Evaluation of the chondroprotective action of N-acetylglucosamine in a rat experimental osteoarthritis model

DAIKI KUBOMURA[1], TOMOYA UENO[1], MASANORI YAMADA[1] and ISAO NAGAOKA[2]

[1]Yaizu Suisankagaku Industry Co., Ltd., Yaizu, Shizuoka 425-8570; [2]Department of Host Defense and Biochemical Research, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan

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Abstract. It has been demonstrated that oral administration of N-acetylglucosamine (GlcNAc) alleviates the symptoms of osteoarthritis (OA). The aim of the present study was to elucidate the molecular mechanisms for the chondroprotective action of GlcNAc in OA. Biomarkers for type II collagen degradation and synthesis were evaluated, as were histopathological changes, using a rat anterior cruciate ligament transection (ACLT)-induced OA model. Changes in the expression of genes in the cartilage were assessed via DNA microarray and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The results indicated that ACLT induced histopathological changes of articular cartilage, whereas oral administration of GlcNAc (1,000 mg/kg/day for 28 days) significantly suppressed these changes. Additionally, GlcNAc significantly decreased levels of a type II collagen degradation marker in sera compared with that in the ACLT group, although there were no significant changes in the levels of a type II collagen synthesis marker. Furthermore, DNA microarray and reverse transcription-quantitative polymerase chain reaction results demonstrated that GlcNAc treatment downregulated the expression of periostin, which is likely involved in the degradation of cartilage, whereas GlcNAc upregulated the expression of lipocalin 2, which is involved in the regulation of chondrocyte proliferation and differentiation. In conclusion, the results of the present study suggest that GlcNAc is able to suppress the histopathological changes induced by OA and exhibits a chondroprotective action by inhibiting type II collagen degradation in the articular cartilage, possibly via modulation of the expression of inflammatory and chondroprotective molecules, including periostin and lipocalin 2.

Correspondence to: Dr Daiki Kubomura, Yaizu Suisankagaku Industry Co., Ltd., 5-8-13 Kogawa-Shimmachi, Yaizu, Shizuoka 425-8570, Japan
E-mail: kubomura@yskf.co.jp

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Introduction

Knee osteoarthritis (OA) is the most prevalent disease of the joints. The prevalence of knee OA is estimated at 9.6% in men and 18% in women aged 60 years or older (1). OA is characterized by a progressive degradation of cartilage and the symptoms of joint pain and severe impaired mobility of the knee as the knee is a weight-bearing joint (2). An imbalance between the synthesis and degradation of articular cartilage leads to joint pain, stiffness and severely impaired joint mobility (3). Pharmacological treatments for knee OA typically utilize analgesic agents, such as nonsteroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors, which are aimed at controlling pain and limiting loss of function. However, previous studies have suggested that the benefits of these pharmaceutical agents are often insufficient and have an associated risk of adverse reactions (4,5). Therefore, the development of alternative strategies for the treatment of knee OA is necessary.

Glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) are naturally occurring amino monosaccharides and are the key components of glycosaminoglycans contained in articular cartilage, such as chondroitin sulfate and hyaluronan; due to their crucial role in glycosaminoglycan formation in cartilage (6,7), GlcN and GlcNAc have been widely used as dietary supplements or alternative treatments for the management of OA (8). Furthermore, clinical studies and a meta-analysis investigating the effect of GlcN on OA have demonstrated that glucosamine sulfate or hydrochloride has potential benefits for relieving pain associated with OA (9-11). Additionally, it has previously been demonstrated that oral administration of GlcNAc alleviated symptoms in patients with OA (12,13).

It has previously been demonstrated that GlcN is incorporated into cells via glucose transporters (14) and used by synovial cells and chondrocytes to produce hyaluronic acid via the upregulation of hyaluronic acid-synthesizing enzymes (8,15). GlcN has also been suggested to exert anti-inflammatory actions by inhibiting nitric oxide production, and downregulating cyclooxygenase-2 and the expression of pro-inflammatory cytokines (16,17). Based on these biochemical and pharmacological findings, it has been suggested that the administration of GlcN recovers cartilage metabolism by inhibiting degradation and stimulating the synthesis of proteoglycans (18). However, the underlying molecular mechanisms of the chondroprotective action of GlcNAc in patients with OA remain to be elucidated.

In the present study, serum biomarkers for type II collagen degradation (type II collagen cleavage neoepitope; C2C) and synthesis (carboxyterminal propeptide of type II procollagen, PIICP) were evaluated in addition to histopathological changes in a rat anterior cruciate ligament transection (ACLT)-induced OA model. The ACLT model has been widely used to analyze histological and biochemical changes that occur during the progression of OA (19). Changes in gene expression in the cartilage were also investigated using a DNA microarray and reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Materials and methods

Animal model. All procedures were carried out at I Tech Lab (Kaizu, Japan) according to the institutional guidelines for animal experiments based on the Guidelines for Proper Conduct of Animal Experiments, Act on Welfare and Management of Animals, and standards for the care and management of experimental animals. The study protocol was approved by the Institutional Animal Care and Use Committee at I Tech Lab. In the present study, a total of 37 male Slc:SD rats (8-weeks-old, initially weighing 307.5±17.5 g; Japan SLC, Inc., Hamamatsu, Japan) were used. All animals were housed in a room maintained at 23±3˚C with 62±5% humidity and a 12-h light/dark cycle. Sterile food (FR-2; Funabashi Farm Co., Ltd., Chiba, Japan) and water were freely available. Rats were acclimated to the environmental conditions for 1 week prior to the experiment. Rats, with the exception of 3 heavy and 4 lightweight rats, were randomly divided into three groups based on body weight: Sham-operated group (sham; n=10), ACLT group without GlcNAc (ACLT; n=10) and ACLT group with GlcNAc (GlcNAc; n=10).

OA was surgically induced in the knee joint. Each rat was anesthetized via intramuscular injection of 0.8 ml/kg each of ketamine (Ketalar 500 mg; Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan) and xylazine (Selactar 2%; Bayer Yakuhin, Ltd., Osaka, Japan). The surgical area was shaved and disinfected with povidone iodine, and the right knee joint was subsequently exposed by dissecting the skin on the inside of the patella along the patellar tendon. The patella was dislocated laterally, the knee was placed in full flexion, and the anterior cruciate ligament was subsequently transected. A positive anterior drawer test ensured complete transection of the ligament. Following surgery, the joint surface was washed with sterile saline and disinfected with 4 ml of antibiotics solution (250 mg/ml ampicillin sodium salt). The knee joint and patella were then returned to their original position. The capsule and skin were subsequently sutured using Vicryl 4-0 absorbable sutures (Ethicon, Somerville, NJ, USA) and monofilament 4-0 nylon threads (Ethicon), respectively. For rats in the sham group, the right knee joint was exposed and washed with saline, following which the incisions were sutured.

GlcNAc was supplied by Yaizu Suisankagaku Industry Co., Ltd. (Yaizu, Japan). On days 1-28 post-surgery, 1,000 mg/kg GlcNAc (200 mg/ml GlcNAc solution) dissolved in sterile water was orally administered to rats in the GlcNAc group daily. An equivalent volume of sterile water was orally administered to rats in the sham and ACLT groups daily.

Blood samples (1 ml/rat) were harvested from the carotid artery at 1, 14 and 28 days post-surgery. Sera were prepared by centrifugation at 1,700 x g for 15 min at 4˚C and stored in aliquots at -80˚C. Rats were sacrificed at 29 days post-surgery for histopathological and genetic analyses.

Enzyme-linked immunosorbent assay (ELISA) of serum biomarkers. Serum samples obtained on days 1, 14 and 28 post-surgery were analyzed for each biomarker. The biomarkers investigated were C2C and PIICP. C2C was measured using a competitive inhibition ELISA (Collagen Type II Cleavage ELISA kit; IBEX Technologies, Inc., Montreal, QC, Canada). PIICP was assessed using an ELISA kit for Procollagen II C-terminal Propeptide (USCN Life Sciences, Inc., Wuhan, China).

Histopathological evaluation. Histopathological evaluation was performed on sagittal sections of cartilage in the weight-bearing area of the femoral condyle and medial tibia plateau from 4 rats per group. Knee joint samples were dissected, fixed in 10% formalin for 24 h at 20-25˚C, decalcified using 10% ethylenediaminetetraacetic acid solution for 2 weeks, and embedded in paraffin. Sections with a thickness of 5 μm were stained with 0.05% toluidine blue (pH 4.1), and the severity of OA lesions was graded on a scale of 0-13, using the modified Mankin scoring system (18), with a combined score of structure (0-6 points), matrix staining (0-4 points) and cellular abnormalities (0-3 points).

RNA isolation. RNA was prepared as described previously (20). Briefly, total RNA was extracted from the harvested the sections of cartilage in the weight-bearing area of the medial tibia plateau using an RNAiso plus reagent (Takara Bio Inc., Otsu, Japan) and the RNase-Free DNase Set (Qiagen, Inc., Valencia, CA, USA). The extracted RNA was further purified using an RNeasy MinElute Cleanup kit (Qiagen, Inc.). The quantity and purity of total RNA were evaluated photometrically at 260, 280 and 320 nm using a spectrophotometer (Ultrospec 2000; GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The ratio of A260/280 was confirmed to be >2.0, and total RNA was used for the following experiments.

DNA microarray analysis. Total RNA (200 ng) was used to generate cDNA and Cy3-labeled cRNA using a Low Input Quick-Amp Labeling kit (Agilent Technologies, Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. The labeled cRNA was used to photometrically determine the quantity and dye-incorporation ratio with an Ultrospec 2000 spectrophotometer, and hybridized to a Whole Rat Genome Microarray (Ver 3.0; cat. no. G4847B; Agilent Technologies, Inc.) at 65˚C for 17 h. The array was scanned with GenePix 4000B (Molecular Devices, LLC, Sunnyvale, CA, USA), and the resulting image was processed using GenePix Pro 6.0 Software (Molecular Devices, LLC). The features were manually examined, and spots of poor quality were flagged and filtered out according to the method of Shintani *et al* (21) and Chae *et al* (22). The signal data for the features (spots of array) were then imported into GeneSpring 12.6 (Agilent Technologies, Inc.), and further analyzed with the software. The signal data from the arrays were normalized

with the 75th percentile method (23), and baseline transformation was performed with the median of the control samples. Quality control was performed in order to filter out signal data with standard errors >0.2.

To analyze the differentially expressed genes, the fold change (FC) vs. the sham samples was calculated, and genes with an FC >1.5 or <0.75 were extracted for the ACLT group. To analyze the effects of GlcNAc, the genes that were shifted toward the baseline levels (the sham group) in the GlcNAc group compared with the ACLT group were selected.

RT-qPCR. RT-qPCR was performed to confirm the expression of 41 target genes selected on the basis of the DNA microarray analysis following the method of Shintani *et al* (21) and Chae *et al* (22). cDNA was synthesized from 1 μg total RNA from the cartilage using PrimeScript Reverse Transcriptase (Takara Bio, Inc.), oligo (dT) primer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and dNTP (Promega Corporation, Madison, WI, USA). qPCR was performed with a Mx3000P qPCR System (Agilent Technologies, Inc.) using a SYBR Premix Ex Taq kit (Takara Bio, Inc.), ROX (Takara Bio, Inc.) and specific primer sets designed using Primer-BLAST program (The sequences of the primers are available upon request) (24). PCR conditions were: 95˚C for 15 sec, followed by 35 cycles of 95˚C for 10 sec and 63˚C for 30 sec. In each run, a standard curve was generated by a serially diluted known GAPDH amplicon to calculate the cDNA copy number of the genes, as described previously (20). The quantity of the mRNA of interest was expressed as its ratio against that of a suitable reference gene, peptidylprolyl isomerase A (25).

Statistical analyses. Values are expressed as the mean ± standard deviation or mean + standard deviation. Student's unpaired t-test was used to assess the intergroup differences in histopathological evaluation and serum biomarkers. Student's paired t-test was used to assess the chronologic changes in serum biomarkers. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using EZR version 1.31 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (26), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 3.22).

Results

Effect of GlcNAc administration on the gross morphology and the histopathological changes in articular cartilage. Images of the articular cartilage from rats in the three groups are shown in Fig. 1. In the sham group, no macroscopic abnormalities were detected on the articular surfaces of femoral condyles (Fig. 1A) and tibial plateau (Fig. 1D). However, the rats of the ACLT group exhibited marked erosive changes on the surfaces of these sites (Fig. 1B and E). The degenerative changes were markedly suppressed in the GlcNAc group (Fig. 1C and F).

The histopathological changes in articular cartilage were also assessed, and representative images are shown in Fig. 2. When compared with cartilage from the sham group (Fig. 2A and E), cartilage from rats in the ACLT group (Fig. 2B and F) displayed histopathological changes including surface depletion, a marked reduction in the number of chondrocytes and reduced toluidine blue staining. Furthermore, treatment with GlcNAc markedly suppressed these structural abnormalities (Fig. 2C and G). These structural changes were further evaluated using the modified Mankin scoring system. The total score was significantly higher in the femoral condyles and tibial plateau of the ACLT group compared with the sham group (both P<0.01). Treatment with GlcNAc markedly reduced this effect in the femoral condyles (Fig. 2D) and significantly reduced the effect in the tibial plateau (P<0.05 vs. the ACLT group; Fig. 2H).

Evaluation of biomarkers. The effect of GlcNAc administration on cartilage metabolism was assessed using biomarkers for type II collagen degradation and synthesis. Serum C2C levels were significantly decreased from the baseline (day 1) on days 14 and 28 post-surgery in the ACLT and GlcNAc groups (Fig. 3A). Furthermore, on day 28 the C2C level was significantly lower in the GlcNAc group compared with the ACLT group (P<0.05; Fig. 3A). No significant differences in PIICP levels were observed between the sham, ACLT and GlcNAc groups. However, the PIICP levels in the sham and GlcNAc groups were demonstrated to be significantly decreased from the baseline on day 28 (P<0.05; Fig. 3B). Consequently, the C2C/PIICP ratio in the GlcNAc group was significantly decreased from the baseline on days 14 and 28 post-surgery (Fig. 3C). Together, these observations suggest that the administration of GlcNAc suppresses the degradation of type II collagen in the articular cartilage of ACLT rats.

DNA microarray analysis. To elucidate the mechanism for the changes of histopathological findings and biomarkers, the effect of GlcNAc administration was investigated further using microarray analysis. The results indicated that 74 genes were upregulated by ACLT (>1.5-fold) compared with the sham group and downregulated in the GlcNAc group compared with the ACLT group (Table I). By contrast, 18 genes were downregulated in the ACLT group (<0.75-fold) compared with the sham group and upregulated in the GlcNAc group compared with the ACLT group (Table II). Pathway analysis indicated that the majority of the modulated genes are associated with synthesis of the components of extracellular matrix in articular cartilage: Tenascin, collagen, laminin and thrombospondin (data not shown).

RT-qPCR analysis. In order to confirm the results of DNA microarray, 41 genes associated with the extracellular matrix components in the articular cartilage were selected and subjected to RT-qPCR. As shown in Table III, 14 genes were upregulated by ACLT (>1.3-fold) and downregulated by GlcNAc (<0.9-fold): Periostin, osteoblast specific factor (*Postn*), fibrillin 2 (*Fbn2*), collagen type I, α1 (*Col1a1*), tenascin N (*Tnn*), sortilin-related VPS10 domain containing receptor 2 (*Sorcs2*), WNT1 inducible signaling pathway protein 2 (*Wisp2*), matrix metalloproteinase 2 (*Mmp2*), vascular endothelial growth factor A (*Vegfa*), collagen type V, α2 (*Col5a2*), Mmp13, A disintegrin and metalloproteinase with thrombospondin motifs 4 (*Adamts4*), Sushi repeat-containing protein, X-linked 2 (*Srpx2*) and tissue inhibitor of metalloproteinase-1 (*Timp1*). Furthermore, 6 genes were downregulated by ACLT (<0.7-fold) and upregulated by GlcNAc (>1.3-fold): Lipocalin-2 (*Lcn2*), uncharacterized anionic trypsin II like (*Prss21*),

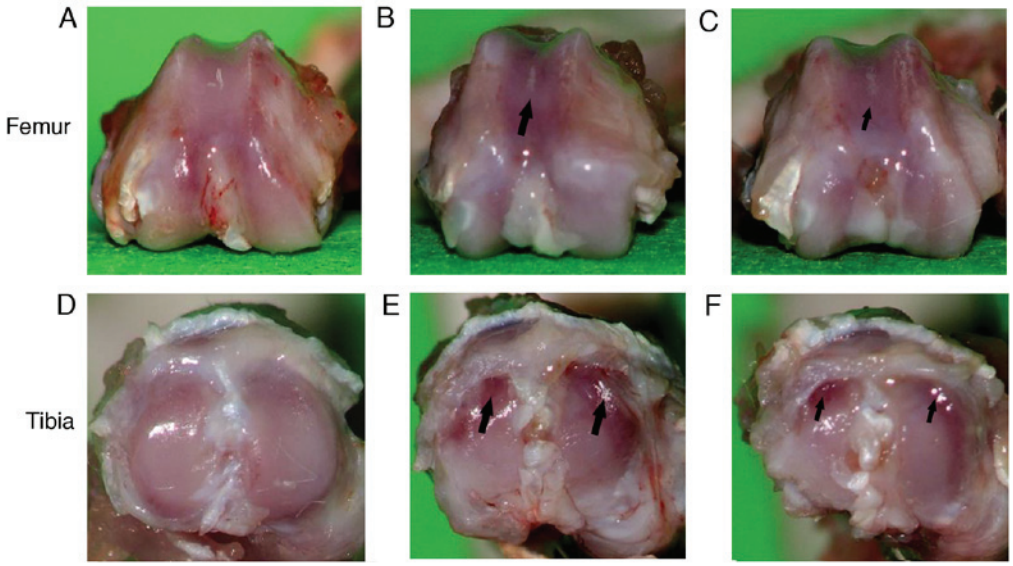

Figure 1. Macroscopic changes of femoral condyles and the tibial plateau at 29 days following ACLT surgery. Femurs from the (A) sham, (B) ACLT and (C) GlcNAc groups. Tibias from the (D) sham, (E) ACLT and (F) GlcNAc groups. Sham-operated joints showed no macroscopic changes, whereas ACLT induced erosive changes on the joint surfaces (indicated by large arrowheads) and GlcNAc administration substantially suppressed these degenerative changes (indicated by small arrowheads). ACLT, anterior cruciate ligament transection; sham, sham-operated; GlcNAc, N-acetylglucosamine.

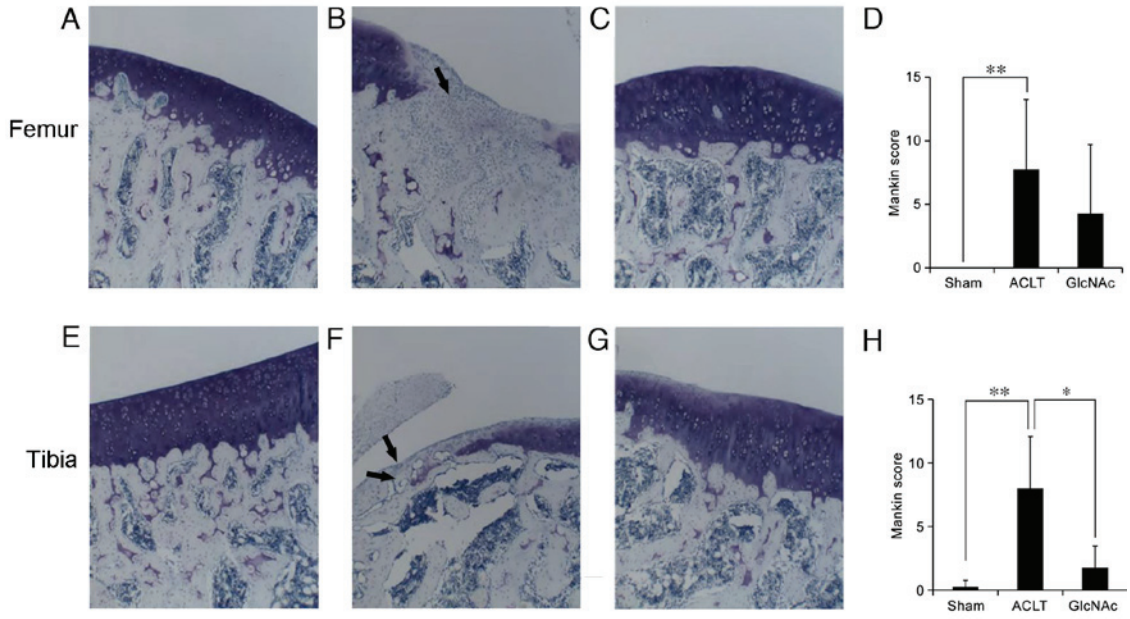

Figure 2. Histopathological evaluation of articular cartilage in a rat OA model. Knee joints were dissected at 29 days following ACLT surgery. Sagittal sections of cartilage in the weight-bearing area of the femoral condyle and the medial tibia plateau were stained with toluidine blue. Femurs from the (A) sham, (B) ACLT and (C) GlcNAc groups, and (D) the severity of OA lesions in femurs. Tibias from the (E) sham, (F) ACLT and (G) GlcNAc groups, and (H) the severity of OA lesions in tibias. ACLT induced surface depletion, a marked reduction in the number of chondrocytes and a reduction in toluidine blue staining of the cartilage from femurs and tibias (indicated by arrowheads). The severity of OA lesions was graded on a scale of 0-13 using the modified Mankin scoring system. Data are presented as the mean ± standard deviation (n=4 per group). *P<0.05 and **P<0.01 as indicated. OA, osteoarthritis; ACLT, anterior cruciate ligament transection; sham, sham-operated; GlcNAc, N-acetylglucosamine.

mesotrypsin (*Try4*), trypsin 10 (*Try10*), elongation factor RNA polymerase II-like 3 (*EII3*) and atlastin GTPase 2 (*Atl2*).

Discussion

Several clinical trials have demonstrated that oral administration of GlcNAc has significant symptom-modifying effects and improves type II collagen biomarkers in patients with OA (12,13,27,28). However, to the best of our knowledge, no previous research exists in which the molecular mechanisms underlying the action of GlcNAc on articular cartilage metabolism are elucidated. Therefore, in the present study an ACLT surgical model was used to investigate the changes in biochemical markers of type II collagen and the expression of cartilage metabolism-related genes in addition to histopathological criteria.

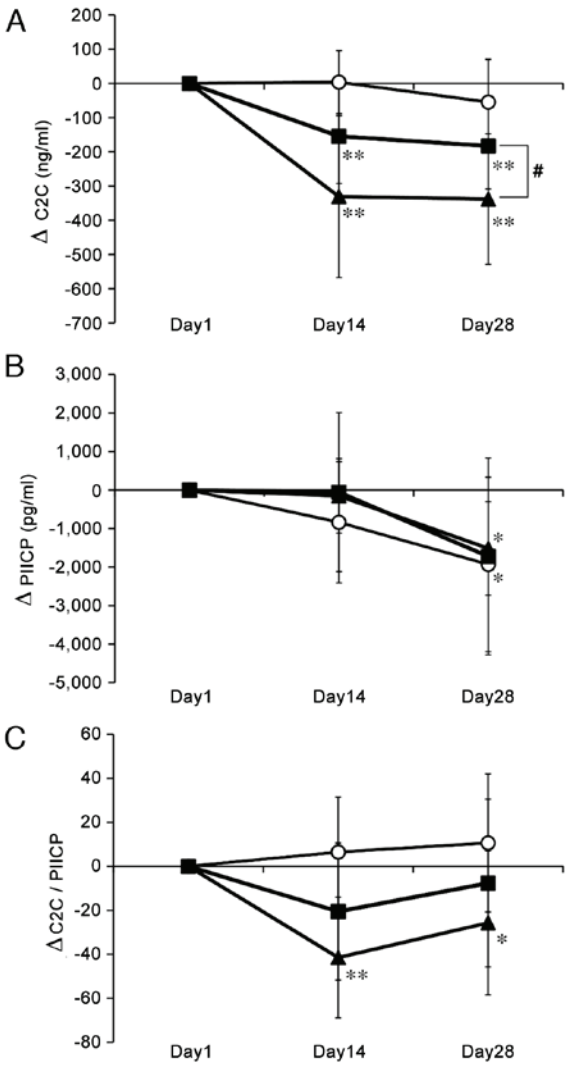

Figure 3. Changes in the biomarkers for type II collagen during GlcNAc treatment for rats with surgically induced ACLT. Changes in (A) C2C, (B) PIICP and (C) the C2C/PIICP ratio from the baseline were calculated for sham (open circle), ACLT (filled square) and GlcNAc (filled triangle) groups on days 14 and 28 post-surgery. Baseline levels on day 1 post-surgery were as follows: C2C, 621.09±139.20, 764.27±50.18 and 881.21±91.97 ng/ml in the sham, ACLT and GlcNAc groups, respectively; PIICP, 8,449.32±1,873.34, 8,698.22±1,977.0 and 8,081.33±765.5 ng/ml in the sham, ACLT and GlcNAc groups, respectively; C2C/PIICP, 77.84±29.46, 92.39±22.82 and 109.91±15.08 in the sham, ACLT and GlcNAc groups, respectively. Data are presented as the mean ± standard deviation. *P<0.05 and **P<0.01 vs. day 1; $^{\#}$P<0.05 as indicated. GlcNAc, N-acetylglucosamine; ACLT, anterior cruciate ligament transection; C2C, type II collagen cleavage neoepitope; PIICP, carboxyterminal propeptide of type II procollagen; sham, sham-operated.

The results of the present study demonstrated that ACLT-induced histopathological changes, such as erosion and degeneration, in articular cartilage, and GlcNAc administration significantly suppressed these changes. Furthermore, GlcNAc administration significantly decreased levels of C2C, a type II collagen degradation marker, in rats with ACLT, although there were no significant changes in serum PIICP levels. Notably, administration of GlcNAc significantly decreased the ratio of C2C and PIICP from the baseline. These observations suggest that GlcNAc exerts a chondroprotective action by suppressing ACLT-associated histopathological changes, possibly via

Table I. List of genes upregulated by ACLT (>1.5-fold) compared with the sham group and downregulated by GlcNAc administration compared with the ACLT group.

	Fold-change	
Gene symbol	ACLT/sham	GlcNAc/ACLT
Tnn	7.009	0.584
LOC685106	3.782	0.255
Postn	3.311	0.364
Crabp2	2.933	0.714
Fbn2	2.688	0.628
RGD1565772	2.594	0.642
Sorcs2	2.572	0.612
RGD1311501	2.215	0.575
Wisp2	2.179	0.718
Vwa1	2.088	0.687
N/A (Q40555_TOBAC (Q40555) Peroxidase, partial (5%) [TC599198])	2.055	0.590
LOC100363229	2.040	0.709
Qprt	2.034	0.524
Smoc2	1.994	0.696
Scx	1.899	0.599
Col18a1	1.881	0.697
H19	1.879	0.596
Nanos3	1.864	0.615
Prrx2	1.845	0.636
Apln	1.838	0.649
Mmp2	1.831	0.746
Nnmt	1.812	0.641
LOC500846	1.786	0.708
Tmem119	1.755	0.679
Srpx2	1.745	0.716
Slc6a8	1.742	0.687
Col12a1	1.740	0.650
Vegfa	1.737	0.680
Cacnb3	1.725	0.604
RGD1305689	1.712	0.626
Timp1	1.708	0.732
A2m	1.708	0.600
Sp1	1.683	0.531
Tnc	1.675	0.725
Cyb561	1.674	0.587
Mmp14	1.670	0.695
Col1a1	1.667	0.714
Aspn	1.647	0.499
Igsf10	1.643	0.717
Pvalb	1.640	0.137
Actg2	1.637	0.558
Dchs1	1.630	0.739
Aifm2	1.628	0.683
Col5a2	1.627	0.688
Col8a1	1.626	0.511
LOC100361571	1.622	0.688
Pdpn	1.619	0.737

Table I. Continued.

Gene symbol	Fold-change	
	ACLT/sham	GlcNAc/ACLT
Hoxa10	1.612	0.599
Mxra7	1.610	0.572
Dapk3	1.610	0.664
Wtip	1.608	0.700
Eps8l2	1.596	0.656
Cpxm2	1.594	0.512
Ano1	1.593	0.700
Vkorc1	1.591	0.747
Plat	1.590	0.746
Thbs4	1.590	0.733
Mmp13	1.575	0.734
Lama5	1.573	0.559
C1qtnf6	1.570	0.704
Sertad4	1.565	0.639
Rcn3	1.562	0.700
Fibin	1.555	0.610
Adamts1	1.552	0.741
Alkbh	1.543	0.383
Rftn2	1.542	0.674
Mcts2	1.537	0.704
LOC287167	1.523	0.717
Fxyd6	1.521	0.462
Pmepa1	1.515	0.728
LOC679958	1.512	0.682
Mmp16	1.509	0.663
Cyp26b1	1.509	0.731
Ppp1r12a	1.507	0.462

ACLT, anterior cruciate ligament transection; GlcNAc, N-acetylglucosamine; sham, sham-operated.

Table II. List of genes downregulated by ACLT (<0.75-fold) compared with the sham group and upregulated by GlcNAc administration compared with the ACLT group.

Gene symbol	Fold-change	
	ACLT/sham	GlcNAc/ACLT
Usp25	0.245	3.446
Inpp5f	0.345	2.191
Try5	0.370	1.991
Dnajc22	0.391	2.947
Lrrc28	0.399	1.553
Lcn2	0.425	2.772
Nip30	0.448	2.238
Atad2	0.352	2.347
Prss2l	0.531	2.524
Shfm1	0.544	2.118
Rnls	0.546	2.340
Try4	0.565	2.317
Try10	0.618	2.250
Ell3	0.627	1.537
Znf282	0.635	1.972
Larp5	0.688	1.739
Atl2	0.729	1.529
LOC682968	0.749	1.508

ACLT, anterior cruciate ligament transection; GlcNAc, N-acetylglucosamine; sham, sham-operated.

inhibiting type II collagen degradation rather than increasing type II collagen synthesis in the arthritic cartilage.

To further clarify the action of GlcNAc on arthritic cartilage, genetic analyses were performed via DNA microarray and RT-qPCR. The results revealed that GlcNAc upregulated or downregulated the expression of several genes associated with the extracellular matrix in articular cartilage. In particular, *Postn* was substantially upregulated by ACLT, and this upregulation was suppressed by GlcNAc administration. The *Postn* gene encodes periostin, a protein that functions as a ligand for integrins to promote cell adhesion and migration (29). Moreover, periostin induces inflammatory cytokines and MMPs via nuclear factor κB signaling, and promotes cartilage degeneration in patients with OA (30,31). Previous studies have demonstrated that the *Postn* expression is upregulated in the damaged cartilage of patients with knee OA (32-34). These observations suggest that GlcNAc has the potential to inhibit the degradation of type II collagen, as evidenced by the suppression of the level of C2C, a type II collagen degradation marker, by downregulating the expression of periostin.

GlcNAc has been reported to stimulate hyaluronan synthesis via the upregulation of hyaluronan synthase-2 (HAS2) in human articular chondrocytes (11). Furthermore, hyaluronan exhibits an anti-inflammatory action by inhibiting the interleukin (IL)-1β-induced MMP-13 expression and IL-1α-induced aggrecanase expression in arthritic chondrocytes (35,36). However, in the present study HAS2 expression was not found to be affected by GlcNAc (data not shown). Therefore, it is unlikely that HAS2 expression and synthesis of hyaluronan are associated with the chondroprotective action of GlcNAc observed in the present results.

GlcNAc administration markedly upregulated the expression of *Lcn2*, which was downregulated by ACLT. Lipocalin 2 has a role in the regulation of chondrocyte proliferation and differentiation, and its expression decreases with age in humans (37). It may therefore be speculated that GlcNAc exhibits a chondroprotective action by maintaining chondrocyte proliferation and differentiation via the upregulation of *Lcn2*.

In summary, the results of the present study suggest that GlcNAc has a chondroprotective action in OA, which it achieves by suppressing histopathological changes, possibly via the inhibition of type II collagen degradation in the articular cartilage. Furthermore, it is likely that GlcNAc modulates the expression of inflammatory and chondroprotective molecules,

Table III. List of genes confirmed by reverse transcription-quantitative polymerase chain reaction.

Gene symbol	Description	Fold-change	
		ACLT/sham	GlcNAc/ACLT
Tnn	Tenascin N	4.500	0.809
Postn	Periostin, osteoblast specific factor	2.550	0.675
Adamts4	A disintegrin and metalloproteinase with thrombospondin motifs 4	2.210	0.787
Crabp2	Cellular retinoic acid binding protein 2	1.980	0.934
Fbn2	Fibrillin 2	1.860	0.618
Timp1	Tissue inhibitor of metalloproteinase 1	1.680	0.815
Wisp2	WNT1 inducible signaling pathway protein 2	1.670	0.796
Mmp13	Matrix metalloproteinase 13	1.560	0.891
Mmp2	Matrix metalloproteinase 2	1.500	0.860
Vwa1	Von Willebrand factor A domain containing 1	1.430	0.930
Vegfa	Vascular endothelial growth	1.420	0.880
Dnajc22	DnaJ (Hsp40) homolog, subfamily C, member 22	1.390	0.791
Sorcs2	Sortilin-related VPS10 domain containing receptor 2	1.390	0.842
Col5a2	Collagen type V, α2	1.380	0.848
Col1a1	Collagen type I, α1	1.370	0.635
Srpx2	Sushi-repeat-containing protein, X-linked 2	1.340	0.776
Mmp14	Matrix metalloproteinase 14	1.290	0.853
Pvalb	Parvalbumin	1.210	0.190
Thbs4	Thrombospondin-4	1.190	0.870
Nip30	NEFA-interacting nuclear protein NIP30	1.140	0.886
Smoc2	SPARC related modular calcium binding 2	1.110	0.802
Wtip	Wilms tumor 1 interacting protein	1.100	1.060
Inpp5f	Inositol polyphosphate-5-phosphatase F	1.080	0.852
Pdpn	Podoplanin	1.050	0.930
Col2a1	Collagen type II, α1	0.990	1.071
Usp25	Ubiquitin specific peptidase 25	0.950	0.705
Sertad4	SERTA domain containing 4	0.940	0.680
Lrrc28	Leucine rich repeat containing 28	0.920	1.120
Ctsk	Cathepsin K precursor	0.920	1.000
Slc6a8	Solute carrier family 6, member 8	0.920	0.946
Atad2	ATPase family, AAA domain containing 2	0.840	1.024
Igsf10	Immunoglobulin superfamily, member 10	0.840	0.880
Zfp282	Zinc finger protein 282	0.820	0.902
Shfm1	Split hand/foot malformation type 1	0.780	0.950
Larp4b	La ribonucleoprotein domain family, member 5	0.700	0.971
Ell3	Elongation factor RNA polymerase II-like 3	0.650	1.400
Atl2	Atlastin GTPase 2	0.630	1.349
Prss2l	Prss2-like, anionic trypsin-2-like	0.490	2.327
Try4	Mesotrypsin	0.400	2.150
Try10	Trypsin 10	0.330	1.909
Lcn2	Lipocalin 2	0.260	4.308

ACLT, anterior cruciate ligament transection; sham, sham-operated; GlcNAc, N-acetylglucosamine.

such as periostin and lipocalin 2, which are involved in the degradation and maintenance of cartilage, thereby having a protective effect on the cartilage. However, the detailed mechanism for the protective action of GlcNAc on OA remains to be elucidated in the future.

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