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IL-10 and TGF-β: Roles in chondroprotective effects of Glucosamine in experimental Osteoarthritis?

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ABSTRACT

Objective: Osteoarthritis (OA) is a complex disease of the whole joint. Glucosamine (GlcN) treatment may have a chondroprotective effect on OA. We investigated the mechanism of action of glucosamine treatment through interleukin-10 (Il-10) and transforming growth factor β-1 (TGF β-1).
Methods: Thirty male albino rats were used. A single intraarticular (i.a.) injection of 2 mg of Monosodium Iodoacetate (MIA) was injected into the knee joint of anesthetized rats. GlcN (50 or 100 mg/kg/day, p.o. for 2 month) was administered orally. Serum levels of Il-10 and TGF-β1 were determined by ELISA. Histopathological changes in treated and control joints were examined using hematoxylin-eosin (H & E) staining.
Results: The mean serum level of IL-10 significantly decreased in the OA group compared to control group (P value < 0.0001). On the other hand, mean serum level of IL-10 significantly increased in GlcN treated groups when compared to the OA group (P value < 0.0001). Serum level of TGF β-1 was significantly elevated in OA group compared to control group (P value < 0.0001). On the other hand, the mean serum level of TGF β-1 was significantly decreased in the GlcN treated groups when compared to the OA group (P value < 0.0001). Histopathological evaluation of GlcN treated groups showed different grades of healing, according to Osteoarthritis Research Society International (OARSI) grading system.
Conclusion: Our results showed that IL-10 and TGF-β1 possibly mediate GlcN chondroprotective effects in OA. Both serum biomarkers can be useful in the follow-up of articular cartilage damage in clinical settings.

1. Introduction

Osteoarthritis (OA) is a common degenerative joint disease in the elderly and is a leading cause of physical disability worldwide [1]. It mainly affects the weight– bearing joints such as hips and knees [2]. To date, there is no effective medical treatment for OA and the end stage of disease usually requires joint replacement [3,4].

Glucosamine (GlcN) is a natural product (amino-monosaccharide) that is widely used for its chondroprotective effects. The beneficial effect of GlcN in OA is debatable [5,6]. A clinical study in the UK showed that GlcN treatment was effective in OA of the knee [7], while another study showed that it exhibited only limited efficacy in both knee and hip OA [8]. Several studies tested the potential use of GlcN alone or in combination with other supplements to obtain the desirable effect [9–12]. While GlcN is recommended for the management of OA by several guidelines from international societies, it is not by others [13].

Several cellular mechanisms for the action of GlcN were suggested. For example, GlcN increased type II collagen and proteoglycan synthesis in human articular chondrocytes [13–16]. Also, it was suggested that GlcN reduced cell death and improved the extracellular cartilage matrix (ECM) anabolic/catabolic balance via the production of some pro-inflammatory mediators and proteases [13].

Interleukin-10 (IL-10) is a cytokine that has a chondroprotective effect. It is elevated in cartilage and synovium of OA patients [17]. It may acts as a stimulator of chondrocyte proliferation [18]. Intra-articular injection of IL-10 reduced cartilage degeneration in mice [19].

Transforming growth factor β (TGF-β) is a member of a super-family of cytokines. TGF-β superfamily members regulate a number

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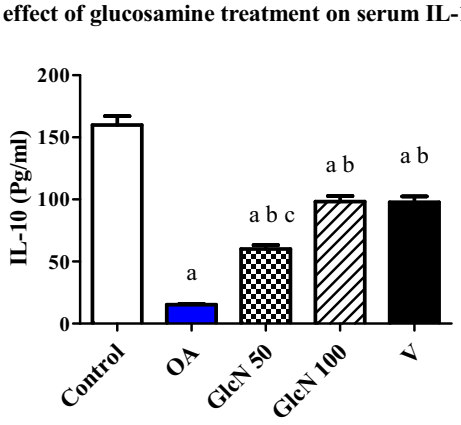

Fig 1. Serum level of IL-10 (pg/ml) in the experimental groups. GlcN treatment raised the serum level of IL-10 in the osteoarthritic rats at the end of 2 months of treatment; C = control; OA = osteoarthritis; GlcN 50 = glucosamine 50 mg/kg; GlcN 100 = glucosamine 100 mg/kg; V = Voltaren. a = significantly different from control at $P < 0.0001$, b = significantly different from OA at $P < 0.0001$, c = significantly different from GlcN 100 at $P < 0.0001$..

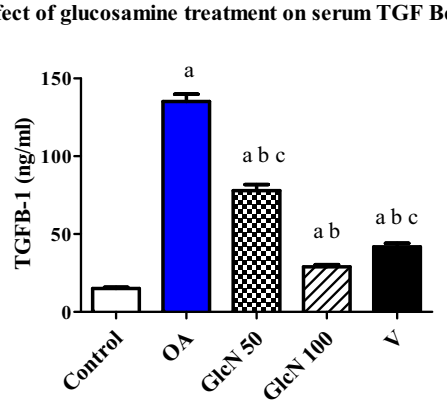

Fig. 2. Serum level of TGF-β1 (ng/ml) in the experimental groups. Glucosamine treatment reduced serum level of TGF-β1 in the osteoarthritic rats at the end of 2 month treatment; C = control; OA = osteoarthritis; GlcN 50 = glucosamine 50 mg/kg; GlcN 100 = glucosamine 100 mg/kg; V = Voltaren. a = significantly different from control at $P < 0.0001$, b = significantly different from OA at $P < 0.0001$, c = significantly different from GlcN 100 at $P < 0.0008$..

of important bone processes. TGF-β1 signaling has been extensively studied in bone since it is the predominant TGF-β isoform expressed in bone [20]. TGF-β1 is involved in various stages of bone formation, for example recruitment and stimulation of osteoblast [21].

Currently, TGF-β1 is thought to play a role in the pathogenesis of OA. TGF-β1 inhibition in animal models of OA reduced degeneration of articular cartilage [22]. Also, it was found that TGF-β1 is activated during osteoclastic activity [23].

In this study we investigate the possible mechanism(s) of action for GlcN in the treatment of OA. We hypothesized that GlcN possibly mediates its chondroprotective effects in a rat model of OA via the modulation of both TGF–β1 and IL-10. We also compared the treatment effectiveness of GlcN with administration of Voltaren™, a conventional pharmacological medication used in OA. Both serum biomarkers can therefore be a used as a tool for the follow up of articular cartilage damage in clinical settings.

2. Material and methods

2.1. Animals

Thirty male albino rats were used, weighting 200 ± 30 g at the start of the experiments. Four rats were housed per cage (size 26 × 41 cm) and placed in the experimental room for acclimatization 24 h before any procedure. The animals were fed with standard laboratory diet and with tap water ad libitum, and kept in an air-conditioned animal room at 23 ± 1 °C with a 12 h light/dark cycle. Animal care and handling conformed to approved protocols of Cairo University and Egyptian Community guidelines for animal care.

2.2. Monosodium iodoacetate injection

A single intra-articular (i.a.) bolus of 2 mg of monosodium iodoacetate (MIA) (Sigma-Aldrich, Egypt) was injected through the infra-patellar ligament into the joint space of the right knee of lightly anesthetized rats (3% isoflurane in O_2 at 1.5 l/min) in a total volume of 50 μl saline [24].

2.3. Experimental groups

Rats were randomly allocated into five groups of six animals each.

Group 1: Normal control group rats injected (i.a) by 50 μl saline in right knee.

Group 2 (OA): Osteoarthritic but untreated rats injected (i.a) with 2 mg of MIA in a total volume of 50 μl saline in right knee.

Group 3 (GlcN 50): Osteoarthritic rats treated with GlcN 50 mg/kg/day, p.o. for 2 months.

Group 4 (GlcN 100): Osteoarthritic rats treated with GlcN 100 mg/kg/day, p.o. for 2 months.

Group 5 (V): Osteoarthritic rats treated with Voltaren™ 30 mg/kg/day [25], p.o. for 2 months. Voltaren™ is one of the pharmacological medications conventionally used in OA. This group served as a positive control.

All tested groups received the designated treatment 3 days after MIA injection [24].

2.4. Drugs

Glucosamine (Eva Pharma, Egypt) and Voltaren (Novartis, Egypt) were dissolved in distilled water and administered orally using the rat feeding tube [26].

2.5. Blood samples and biochemical analysis

At the end of the study, rats were fasted overnight, anesthetized with thiopental sodium (50 mg/kg) [27], and blood samples were collected (5 ml per rat). Blood samples were centrifuged at 3000 rpm for 15 min after 30 min of collection and stored at −80 °C until analyzed. Serum Il-10 and TGF-β1 were determined using the corresponding rat enzyme immunoassay kits (Il-10: RayBiotech, Inc., Norcross GA, USA, TGF- β1: Kamiya Biomedical Company, USA) according to the manufacturer's instructions.

2.6. Histopathological examination

After sacrifice, knee joints were dissected and rinsed in saline. Specimens were fixed in 10% formalin and then decalcified in nitric oxide for 4 days, routinely processed and embedded in paraffin. Five micrometer sections were cut and stained with hematoxylin and eosin (H&E). Histopathological evaluations were done according to Osteoarthritis Research Society International (OARSI) cartilage OA grading system [28].

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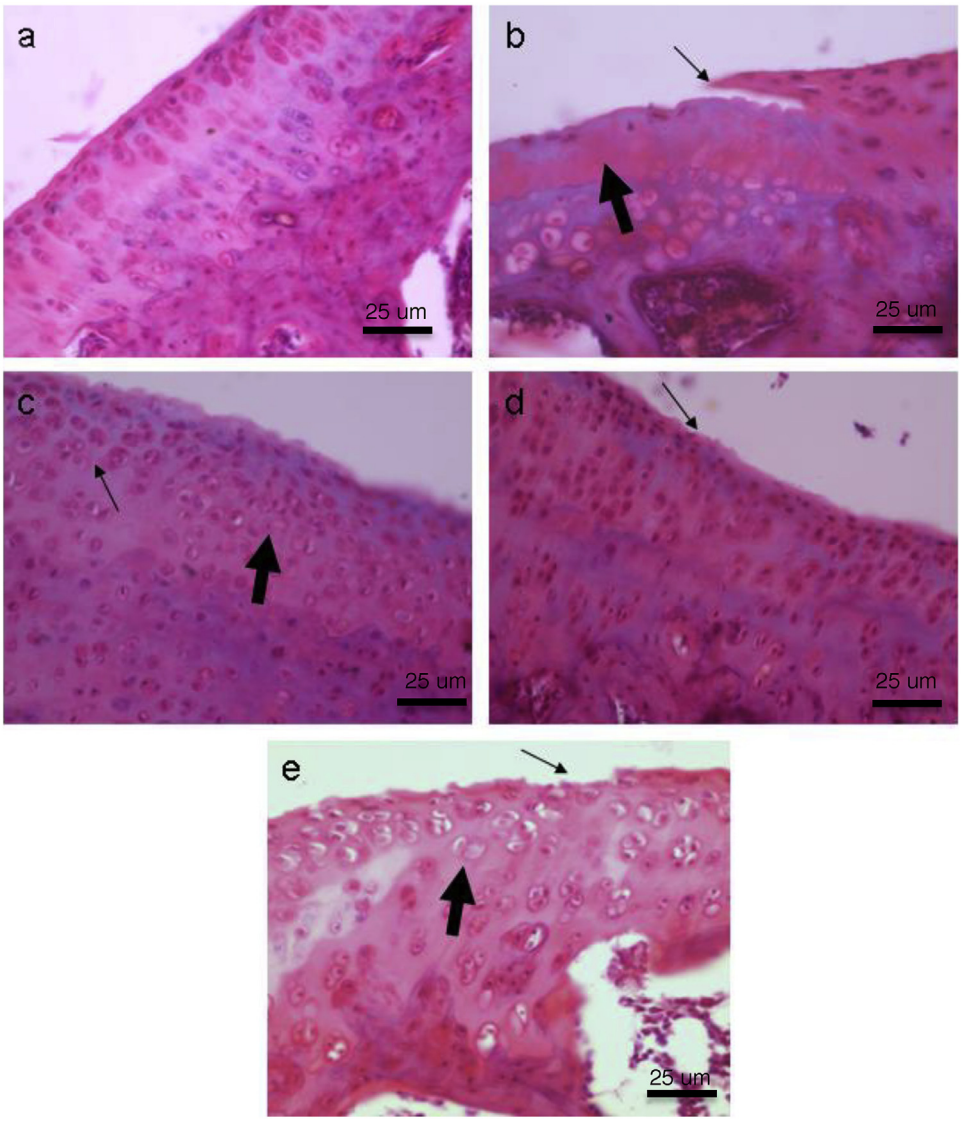

Fig. 3. Histopathological sections of hematoxylin and eosin (H&E) stained rat knee joints of the following study groups: a) **Control** group showing intact superficial layer, normal population and orientation of chondrocytes. b) **OA** group with erosion, clefting (thin arrow), chondrocytes degeneration (thick arrow), matrix changes and fibrosis. c) **GlcN 50** group showing superficial fibrillation, clustering of chondrocytes (thin arrow) and disorientation of chondron columns, d) **GlcN 100** showing superficial fibrillation (thin arrow), no clustering. e) **V group** showing loss of superficial layer (thin arrow), chondrocytes necrosis (thick arrow) and simple fissures. OA = osteoarthritis, GlcN 50 = glucosamine at 50 mg/kg, GlcN 100 = glucosamine at 100 mg/kg, V = Voltaren at 30 mg/kg. Magnification 400, scale bars 25 μm.

2.7. Statistical analyses

All data were expressed as mean ± SEM and analyzed using Prism program version 6. For all parameters, comparisons among groups were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. All *P* values reported are two-tailed and *P &z.lsquo; 0.05* was considered significance.

3. Results

3.1. Effect of GlcN on blood IL-10

The mean serum level of IL-10 was significantly decreased in the OA group compared to the control group (P value < 0.0001). On the other hand, the mean serum level of IL-10 was significantly increased in the GlcN 50, GlcN 100 and V groups when compared to the OA group (P value < 0.0001). The mean serum level of IL-10 was significantly increased in the GlcN 100 group compared to the GlcN 50 group (P value < 0.0001). No significant difference of IL-10 serum levels was found between the GlcN 100 group and the V group (Fig. 1).

3.2. Effect of glucosamine on blood transforming growth factor β-1 marker (TGF β-1)

The mean serum level of TGF-β1 was significantly elevated in the OA group compared to the control group (P value < 0.0001). On the other hand, the mean serum level of TGF β-1 was significantly decreased in the GlcN 50, GlcN 100 and V groups when compared to the OA group (P value < 0.0001). The mean serum level of TGF-β1 was significantly decreased in the GlcN 100 group when compared to the GlcN 50 group (P value < 0.0001) and the V group (P value < 0.0008) (Fig. 2).

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Table 1
Effect of Glucosamine on IL-10 and TGF β-1 in osteoarthritis in rats.

Groups	IL-10 (Pg/ml)	TGF β-1 (ng/ml)
Control	160 ± 7.12	15.1 ± 0.72
OA.	15.2 ± 0.72[a]	135 ± 4.60[a]
GlcN 50	60 ± 3.22[a,b,c]	77.9 ± 3.91[a,b,c]
GlcN 100	98.2 ± 4.55[a,b]	29.0 ± 1.37[a,b]
V	97.8 ± 4.72[a,b]	41.8 ± 2.32[a,b,c]

Glucosamine treatment at different doses significantly raised IL-10 level and decreased TGF β-1 level in osteoarthritic rats. C = control; OA = osteoarthritis; GlcN 50 = glucosamine 50 mg/kg; GlcN 100 = glucosamine 100 mg/kg; V = Voltaren. IL–10 = Interleukin-10; TGF β-1 = transforming growth factor β-1. Results were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Bonferroni's post hoc test.

[a] Significant from control at $P < 0.0001$.
[b] Significant from OA at $P < 0.0001$.
[c] Significant from GlcN 100 at $P < 0.0001$.

3.3. Histopathological changes associated with glucosamine treatment

Sections from the control group showed normal histological appearance and cartilage proteoglycan content with preserved integrity of the articular surface, normal orientation and distribution of chondrocytes, and preservation of superficial layer. The OA group showed histological features of osteoarthritis grade 5 (ORASI grading system), recognized by denudation and erosion of the cartilage with reparative fibrotic changes (clefting, chondrocyte degeneration, calcification, fibrosis and matrix changes).

Sections from the GlcN 50 group showed superficial fibrillation, clustering of chondrocytes with disorientation of chondron columns while sections from the GlcN 100 group showed superficial fibrillation with no clustering. Finally, Voltaren group showed loss of superficial layer, chondrocytes necrosis, and simple fissures. Grading of OA according to ORASI scale for all groups is illustrated in Table 1 and Fig. 3.

4. Discussion

To the best of our knowledge, this study is the first to demonstrate that GlcN treatment altered both IL-10 and TGF-β1 serum levels in a rat OA model. In this study, we showed that GlcN has a chondroprotective effects in a rat model of OA. Our results are in agreement with several other studies that demonstrated a beneficial role for glucosamine in the management of osteoarthritis, both in human and animal model [29–32].

In this study histopatholgical evaluation of GlcN treated groups showed different grades of healing according to Osteoarthritis Research Society International (OARSI) grading system.

GlcN possibly exerts its chondroprotective effects via the stimulation of IL-10 production. IL-10 was significantly decreased in the serum OA group while it was significantly increased with GlcN treatment. This effect was similar to the effect observed with Voltaren™ treatment, although at 50 mg/kg GlcN was more potent than Voltaren™ (Table 1 and Fig. 1). IL-10 reduction of the articular cartilage in OA may account for disease progression [33]. It opposed the TNF-α induced rise of matrix metalloproteinase-13 (MMP-13) and interleukin-1β (IL-1β) in human chondrocyte cell culture [33,34] (both cytokines are implicated in the pathogenesis of OA). IL-10 was shown to have a chondroprotective effect, possibly by enhancing the tissue inhibitor MMP-1 and stimulation of synthesis of IL-1β antagonist (IL-1Ra) [35]. It may acts as a stimulator of chondrocyte proliferation [18]. Also, intra-articular injection of IL-10 reduced cartilage degeneration in mice [19].

On the other hand, TGF-β1 was significantly elevated in the OA group while it was significantly decreased with GlcN treatment at doses of 50 and 100 mg/kg. This effect was similar to the effect observed with Voltaren™ treatment, although at 100 mg/kg GlcN was more effective than Voltaren™ (Table 1 and Fig. 2). The TGF-β superfamily of cytokines includes over 30 polypeptide growth factors [20]. TGF-β superfamily members are well expressed in the bone with TGF-β1 as a predominant isoform. TGF-β1 is involved in several regulatory processes of bones, e.g. bone formation, recruitment of osteoblast progenitors, and inhibition of osteoblast apoptosis [20,21].

TGF-β1 is thought to play a role in the pathogenesis of OA. Inhibition of TGF-β1 in animal models of OA reduced degeneration of articular cartilage [22]. Also, it was found that TGF-β1 is activated during osteoclastic activity [23].

In conclusion, our results showed that GlcN treatment indeed has beneficial effects in the treatment of OA compared to a standard anti-inflammatory treatment. GlcN treatment of OA is possibly mediated via modulation of both IL-10 and TGF β1. Both serum biomarkers can be a used as tools for the follow up of articular cartilage damage in the clinical setting. These biomarkers can be useful in confirming the diagnosis as well as monitoring the disease progression and severity. Further studies are needed to identify the best clinical application for these biomarkers (Table 2).

Table 2
Histopathological changes associated with Glucosamine (GlcN) treatment.

Group	Score	Histological findings
Control (C)	0	Normal findings with normal orientation and distribution of cells and preservation of superficial layer
Osteoarthritis (OA)	4–5	Denudation, erosion, clefting, chondrocytes degeneration, matrix changes, calcification, fibrosis
GlcN 50	2	Superficial fibrillation, clustering of chondrocytes with disorientation of chondron columns
GlcN 100	1	Superficial fibrillation, no clustering
V	3	Loss of superficial layer, chondrocytes necrosis, simple fissures

A table showing Histopathological changes associated with different doses of glucosamine (GlcN) treatment versus the control and Voltaren (V). Groups showed different grades of OA according to ORASI scale.

5. Conclusion

Our results showed that IL-10 and TGF-β1 are possible mediators of GlcN chondroprotective effects in OA. Both serum biomarkers can be a used as tools for the follow up of articular cartilage damage in the clinical setting.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Animal care and handling was performed in conformance with approved protocols of Cairo University and Egyptian Community guidelines for animal care.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

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