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Application of glucosamine on human disease—Osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a disease that affects various joints in elderly population. Wear of articular cartilage and syonovitis are characteristic feature of OA. Cartilage matrix is mainly composed of collagen and proteoglycan in which glucosamine is a major element. In OA, inflammatory mediators (prostaglandin E_2 (PGE_2), nitric oxide (NO)) and proteases (matrix metalloproteinases (MMPs), aggrecanase) are produced by chondrocytes and synoviocytes. These mediators cause inflammation, pain and degradation of cartilage. A large amount of *in vitro* studies showed that glucosamine suppressed these mediators in stimulated chondrocytes, synoviocytes or cartilage explants. Mechanism of suppressive effects of glucosamine is under the investigation. Glucosamine has been used to treat OA since 1960s and got popularity in North America and all over the world in the end of 20th century. A number of randomized controlled trials were conducted and analyzed scientifically. Glucosamine seems to have both symptom-modifying effects and structure-modifying effects. Though there is some heterogeneity during the trials, glucosamine remains to be a potential remedy for OA at present.

1. Introduction

Osteoarthritis is a disease that affects various joints such as the knee, hand, hip and spine in elderly population. It causes pain, aching, stiffness, limitation of motion and deformity. Among joints, the knee is frequently affected. Knee OA accounts for more dependency in walking, stair climbing and other lower extremity tasks than any other diseases and is one of the reasons of disability and morbidity in elderly people (Felson & Zhang, 1998). Clinically, OA is diagnosed by standard radiographs and symptoms such as pain, stiffness and crepitus. The prevalence of radiological knee OA was reported as 33% in the population older than 63 years, while symptomatic OA was 9.5% (Lawrence et al., 1998). OA is characterized by wear of articular cartilage and no radical treatment that compensates the loss of cartilage or prevents the progression of OA is available at present. Thus, the current treatment merely provides pain reduction and improvement of function without structural recovery. Nonsteroidal anti-inflammatory drugs (NSAIDs) are recommended, however, they are associated with adverse effects such as gastrointestinal bleeding and cardiovascular events. Therefore, people become to rely on complementary alternative medicine (CAM) that is seemingly safer and allegedly more effective than NSAIDs. Representative CAM for OA is glucosamine preparations (Vangsness, Spiker, & Erickson, 2009). In this article, the etiology and the molecular mechanism of OA will be described first. Then, *in vitro* effects of glucosamine on articular components and clinical evidence of glucosamine on OA are reviewed.

2. Etiology and molecular mechanism of OA

Articular cartilage is the specialized connective tissue firmly attached to the end of bone, which acts as shock absorber and provides joints with smooth motion. The joint capsule is a fibrous tissue that encapsulates joints and defines the outer boundary. The thin membrane called synovium or synovial membrane runs beneath the capsule (Fig. 1). Synovial fluid from synovium lubricates joint surface and provides cartilage with oxygen and nutrition. In rheumatic diseases including OA, inflammation of synovial membrane (synovitis) occurs very often (Nakamura, Yoshino, Kato, Tsuruha, & Nishioka, 1999). Articular cartilage has a vast preponderance of extracellular matrix composed of collagen and proteoglycan, in which chondrocytes are distributed sparsely (Fig. 1). Collagen, mainly type II is a building block of cartilaginous matrix. The proteoglycan aggregate is an aggregation of proteoglycan monomers attaching to the filamentous hyaluronan backbone and fills the space of collagen net work. It consists of core proteins from which many chondroitin sulfate and keratan sulfate side chains arise. Chondroitin sulfate and keratan sulfate are linear polymers composed of sugar residues. They are composed of repeating unit of N-acetylgalactosamine and glucuronic acid in chondroitin sulfate and N-acetylglucosamine and galactose in keratan sulfate (Fig. 1). They are members of glycosaminoglycan that are negatively charged, so they attract a large quantity of water molecule.

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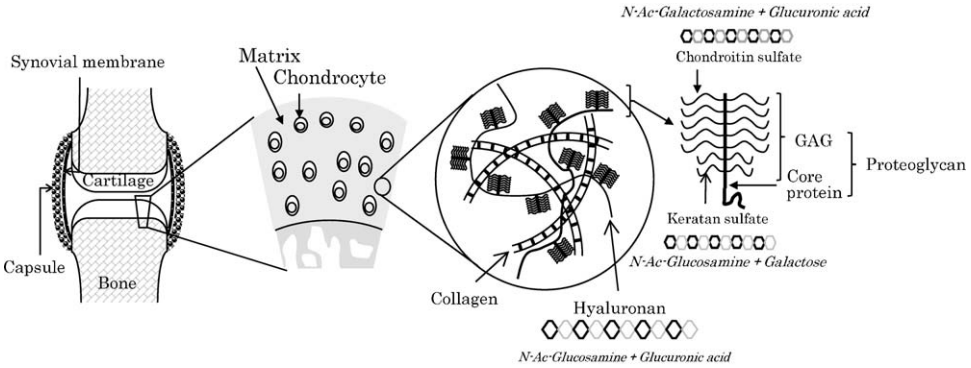

Fig. 1. Articular cartilage is attached to the end of bone. Synovial membrane runs beneath the capsule that encapsulates the joint. Articular cartilage has extracellular matrix composed of collagen and proteoglycan, in which chondrocytes are distributed sparsely. Collagen is a building block of cartilaginous matrix and proteoglycan aggregate is an aggregation of proteoglycan monomers attaching to the filamentous hyaluronan backbone. Chondroitin sulfate and keratan sulfate are linear polymers composed of repeating unit of N-acetylgalactosamine and glucuronic acid in chondroitin sulfate and N-acetylglucosamine and galactose in keratin sulfate. They arise from a core protein.

Thus, water consists no less than 70% of the net weight of cartilage (Rosenberg, 1989).

In OA, inflamed synovial cells and chondrocytes produce matrix metalloproteinases (MMPs) and aggrecanases that specifically degrade collagen and proteoglycan molecules (Bayliss, Hutton, Hayward, & Maciewicz, 2001; Bondeson, Wainwright, Lauder, Amos, & Hughes, 2006). Nitric oxide (NO), synthesized by these cells enhances inflammation and induces apoptosis in chondrocytes (Abramson, 2008). Prostaglandin E_2 (PGE_2), an inflammatory lipid mediator, known to enhance pain and inflammation is also synthesized both by synoviocytes and chondrocytes (Pelletier, Martel-Pelletier, & Abramson, 2001). These molecules are involved in the degradation of cartilage as well as the development of synovitis, resulting in pain and dysfunction. As interleukin-1 (IL-1) is expressed in chondrocytes and inflamed synoviocytes in OA, and stimulates production of the relevant molecules, this proinflammatory cytokine is believed to play a central role in the pathogenesis of OA (Pujol et al., 2008).

3. Effects of glucosamine on chondrocytes and synoviocytes

The effects of glucosamine were evaluated *in vitro* with various specimens like chondrocytes, cartilage explants or synoviocytes. Table 1 shows the representative studies of glucosamine on articular components (Bassleer, Rovati, & Franchimont,1998; Byron, Orth, Venta, Lloyd, & Caron, 2003; de Mattei et al., 2002; Dechant, Baxter, Frisbie, Trotter, & McIlwraith, 2005; Dodge & Jimenez, 2003; Gouze et al., 2001, 2002, 2006; Homandberg, Guo, Ray, & Ding, 2006; Hua et al., 2007; Largo et al., 2003; Nakamura, Shibakawa, Tanaka, Kato, & Nishioka, 2004; Nakatani, Mano, Ryanghyok, Shimizu, & Wada, 2007; Neil, Orth, Coussens, Chan, & Caron, 2005; Piperno et al., 1998; Shikhman, Kuhn, Alaaeddine, & Lotz, 2001; Varghese et al., 2007). Bassleer et al. (1998) investigated the effects of glucosamine sulfate on proteoglycan production using radioimmunoassay and showed that 10–100 μg/ml of glucosamine sulfate increased the production of proteoglycan. As mentioned later, we performed a clinical trial using glucosamine on knee OA and showed that 12-week treatment of glucosamine decreased the serum levels of PGE_2 significantly (Nakamura & Nishioka, 2002). This result prompted us to conduct *in vitro* experiments using chondrocytes. In brief, human chondrocytes were isolated from cartilage obtained during orthopedic surgery and cultured in complete medium. PGE_2 levels in medium, which was very low in an unstimulated condition, became significantly higher with the stimulation of IL-1β and partially reduced by the addition of glucosamine hydrochloride (Nakamura et al., 2004). Similar results were reported by other authors (Byron, Orth, Venta, Lloyd, & Caron, 2003; Largo et al., 2003). NO, another inflammatory mediator and nitric oxide synthase (NOS) that synthesize NO from arginine were down-regulated in stimulated by glucosamine chondrocytes. The inhibition of these inflammatory mediators by glucosamine is possibly relevant to clinical features such as pain relief. Glucosamine also suppressed the production of proteases (MMP-1, MMP-3, MMP-13 and aggrecanase) by stimulated chondrocytes. As each protease specifically degrades collagen or proteoglycan, glucosamine may retard wear of cartilage and prevent the progression of OA.

The molecular mechanism of glucosamine was also intensely investigated. Gouze et al. (2002) showed that glucosamine decreased the activity of nuclear κB (NFκB) that mediates intracellular signaling of cytokines and MMPs in stimulated chondrocytes. Neil et al. (2005) elucidated that glucosamine at a concentration of 10 μg/ml significantly reduced IL-1β-induced mRNA expression of c-Jun-N-terminal kinase (JNK), NOS and cyclooxygenase-2 (COX-2) that synthesizes PGE_2 from arachidonic acid, in equine chondrocytes. Hua et al. (2007) showed that glucosamine suppressed the IL-1β-induced phosphorylation of p38 mitogen-activated protein kinase (MAPK) in human synoviocytes.

Glucosamine also modulates cell function. Glucosamine hydrochloride inhibited chondrocytes proliferation at a high concentration (e.g. >5 mM) depending on its culture condition, however, it enhanced expression of matrix components (Varghese et al., 2007). Glucosamine was revealed to inhibit ossification of mouse chondrogenic cells (ATDC5) and induced sulfated glucosaminoglycan by regulating chondrogenic master genes, Smad2 and Smad4 (Nakatani, Mano, Ryanghyok, Shimizu, & Wada, 2007).

Taken together, glucosamine has a large amount of *in vitro* evidence that supports symptom-modifying and structure-modifying effects in clinical use.

4. Clinical study of glucosamine on human OA

Glucosamine is now classified as a neutraceutical supplement or medical drug in some countries. Antecedent to *in vitro* proof of chondroprotective and anti-inflammatory effects of glucosamine, its clinical use started in 1965 as far as I looked through (Vetter, 1965). Bohne (1969) reported that intraarticular injection of glucosamine was helpful to treat osteoarthritis. The first double-blind clinical trial of glucosamine on OA was conducted in 1980 (Pujalte, Llavore, & Ylescupidez, 1980). Thereafter, clinical use of glucosamine received little attention as a potential therapy of OA. However, the situation significantly changed in the end of 20th century by a book entitled "Maximizing the arthritis cure" (Theodosakis, Adderly, & Fox, 1997). In this book, authors recommended to take glucosamine and chondroitin sulfate together with

Table 1
In vitro study of glucosamine on articular components.

Author	Year	Specimen	Stimulant	Effects of glucosamine	
				Down-regulation	Up-regulation
Bassleer C	1998	HOC	Non	PG production	
Piperno M	2000	HOC	Non	PLA_2, collagenase activity	PKC
Gouze JN	2001	RC	IL-1	NO, PGE_2, MMP-3	GlcAT-1
Shikhman AR	2001	HC	IL-1	NO,iNOS,COX-2	
Gouze JN	2002	RC	IL-1	NFκB	IL-1RII
			ROS	NFκB	
De Mattei M	2002	BEx	Non	^{35}S uptake, cell viability	
Doge GR	2003	HOC	Non	MMP-3, collagenolytic activity	Aggrecan, GAG content
Largo R	2003	HOC	IL-1	NFκB,COX-2	
Byron CR	2003	BEx	LPS	MMP-1,3,13	
Nakamura H	2004	HC	IL-1	NO,MMP-1,3,13	
		HS	IL-1	PGE_2,MMP-1,3,13	
Neil KM	2005	EC	IL-1	MMP-13, aggrecanase-1, JNK, NOS,COX-2	
Dechant JE	2005	EEx	IL-1	GAG release	
Gouze JN	2006	RC	IL-1	NO,PGE_2, chemokine	
Homandberg GA	2006	BEx	Fn-f	MMP-3,MMP-13	
Hua J	2007	HS	IL-1	IL-8, PGE_2, p38	
Nakatani S	2007	ATDC5	Non	Ossification	Sulfated GAG
Varghese S	2007	BC	Non	Cell proliferation	Aggrecan, Type II collagen, TGF-β1

HOC: human OA chondrocyte; HC: human chondrocyte; RC: rat chondrocyte; BC: bovine chondrocyte; BEx: bovine explant; EC: equine chondrocyte; EEx: equine explant; IL-1: interleukin-1; ROS: reactive oxygen species; Fn-fs: fibronectin fragment; HS: human synoviocyte; PG:proteoglycan; PLA_2:phospholipase A_2; PKC: protein kinase C; NO: nitric oxide; NOS; nitric oxide synthase; PGE_2:prostaglandin E_2; MMP: matrix metalloprotease; GlcAT-1: galactose-beta-1,3-glucuronosyltransferase; IL-1RII: type II IL-1 receptor (decoy receptor of IL-1); NFκB: nuclear factor κB; COX-2: cyclooxygenase; JNK: c-Jun-N-terminal kinase.

other remedies such as exercise, weight management and antioxidant foods. Thereafter, glucosamine allegedly became explosively popular during the general population who had joint pain or who wanted to protect joints in North America and then all over the world. McAlindon, LaValley, Gulin, and Felson (2000) examined the previous glucosamine trials and conducted a meta-analysis. In his meta-analysis, the effect size of glucosamine was rated as 0.42 which implied that glucosamine had intermediate effects on OA.

Reginster et al. (2001) issued impressive results of a glucosamine trial. Two hundred and thirteen patients with knee OA were allocated into the glucosamine and placebo group and each treatment was continued for 3 years. At the end of the study, pain and function assessed by WOMAC score system (Bellamy, Buchanan, Goldsmith, Campbell, & Stitt, 1988) improved significantly in the glucosamine group. Moreover, joint space narrowing was less in the glucosamine group compared to that in the placebo group, which indicated that glucosamine suppressed the progression of articular degradation. As soon as this issue was published, lots of contradictions were raised (Chard & Dieppe, 2001). Their major concern was industry sponsorship that affects likelihood of positive results and publication bias. Regardless of controversy over the efficiency on OA, glucosamine continued to gain popularity among the general population.

To settle this issue, a large scale randomized trial, glucosamine/chondroitin arthritis intervention trial (GAIT) was conducted with the support of national center for complementary alternative medicine (NCCAM), the federal government's lead agency (Clegg et al., 2006). In this trial, 1583 patients with symptomatic knee OA were randomly allocated into 5 groups, glucosamine hydrochloride, chondroitin sulfate, combination of these, celecoxib (a COX-2 selective inhibitor) or placebo. The primary outcome measure was a 20% decrease in knee pain from baseline to week 24. Overall, the rate of response to celecoxib was significantly higher compared to placebo. However, in patients with moderate-to-severe pain at the baseline, the rate of response was significantly higher with combination of glucosamine and chondroitin than placebo. The problem of this study is a high placebo rate reaching no less than 60%, which usually is around 30%. This high placebo effect is supposedly attributed to 5 therapeutic branches in the trial, in which 4 were active preparations, and popular consensus that glucosamine was effective. Despite the largest trial of

Table 2
Randomized controlled trials of glucosamine on knee OA.

Author	Year	Salts[a]	*n*	Period	Result[b]	Comments
Pujalte JM	1980	S	24	8 W	E	
Noack W	1994	S	252	4 W	E	
Houpt JB	1999	H	118	8 W	N[c]	
Rindone JP	2000	S	114	60 D	N	Older patients with severe OA
Reginster JY	2001	S	212	156 W	E	
Pavelka K	2002	S	202	156 W	E	
Huges R	2002	S	80	24 W	N	Severer patients with previous medication
Cibere J	2004	S	137	6 M	N	Discontinuation study
McAlindon T	2004	S/H	205	12 W	N	Online trial
Clegg D	2006	H	630[d]	24 W	N[e]	Placebo effects > 60%
Herrero-Beaumont G	2007	S	210[d]	26 W	E	

[a] S: sulfate; H: hydrochloride.
[b] E: effective by the primary outcome; N: ineffective.
[c] Effective in the cumulative pain deduction by a daily diary.
[d] Number allocated to branches including glucosamine and placebo.
[e] Combination of glucosamine and chondroitin sulfate was effective in the patients with moderate-to-severe pain.

glucosamine, no reliable answer whether glucosamine was effective or not was obtained.

Another positive trial, in which 1500 mg of glucosamine sulfate was administered once daily for 6 months was issued (Herrero-Beaumont et al., 2007). Three-hundred and eighteen patients with symptomatic knee OA were assigned to receive glucosamine sulfate, acetaminophen or placebo. The primary efficacy outcome measure was the change in the Lequesne index (Lequesne, 1991) after 6 months after the treatment. Glucosamine sulfate but not acetaminophen was more effective than placebo in improving the Lequesne index.

A number of negative results were also reported (Cibere et al., 2004; Houpt, McMillan, Wein, & Paget-Dellio 1999; Hughes & Carr, 2002; McAlindon, Formica, LaValley, Lehmer, & Kabbara, 2004; Rindone, Hiller, Collacott, Nordhaugen, & Arriola, 2000) (Table 2). However, the effect of glucosamine is not necessarily negated in these issues. Houpt et al. (1999) showed that the result of cumulative pain reduction measured by daily diary was favorable to glucosamine hydrochloride. Trials conducted by Rindone et al. (2000) and Hughes & Carr (2002) included severer patients compared to the trial by Reginster et al. (2001). A trial by Cibere et al. (2004) was conducted as a discontinuation study. Present glucosamine users were recruited and allocated randomly into glucosamine group and placebo group. The outcome was assessed by the proportion of disease flare, the period until flare and the usage of rescue medicine. Though significant difference was not found between glucosamine and placebo, the result is reasonable because of carry-over effects of glucosamine (Thie, Prasad, & Major, 2001). The purpose of the study by McAlindon et al. (2004) was to establish the clinical trial on the web.

Recently, meta-analysis of glucosamine was conducted to identify the factors that explain heterogeneity in these trials (Vlad, LaValley, McAlindon, & Felson, 2007) and showed that the effect size of glucosamine was 0.35 which was smaller compared to previous analysis (McAlindon, LaValley, Gulin, & Felson, 2000). They also showed that the effect size was higher when the study was conducted with support of an industry and lower when glucosamine hydrochloride was used. In fact, studies of prescription drugs have been often supported by industries and these results have been justified, if properly reviewed. Glucosamine hydrochloride was proven to alter metabolism of chondrocytes *in vitro* just as glucosamine sulfate. Moreover, both glucosamine sulfate and hydrochloride salts are dissociated in the stomach and free glucosamine is absorbed in the small intestine (Anderson, Nicolosi, & Borzelleca, 2005). Our open-label trial using glucosamine hydrochloride showed that the preparation was effective for pain reduction in more than 2/3 of patients with knee OA (Nakamura & Nishioka, 2002).

Finally, glucosamine had a structure-modifying effect indicated by joint space narrowing in radiographs. Reginster et al. (2001) and Pavelká et al. (2002) could show the delay of progression of knee OA. The recent report also showed the trend toward improvement of disease progression in patients with earlier stage (Sawitzke et al., 2008). The fact that long-term treatment with glucosamine prevents joints from total joint replacement (Bruyere et al., 2008) is possibly attributed to the above effects.

5. Conclusion

In conclusion, glucosamine has enough *in vitro* evidence that supports anti-inflammatory and chondro-protective effects on joints. Quite a few randomized controlled trials were conducted and analyzed scientifically. Glucosamine seems to have both symptom-modifying effects and structure-modifying effects. Though, there is some heterogeneity during the clinical trials of glucosamine and controversy has been raised regarding publication bias and glucosamine moiety, it remains to be a potential remedy for OA at present.

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