Mackay Memorial Hospital (94MMH-TMU-14) is highly appreciated.

References

1. Abramson SB, Amin A (2002) Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. Rheumatology (Oxford) 41:972–980
2. Lotz M (2001) Cytokines in cartilage injury and repair. Clin Orthop Relat Res 391(Suppl):S108–115
3. Vincenti MP, Brinckerhoff CE (2001) Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1beta. Arthritis Res 3:381–388
4. Gouze JN, Bianchi A, Becuwe P, Dauca M, Netter P, Magadalou J, Terlain B, Bordji K (2002) Glucosamine modulates IL-1-induced activation of rat chondrocytes at a receptor level, and by inhibiting the NF-kappa B pathway. FEBS Lett 510:166–170
5. Ciaraldi TP, Carter L, Nikoulina S, Mudallar S, McClain DA, Henry RR (1999) Glucosamine regulation of glucose metabolism in cultured human skeletal muscle cells: divergent effects on glucose transport/phosphorylation and glycogen synthase in non-diabetic and type 2 diabetic subjects. Endocrinology 140:3971–3980
6. Ninomiya-Tsuji J, Kishimoto K, Hiyama A, Inoue J, Cao Z, Matsumoto K (1999) The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. Nature 398:252–256
7. Firestein GS, Manning AM (1999) Signal transduction and transcription factors in rheumatic disease. Arthritis Rheum 42:609–621
8. Gouze JN, Bordji K, Gulberti S, Terlain B, Netter P, Magdalou J, Fournel-Gigleux S, Ouzzine M (2001) Interleukin-1beta downregulates the expression of glucuronosyltransferase I, a key enzyme priming glycosaminoglycan biosynthesis: influence of glucosamine on interleukin-1beta-mediated effects in rat chondrocytes. Arthritis Rheum 4:351–360
9. Gomez-Garre D, Largo R, Tejera N, Fortes J, Manzarbeitia F, Egido J (2001) Activation of NF-kappaB in tubular epithelial cells of rats with intense proteinuria: role of angiotensin II and endothelin-1. Hypertension 37:1171–1182
10. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156
11. Dodge GR, Jimenez SA (2003) Glucosamine sulfate modulates the levels of aggrecan and matrix metalloproteinase-3 synthesized by cultured human osteoarthritis articular chondrocytes. Osteoarthr Cartilage 11:424–432
12. Reddy SA, Huang JH, Liao WS (1997) Phosphatidylinositol 3-kinase in interleukin 1 signaling. Physical interaction with the interleukin 1 receptor and requirement in NFkappaB and AP-1 activation. J Biol Chem 272:29167–29173
13. Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB (1999) NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature 401:82–85
14. Largo R, Alvarez-Soria MA, Diez-Ortego I, Calvo E, Sanchez-Pernaute O, Egido J, Herrero-Beaumont G (2003) Glucosamine inhibits IL-1beta-induced NFkappaB activation in human osteoarthritic chondrocytes. Osteoarthr Cartilage 11:290–298
15. Borden P, Heller RA (1997) Transcriptional control of matrix metalloproteinases and the tissue inhibitors of matrix metalloproteinases. Crit Rev Eukaryot Gene Expr 7:159–178
16. Bond M, Baker AH, Newby AC (1999) Nuclear factor kappaB activity is essential for matrix metalloproteinase-1 and -3 upregulation in rabbit dermal fibroblasts. Biochem Biophys Res Commun 264:561–567
17. Liacini A, Sylvester J, Li WQ, Zafarullah M (2002) Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. Matrix Biol 21:251–262
18. Mengshol JA, Vincenti MP, Coon CI, Barchowsky A, Brinckerhoff C (2000) Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappaB: differential regulation of collagenase 1 and collagenase 3. Arthritis Rheum 43:801–811
19. Thomas B, Thirion S, Humbert L, Tan L, Goldring MB, Bereziat G (2002) Differentiation regulates interleukin-1beta-induced cyclo-oxygenase-2 in human articular chondrocytes: role of p38 mitogen-activated protein kinase. Biochem J 362:367–373

20. Masuko-Hongo K, Berenbaum F, Humbert L, Salvat C, Goldring MB, Thirion S (2004) Up-regulation of microsomal prostaglandin E synthase 1 in osteoarthritic human cartilage: critical roles of the ERK-1/2 and p38 signaling pathways. Arthritis Rheum 50:2829–2838
21. Liu R, Liote F, Rose DM, Merz D, Terkeltaub R (2004) Proline-rich tyrosine kinase 2 and Src kinase signaling transduce monosodium urate crystal-induced nitric oxide production and matrix metalloproteinase 3 expression in chondrocytes. Arthritis Rheum 50:247–258
22. Chan PS, Caron JP, Rosa GJM, Orth MW (2005) Glucosamine and chondroitin sulfate regulate gene expression and synthesis of nitric oxide and prostaglandin E(2) in articular cartilage explants. Osteoarthr Cartilage 13:387–394
23. Ichijo H, Nishida E, Irie K, Ten Dijke P, Saltoh M (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science 275:90–94
24. Starkman BG, Cravero JD, DelCarlo M, Loeser RF (2005) IGF-I stimulation of proteoglycan synthesis by chondrocytes requires activation of the PI 3-kinase pathway but not ERK MAPK. Biochem J 389(Pt 3):723–729