European Review for Medical and Pharmacological Sciences 2020; 24: 4642-4651

NF-κB inhibitor QNZ protects human chondrocyte degeneration by promoting glucose uptake through Glut4 activation

S.-B. ZHU[1], Y.-Q. XU[2], H. GAO[1], Y. DENG[1]

[1]Department of Orthopaedic Trauma and Microsurgery, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, People's Republic of China
[2]Department of Orthopaedics, Hunan Provincial People's Hospital, Changsha, Hunan, People's Republic of China

Abstract. – OBJECTIVE: Glucose is not only an energy source but also raw material for proteoglycan biosynthesis of chondrocyte. The aim of the present study is to explore the role of QNZ in the progress of chondrocytes glucose uptake and investigate whether it will improve the chondrocytes degeneration through Glut4 activation.

PATIENTS AND METHODS: We isolated human chondrocytes from the cartilage by the patients who underwent total knee arthroplasty operations. Chondrocytes were pretreated with insulin or QNZ for 24 h. The uptake of glucose with stimulation, as well as the expression of Glut4, collagen II, aggrecan, MMP13, TNF-α, PCNA, and the p16 levels were determined by Western blot, quantitative reverse-transcription polymerase chain reaction (qPCR), or immunofluorescence.

RESULTS: Both insulin and QNZ stimuli to chondrocytes contributed to the expression of Glut4 and glucose uptake compared to the normal cells. Additionally, collagen II and aggrecan expression was detected to a significant increase, along with the reduced levels of MMP13 and TNF-α after exposed to QNZ. Moreover, QNZ protected chondrocytes degeneration *via* promoting proliferation and delaying aging. After blocking Glut4, the glucose uptake significantly reduced in QNZ treatment, as well as the expression of collagen II and aggrecan. However, no significant changes were noticed in the MMP13 and TNF-α levels.

CONCLUSIONS: The present study demonstrates that inhibition of NF-κB activation by QNZ would improve the glucose uptake through Glut4 activation, which plays an important role in the protection of chondrocytes degeneration.

Key Words:
NF-κB inhibitor, QNZ, Chondrocyte degeneration, Glut4, Glucose uptake.

Introduction

Osteoarthritis (OA) is one of the most common joint diseases embodied in physiological changes mainly in degeneration of articular cartilage, the formation of osteophytes, and microenvironment inflammation[1]. Nearly 20% of the worldwide adult population suffers from OA pain at some point in life, in the meantime, which also produces a considerable social-economic burden[2]. Increasing factors have been verified to be related to the progress of OA, such as joint trauma, immunopathy, aging, among this cartilage destruction is supposed to be a core risk in the development of OA[3]. Pathologically, cartilage degeneration involves chondrocyte senescence, oxidative stress, and the excess inflammatory factors such as matrix metalloproteinases (MMPs), tumor necrosis factor-α (TNF-α), and prostaglandins (PG)[4-6]. The nutrient supply of cartilage mainly comes from the diffusion of joint fluid. Therefore, it is difficult for self-repair compared to other connective tissues[7,8].

Limit vessels and oxygen supplied but high glucose-derived demand for matrix proteoglycans production leave cartilage a unique challenge in balancing glucose metabolism[9]. The main way for chondrocytes ATP production under hypoxic condition is anaerobic glycolysis with the transport of glucose into cells[10]. Thus, it can be seen that enough glucose uptake is essential to the chondrocyte's metabolism. Glucose uptake relies on glucose transporters (GLUTs), which help glucose to transport across the membrane *via* the electrochemical gradient. Therein the GLUT family members, Glut1-4 are found to produce in the chondrocytes[11], among which Glut4 is men-

Corresponding Author: Yu Deng, MD; e-mail: dy-198172@163.com

tioned to sensitive to insulin (a peptide hormone regulate glucose metabolism). Glut4 mRNA level has been verified to overexpress in proliferating chondrocytes[12].

Inflammatory cytokines activation has been suggested to lead to a decreased glycosaminoglycans synthesis in chondrocytes, which affects chondrocytes glucose uptake[13,14]. NF-kB, a core regulator of inflammatory responses, is reported to limit glucose transport in human articular cartilage, including the suppression of Glut4 expression[15]. In addition to this, the inhibition of NF-κB signaling pathway is also confirmed to improved glucose uptake by inhibition of ROS and anti-inflammatory effect in diabetic mice[16]. QNZ, a quinazoline derivative, exhibits high anti-inflammatory activity which characterized as a specific NF-κB inhibitor[17]. Even previous studies show QNZ attenuated inflammation, the role of QNZ in promoting glucose uptake by Glut4 activation remains essentially unknown.

The present study aims to evaluate QNZ on glucose uptake and GLUT4 expression in human chondrocytes and to declare whether it contributes to the protection of the chondrogenic degeneration.

Patients and Methods

Patient Tissue Samples Collection

This investigation was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University. A total of 5 patients (3 males, 2 females; every age: 53 years, from 46 to 73 years) undergoing total knee arthroplasty operations in our hospital from December 2017 to July 2018 participated in the study. Written informed consents were obtained from patients or relatives before the operations. We conserved the tissues in a sterile cell culture medium immediately after cutting from patients for the following chondrocytes isolation. This study was conducted in accordance with the Declaration of Helsinki.

Chondrocyte Cells Isolation and Cell Culture

Cut the cartilage into small pieces with a scalpel from the joint tissue and wash with phosphate-buffered saline (PBS) to remove erythrocytes. Incubate cartilage with Collagenase XI (1500 U/ml), Dispase II (2 .4 U/ml), and cell culture medium at 37°C overnight while shaking on the magnetic stirrer. Filter the cell solution onto a 100 μm pore size cell strainer and centrifuge to get the chondrocytes. Culture the cells in the medium [Dulbecco's Modified Eagle's Medium (DMEM)+GlutaMAX], 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin, Amphotericin B (Thermo Fisher Scientific, Waltham, MA, USA) and change the medium twice a week. The passage 1 chondrocytes were grouped in the following experiment: 1) Control group (free from intervention); 2) QNZ group (treated with 10 nM QNZ; Selleck, Houston, TX, USA); 3) Insulin group (treated with 100 nM insulin; Sigma-Aldrich; St. Louis, MO, USA). 4) siGlut4 group (silencing of Glut4 gene), 5) QNZ+siGlut4 group (silencing of chondrocytes Glut4 gene and treated with 10 nM QNZ). These cells were cultured for 3 days for the next steps.

Silencing of GLUT4 Gene Expression

Glut4 gene silencing was performed using small interfering RNA (siRNA: sense 5'-GAACACAAUAGUACAUACTT-3'; antisense 5'-GUAUGUACUAUUGUGUUCCTT-3' (Cell Signaling Technology, Danvers, MA, USA). Chondrocytes (10^5 cells/ml) were pre-cultured for 24 h and then washed with PBS and incubated with the TransMessenger-siRNA mixture at a final volume of 200 μl of FBS-deficient culture medium for 4 h. At the end of the incubation, cells were washed with PBS and resuspended in FBS-containing culture medium. SiRNA efficiency was detected by Glut4 immunohistochemistry.

Western Blot Analysis

Endogenous expressions of collagen II, aggrecan, and p16 proteins in human chondrocytes were assessed by Western Blot analysis. Total protein was isolated from cell lysates with the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). Samples of 10 μg of proteins were added in the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), then blocked with 5% milk. Membranes were incubated with desired primary antibodies: collagen II (1:1000; Cell Signaling, Danvers, MA, USA), aggrecan (1:1000; Cell Signaling, Danvers, MA, USA), p16 (1:1000; Abcam, Cambridge, MA, USA), and β-Actin (as loading control, 1:1000; Abcam, Cambridge, MA,

USA) at 4°C overnight. After that, membranes were washed three times with phosphate-buffered saline and tween-20 (PBST) and incubated with secondary antibody for 1 h at room temperature. Finally, membranes were detected by enhanced chemiluminescence (ECL) substrate (Beyotime, Shanghai, China), and analyzed using an ImageQuant LAS4000 imager and Total Lab program, respectively.

Immunofluorescence (IF)

After treatment, chondrocytes on coverslips were washed three times with PBS and fixed with 4% paraformaldehyde for 15 min, then permeabilized using 0.1% Triton-X for 10 min at room temperature. To avoid non-specific binding, coverslips were following blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature. Afterward, coverslips were washed and incubated with primary antibodies: PCNA (1:200; Cell Signaling, Danvers, MA, USA), Glut4 (1:250; Abcam, Cambridge, MA, USA) at 4°C overnight. Wash the coverslips with PBS three times, and incubate them with Alexa Fluor488 secondary antibody (1:1000; Invitrogen, Carlsbad, CA, USA) subsequently for 1 h at room temperature avoid light. The staining intensity was analyzed using the Image-Pro software (Version 4.5, Media Cybernetics, Inc., Silver Springs, MD, USA).

Quantitative Reverse-Transcription Polymerase Chain Reaction (qPCR)

Briefly, total RNAs of chondrocytes were extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity of total RNAs was analyzed by Nanodrop 2000. Then, RNAs were reverse transcribed into cDNA with PrimeScript RT Master Mix (TaKaRa Bio, Otsu, Shiga, Japan). QPCR was performed to the analysis of collagen II, aggrecan, MMP13, TNF-α, and Glut4 RNA expressions using SYBR Green Master (Beyotime, Shanghai, China) according to the manufacturer's instructions. Melting curve analysis was carried out at the end of the cycling program. Relative gene expressions were normalized by the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and calculated according to the method of $2^{-\Delta\Delta Ct}$. All experiments were performed in triplicate. The primers used for qPCR are listed in Table I.

2-Deoxyglucose (2-DOG) Uptake

To assay 2-DOG uptake, chondrocytes were starved (FBS-deprived medium) for 2 h, washed with Krebs-Ringer phosphate (KRP) buffer, and treated with insulin or cytochlasin B (CB; Sigma-Aldrich; St. Louis, MO, USA) as blank for 3 h at room temperature. [^{3}H]2-DOG mixture [hot 2-deoxyglucose (GE Healthcare; Amersham, UK) dissolved in cold 2-DOG (Sigma-Aldrich; St. Louis, MO, USA)] was added in the culture medium for an additional 30 min. The reaction was stopped by stopping solution [CB and phloretin (Sigma-Aldrich; St. Louis, MO, USA) in SDS] washing. Radioactivity was counted in the presence of 3 ml of Ultima Gold scintillation liquid (Packard BioScience, Wellesley, MA, USA), and normalized to the protein content determined in Bradford analysis.

Statistical Analysis

Data were presented as the mean ± standard deviation (SD). Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). All statistical analysis was performed using SPSS Version 22.0 software package (SPSS IBM, Armonk, NY USA). p-value <0.05 was considered statistically significant for all tests.

Table I. Primer sequences of the genes for qPCR.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
Aggrecan	GGTGAACCAGTTGTGTTGTC	CCGTCCTTTCCAGCAGTC
Collagen II	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
MMP13	ACTGAGAGGCTCCGAGAAATG	GAACCCCGCATCTTGGCTT
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
Glut4	CCACCTCCTATGCCCAAGAC	GCTGCTTCGAGACATGATGGAA
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

qPCR, quantitative reverse-transcription polymerase chain reaction.

Results

Effects of Insulin and QNZ on Cellular Glut4 Levels and Glucose Uptake

Based on the previous studies, insulin has been found to regulate the Glut4 expression, but the function of QNZ in Glut4 mediation is still not clear. We determined the effects of QNZ on the expression of Glut4 and glucose metabolism in human chondrocytes *in vitro*. In dose-response studies[18,19], the optimized doses were 10 and 100 nM for insulin and QNZ treatments, respectively. Immunofluorescence analysis of Glut4 suggested that pretreatment with either QNZ or insulin for 24 hours increased the levels of Glut4 protein in the human chondrocytes (Figure 1A, 1B). To further verify the effects on the expression within mRNA levels, qPCR was used to determine the differential expression of Glut. QNZ and insulin upregulated Glut4 mRNA levels over 160 and 220 % compared with controls, respectively (Figure 1C). Additionally, compared with controls, these two drugs simulation significantly enhanced 2-DOG glucose uptake (Figure 1D). These results corroborated the NF-κB inhibitor QNZ could promote Glut4 for glucose metabolism in the human chondrocytes.

Effects of Insulin and QNZ on Cellular Collagen II, Aggrecan, MMP-13, and TNF-α Levels

In early developmental life, insulin acts as local growth factors. To further explore whether QNZ plays a role in the protection of chondrocytes ECM. We used Western blot method to measure the collagen II, aggrecan, MMP-1, and TNF-α levels under insulin and QNZ treatments mentioned above. Figure 2A indicated

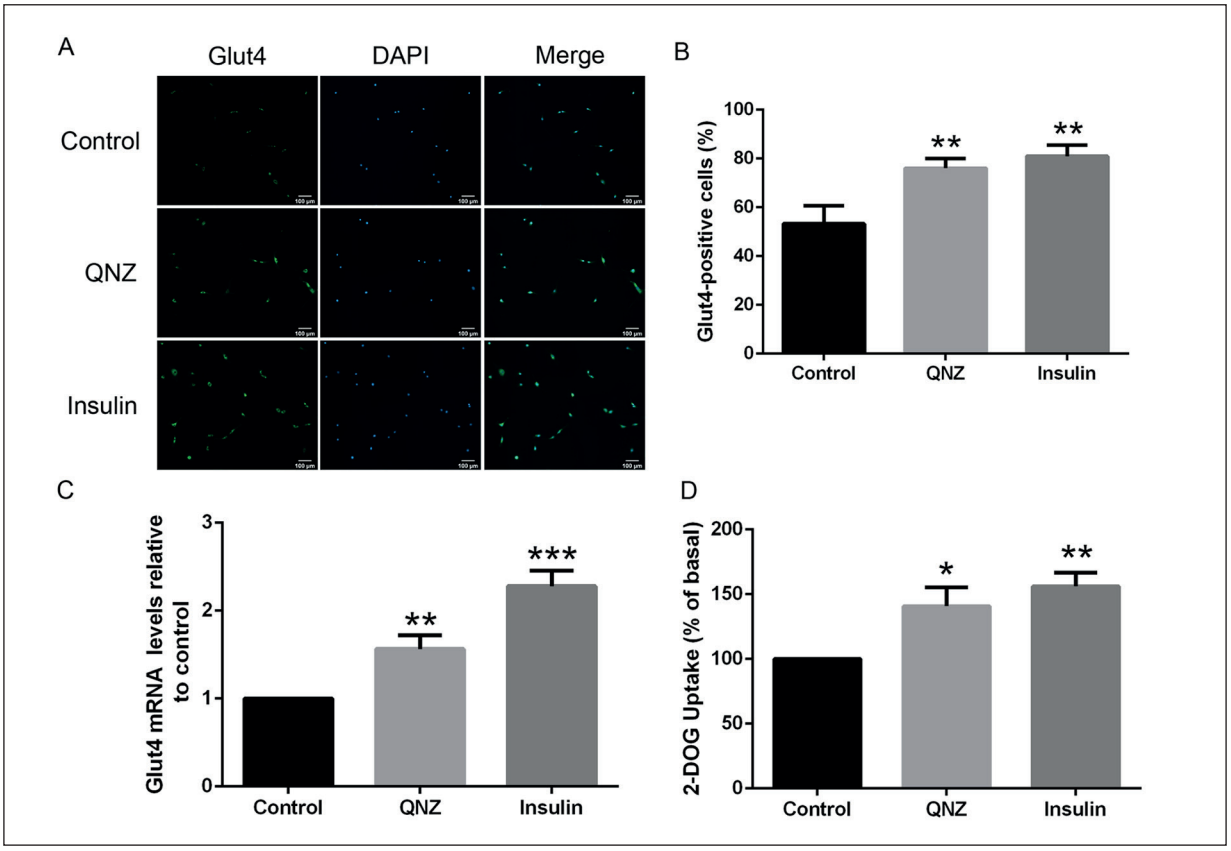

Figure 1. Effects of QNZ and insulin on Glut4 expression in human chondrocytes. Cultures were incubated for 3 days in the presence of 10 nM of QNZ or 100 nM of insulin. **A, C,** Expression of Glut4 was determined using both IF (magnification: 100X) (**A**) and qPCR (**C**). **B,** Positive-Glut4 chondrocytes were measured by densitometry. **D,** Effects of QNZ and insulin on 2-deoxy-[3H] glucose (2-DOG) uptake glucose. All data were expressed as means ± SDs. *p< 0.05 *vs.* control, **p< 0.01 *vs.* control, ***p< 0.001 *vs.* control.

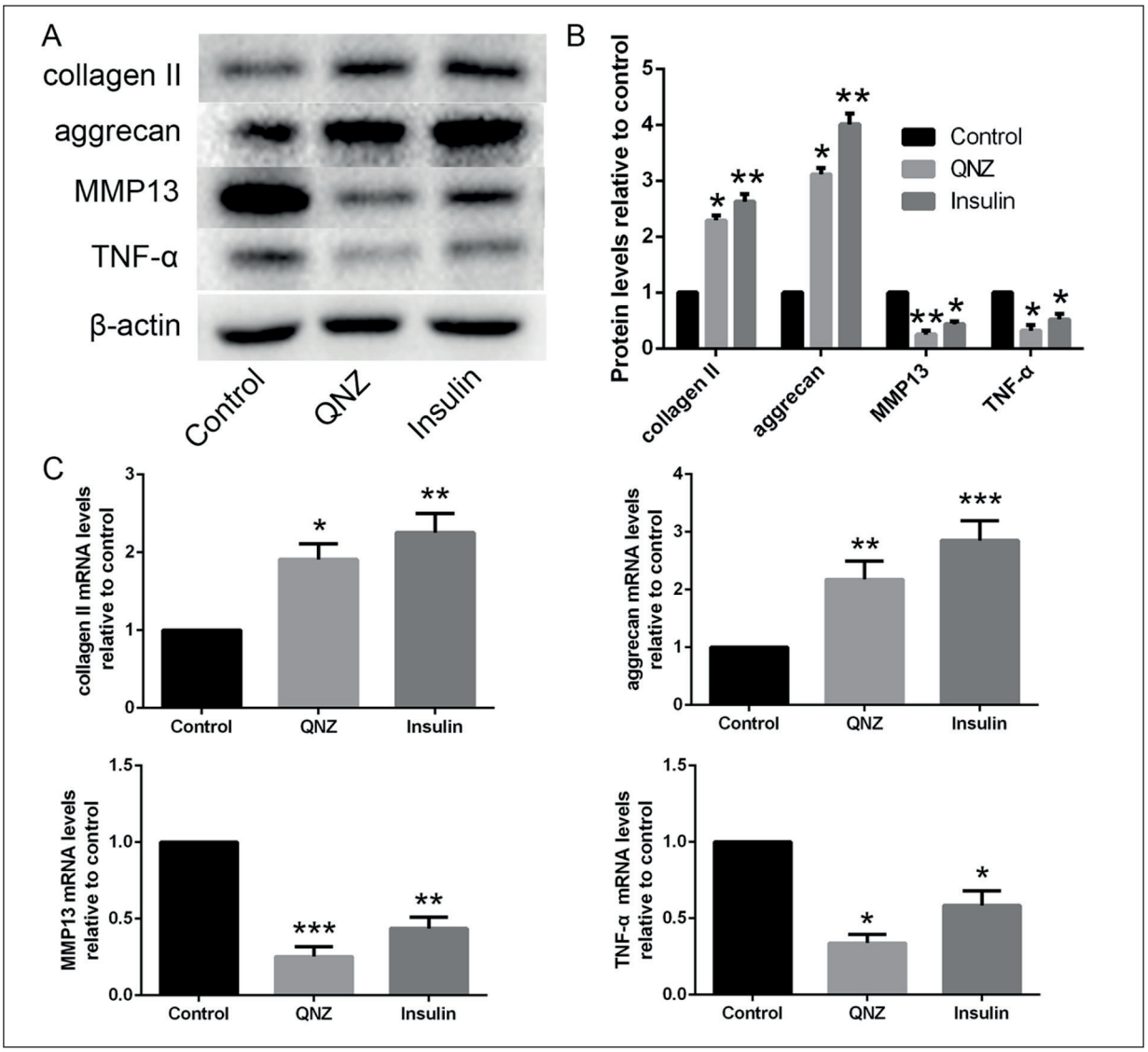

Figure 2. Effects of QNZ and insulin on extracellular matrix of human chondrocytes. Cultures were incubated for 3 days in the presence of 10 nM of QNZ or 100 nM of insulin. **A, C,** Expression of collagen II, aggrecan, MMP13, and TNF-α were determined using both WB (**A**) and qPCR (**C**). **B,** Quantification of these protein by Image J. All data were expressed as means ± SDs. *$p< 0.05$ *vs.* control, **$p< 0.01$ *vs.* control, ***$p< 0.001$ *vs.* control.

that insulin and QNZ not only upregulated the expression of collagen II and aggrecan protein content but also decreased levels of MMP-13 and TNF-α, the catabolic hallmarks of the ECM compared to control group. The differences in expression of these positive or negative manners can also be measured by qPCR (Figure 2B). The mRNA levels of collagen II and aggrecan were significantly enhanced by both insulin and QNZ treatment, but MMP-13 and TNF-α decreased compared with the control (Figure 2C).

Effects of Insulin and QNZ on Cellular PCNA and p16 Levels

To explore the proliferative ability and aging situation of chondrocytes under the stimulation of insulin and QNZ, we chose PCNA (a cofactor of DNA polymerase) and p16 as the hallmarks through immunofluorescence staining. As shown in Figure 3A, 3B, the result indicated a higher level of PCNA within both QNZ or insulin stimulation, suggesting an increase in proliferation activity of the chondrocytes in both treatments. In contrast, compared with untreated chondro-

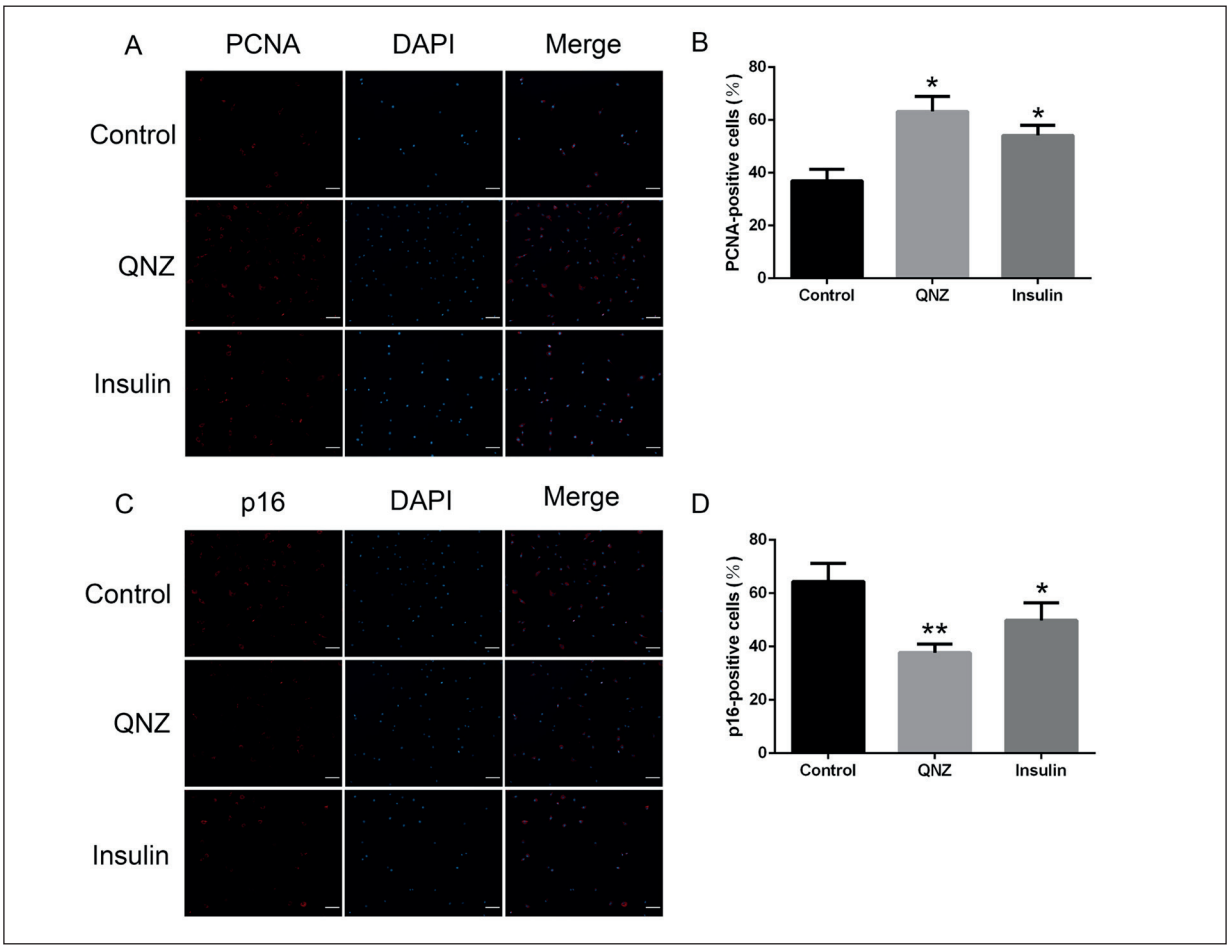

Figure 3. Effects of QNZ and insulin on PCNA and p16 expression in human chondrocytes. Cultures were incubated for 3 days in the presence of 10 nM of QNZ or 100 nM of insulin. **A, C,** Expression of PCNA (**A**) and p16 (**C**) were determined using IF (magnification: 100X). **B, D,** Positive-PCNA chondrocytes (**B**) and positive-p16 chondrocytes (**D**) were measured by densitometry. All data were expressed as means ± SDs. *$p< 0.05$ *vs.* control, **$p< 0.01$ *vs.* control.

cytes, p16 positive cells decreased in the presence of QNZ or insulin treatment, respectively (Figure 3C, 3D). What we found indicated that the QNZ treatment contributed to the proliferative effects but slowed down the aging process to the human chondrocytes, which would supply evidence to explain how QNZ protects the degeneration of chondrocytes.

Glut4 Silencing Abolishes QNZ-Induced Glucose Uptake

To confirm whether QNZ promoted chondrocytes glucose uptake is the result of Glut4 translocation that was shown in previous studies, Glut4 gene was silenced by transfecting with siRNA. The optimal siRNA transfected time was analyzed by densitometric measurement of Glut4 immunofluorescence. Maximum silencing efficiency (64%) was achieved at 36 h post-transfection (Figure 4A). So, we used the silenced chondrocytes after 48 h transfection in the following experiments. To determine the insulin resistance status of each group, we also measured the chondrocytes glucose uptake. Reducing Glut4 cellular levels led to a significant reduction in glucose uptake even after QNZ stimulation compared to the controls, which meant QNZ had on effect on glucose uptake of chondrocytes without Glut4 (Figure 4B). The data suggested that the existence of Glut4 in chondrocytes played a vital role in glucose uptake resulting from QNZ stimulation, which denoted QNZ inhibited insulin resistance *via* Glut4 activation.

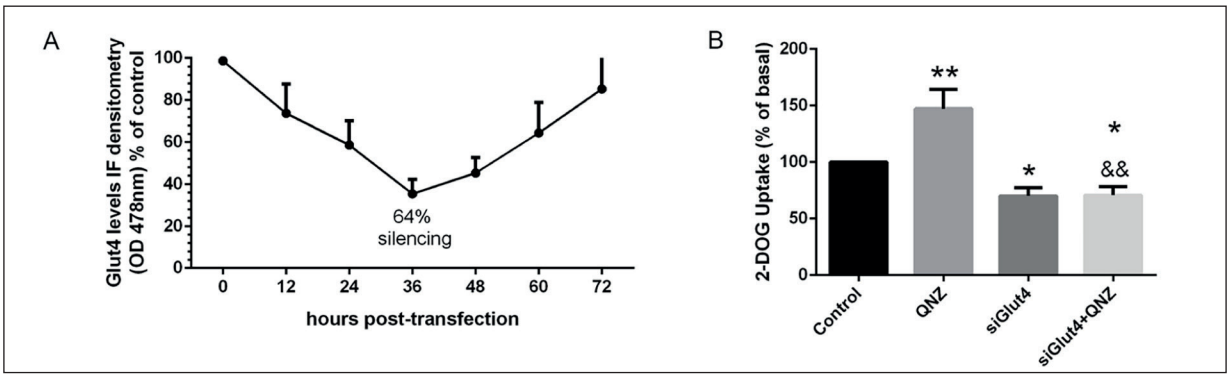

Figure 4. Glut4 gene silencing affects QNZ-stimulated glucose uptake. Chondrocytes were subjected to transfection of Glut4-siRNA. At different times, post-transfection cell culture was subjected to IF. **A,** Time course of Glut4 protein levels. **B,** QNZ-induced uptake of 2-DOG. All data were expressed as means ± SDs. *$p< 0.05$ *vs.* control, **$p< 0.01$ *vs.* control, &&$p< 0.01$ *vs.* QNZ.

Glut4 Silencing Abolishes QNZ-Induced Extracellular Matrix Protection

To determine the role of Glut4 in QNZ-induced extracellular matrix protection, human chondrocytes were divided into two groups (control group and Glut4-silenced group) which both treated with or without QNZ. We used Western blot and qPCR methods to analyze the differences in extracellular matrix related gene expressions. As shown in Figure 5A, 5B, compared with the controls, collagen II and aggrecan protein levels increased due to QNZ stimulation, but reduced after silencing Glut4. After Glut4 was silenced, even if there was QNZ stimulation, collagen II and aggrecan protein levels did not increase significantly. In addition, QNZ significantly reduced MMP-13 and TNF-α levels. The reduction of the protection of the extracellular matrix was further confirmed by mRNA expression measurement, which suggested us a same tendency with the protein levels. As a matter of fact, blocking Glut4 only abolished QNZ stimulatory effects on the collagen II and aggrecan but not on the anti-inflammation pathway. These results further emphasized that QNZ-induced chondrocyte collagen II and aggrecan upregulation were mainly mediated by Glut4. In addition, the effect of anti-inflammation may have nothing to do with the existence of QNZ or not (Figure 5C).

Discussion

Glucose serves as the main source for glycosaminoglycan synthesis in chondrocytes. GLUTs are detected to participate in glucosamine transport, which also plays a role in mediating inflammatory responses of chondrocytes[15]. Inflammatory activation is regarded to result in the decrease of Glut4 which affects glucose uptake[20]. QNZ, 6-amino-4-phenoxyphenylethylamino quinazoline, is an excellent NF-κB inhibitor. Chen et al[17] found that QNZ promoted anti-angiogenic and anti-metastatic effects *via* suppression of NF-κB activation. Sun et al[21] showed that QNZ could modulate inflammation, oxidative stress, and apoptosis in cigarette smoke extract-induced COPD.

This current study reveals that the promotion of glucose uptake in human chondrocytes is stimulated by QNZ mainly through the Glut4 activation. We used insulin as a measure to comprehend the efficiency caused by QNZ through the previous report[22]. We showed for the first time that the ability of QNZ to stimulate Glut4 expression and glucose uptake is similar to insulin effect against chondrocytes, although QNZ is not as effective as insulin. Due to the increased glucose uptake of chondrocytes, we found that the content of collagen II and aggrecan synthesized by chondrocytes increased compared with control group, which makes sense to the extracellular matrix. Similarly, Wu et al[23] have declared that glucose enhances aggrecan expression in chondrocytes. Kim et al[24] reported that glucose treatment significantly induced the type II collagen expression on rabbit articular chondrocytes. Furthermore, the suppression of NF-κB by QNZ inhibited the inflammatory factors MMP-13 and TNF-α expression that contributed to the protection of the chondrocyte's extracellular matrix. Surprisingly, in contrast to the controls, insulin, and QNZ both

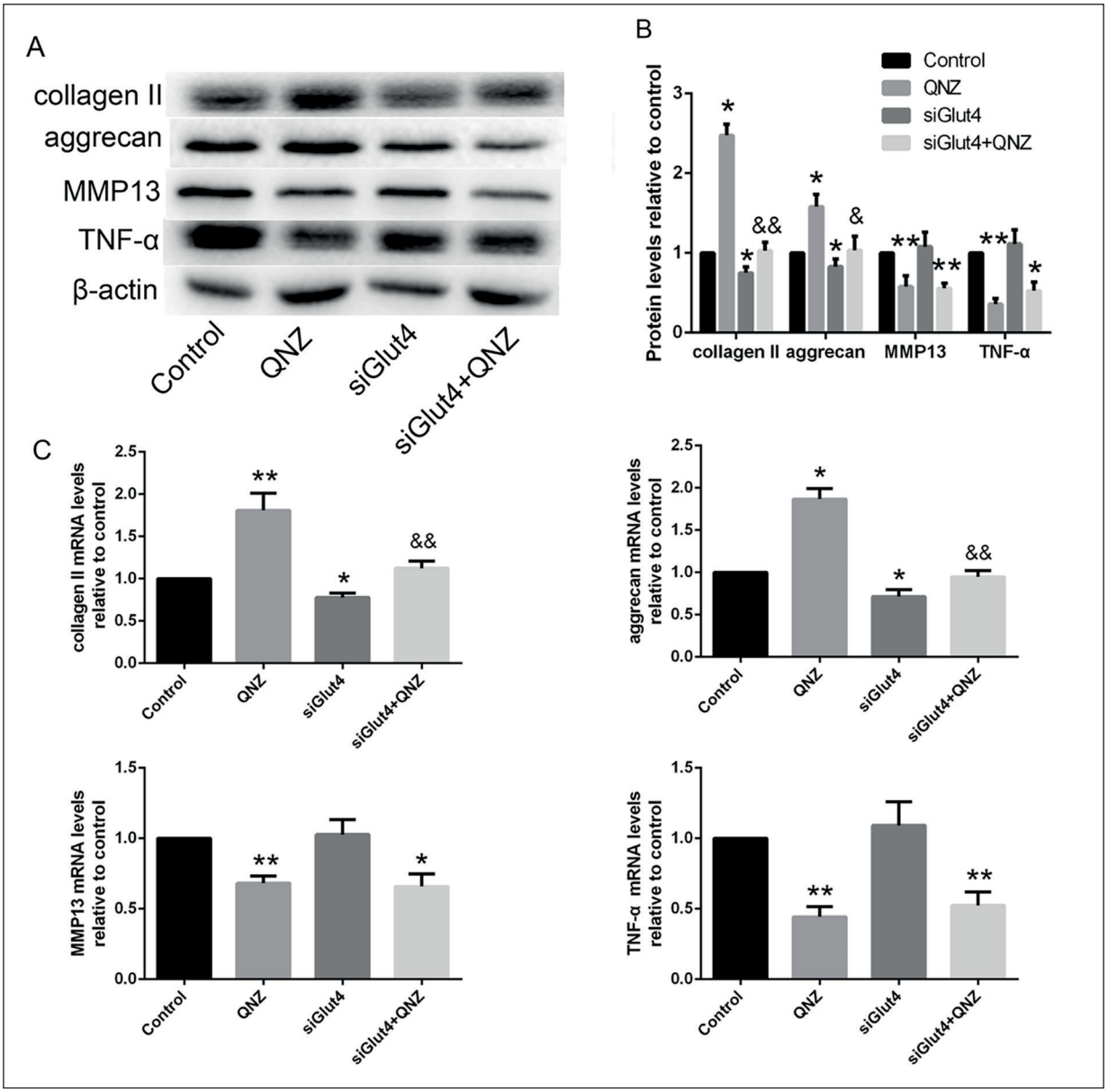

Figure 5. Glut4 gene silencing affects extracellular matrix of human chondrocytes. Chondrocytes were subjected to transfection of Glut4-siRNA. Cultures were incubated for 3 days with or without 10 nM of QNZ. **A, C,** Expression of collagen II, aggrecan, MMP13, and TNF-α were determined using both WB (**A**) and qPCR (**C**). **B,** Quantification of these protein by Image J. All data were expressed as means ± SDs. *p< 0.05 *vs.* control, **p< 0.01 *vs.* control, &&p< 0.01 *vs.* QNZ.

showed to promote the proliferative and alleviate the senescence population of chondrocytes; however, QNZ is more effective than insulin at this time.

Effects of Glut4 on glucose metabolism depending on insulin have been widely reported in various tissues, including articular cartilage[25]. Indeed, blocked Glut4 gene expression would affect the glucose uptake under insulin stimulation[18]. Based on these results, we speculated that QNZ promoted the chondrocytes glucose uptake by Glut4 activation. As a matter of fact, we truly found the effect of QNZ acting on glucose uptake apparently decreased after Glut4 silencing. Meanwhile, the collagen II and aggrecan expression significantly reduced with Glut4 silencing. However, we did not observe significant differences in MMP13 and TNF-α between the groups before and after Glut4 silencing, which also indicated the anti-inflammatory effect of QNZ was

not associated with Glut4. Glucose is not only an energy source for developmental activities but also the raw material for proteoglycan biosynthesis of chondrocyte.

Therefore, the inhibition of NF-κB activation by QNZ could result in the improvement of Glut4 expression in human articular chondrocytes, which contributes to glucose uptake. Glut4 overexpression is accompanied by increased levels of collagen II and aggrecan, as well as by decreased MMP13 and TNF-α expression *via* the inhibition of NF-κB activation. The QNZ accelerates chondrocytes proliferation but delays senescence. Collectively, the data reveal QNZ protects chondrocyte degeneration mainly by promoting glucose uptake through Glut4 activation and inflammation suppression, which suggests new evidence for the understanding of NF-κB inhibitor and new strategies for OA treatment in the future.

Conclusions

The present study demonstrates that inhibition of NF-κB activation by QNZ would improve the glucose uptake through Glut4 activation, which plays an important role in the protection of chondrocytes degeneration.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol 2007; 213: 626-634.
2) Felson DT. Identifying different osteoarthritis phenotypes through epidemiology. Osteoarthritis Cartilage 2010; 18: 601-604.
3) Bay-Jensen AC, Henrotin Y, Karsdal M, Mobasheri A. The need for predictive, prognostic, objective and complementary blood-based biomarkers in osteoarthritis (OA). EBioMedicine 2016; 7: 4-6.
4) Terkeltaub R, Yang B, Lotz M, Liu-Bryan R. Chondrocyte AMP-activated protein kinase activity suppresses matrix degradation responses to proinflammatory cytokines interleukin-1beta and tumor necrosis factor alpha. Arthritis Rheum 2011; 63: 1928-1937.
5) Wang X, Hunter D, Xu J, Ding C. Metabolic triggered inflammation in osteoarthritis. Osteoarthritis Cartilage 2015; 23: 22-30.
6) Lee AS, Ellman MB, Yan D, Kroin JS, Cole BJ, van Wijnen AJ, Im HJ. A current review of molecular mechanisms regarding osteoarthritis and pain. Gene 2013; 527: 440-447.
7) Asanbaeva A, Masuda K, Thonar EJ, Klisch SM, Sah RL. Cartilage growth and remodeling: modulation of balance between proteoglycan and collagen network in vitro with beta-aminopropionitrile. Osteoarthritis Cartilage 2008; 16: 1-11.
8) Asanbaeva A, Tam J, Schumacher BL, Klisch SM, Masuda K, Sah RL. Articular cartilage tensile integrity: modulation by matrix depletion is maturation-dependent. Arch Biochem Biophys 2008; 474: 175-182.
9) Hollander JM, Zeng L. The emerging role of glucose metabolism in cartilage development. Curr Osteoporos Rep 2019; 17: 59-69.
10) Bartrons R, Caro J. Hypoxia, glucose metabolism and the Warburg's effect. J Bioenerg Biomembr 2007; 39: 223-229.
11) Mobasheri A, Vannucci SJ, Bondy CA, Carter SD, Innes JF, Arteaga MF, Trujillo E, Ferraz I, Shakibaei M, Martin-Vasallo P. Glucose transport and metabolism in chondrocytes: a key to understanding chondrogenesis, skeletal development and cartilage degradation in osteoarthritis. Histol Histopathol 2002; 17: 1239-1267.
12) Maor G, Karnieli E. The insulin-sensitive glucose transporter (GLUT4) is involved in early bone growth in control and diabetic mice, but is regulated through the insulin-like growth factor I receptor. Endocrinology 1999; 140: 1841-1851.
13) Kim JJ, Conrad HE. Kinetics of mucopolysaccharide and glycoprotein synthesis by chick embryo chondrocytes. Effect of D-glucose concentration in the culture medium. J Biol Chem 1976; 251: 6210-6217.
14) Sweeney C, Mackintosh D, Mason RM. UDP-sugar metabolism in Swarm rat chondrosarcoma chondrocytes. Biochem J 1993; 290 (Pt 2): 563-570.
15) Long SD, Pekala PH. Regulation of GLUT4 mRNA stability by tumor necrosis factor-alpha: alterations in both protein binding to the 3' untranslated region and initiation of translation. Biochem Biophys Res Commun 1996; 220: 949-953.
16) Wang X, Liu R, Zhang W, Zhang X, Liao N, Wang Z, Li W, Qin X, Hai C. Oleanolic acid improves hepatic insulin resistance via antioxidant, hypolipidemic and anti-inflammatory effects. Mol Cell Endocrinol 2013; 376: 70-80.
17) Chen JH, Chen WL, Liu YC. Amentoflavone induces anti-angiogenic and anti-metastatic effects through suppression of NF-kappaB activation in MCF-7 cells. Anticancer Res 2015; 35: 6685-6693.
18) Maor G, Vasiliver-Shamis G, Hazan-Brill R, Wertheimer E, Karnieli E. GLUT4 in murine bone growth: from uptake and translocation to proliferation and differentiation. Am J Physiol Endocrinol Metab 2011; 300: E613-E623.

19) Shi H, Li Y, Ren X, Zhang Y, Yang Z, Qi C. A novel quinazoline-based analog induces G2/M cell cycle arrest and apoptosis in human A549 lung cancer cells via a ROS-dependent mechanism. Biochem Biophys Res Commun 2017; 486: 314-320.

20) Mattera MSLC, Chiba FY, Lopes FL, Tsosura TVS, Peres MA, Brito VGB, de Oliveira SHP1, Pereira RF, Marani F, Dos Santos RM, Belardi BE, Tessarin GWL, Benites ML, Ervolino E, Heimann JC, Sumida DH. Effect of maternal periodontitis on GLUT4 and inflammatory pathway in adult offspring. J Periodontol 2019; 90: 884-893.

21) Sun X, Feng X, Zheng D, Li A, Li C, Li S, Zhao Z. Ergosterol attenuates cigarette smoke extract-induced COPD by modulating inflammation, oxidative stress and apoptosis in vitro and in vivo. Clin Sci (Lond) 2019; 133: 1523-1536.

22) Wu S, Morrison A, Sun H, De Luca F. Nuclear factor-kappaB (NF-kappaB) p65 interacts with Stat5b in growth plate chondrocytes and mediates the effects of growth hormone on chondrogenesis and on the expression of insulin-like growth factor-1 and bone morphogenetic protein-2. J Biol Chem 2011; 286: 24726-24734.

23) Wu TJ, Fong YC, Lin CY, Huang YL, Tang CH. Glucose enhances aggrecan expression in chondrocytes via the PKCα/p38-miR141-3p signaling pathway. J Cell Physiol 2018; 233: 6878-6887.

24) Kim SJ, Sancheti SA, Sancheti SS, Um BH, Yu SM, Seo SY. Effect of 1,2,3,4,6-penta-O-galloyl-beta-D-glucose on elastase and hyaluronidase activities and its type II collagen expression. Acta Pol Pharm 2010; 67: 145-150.

25) Richardson S, Neama G, Phillips T, Bell S, Carter SD, Moley KH, Moley JF, Vannucci SJ, Mobasheri A. Molecular characterization and partial cDNA cloning of facilitative glucose transporters expressed in human articular chondrocytes; stimulation of 2-deoxyglucose uptake by IGF-I and elevated MMP-2 secretion by glucose deprivation. Osteoarthritis Cartilage 2003; 11: 92-101.