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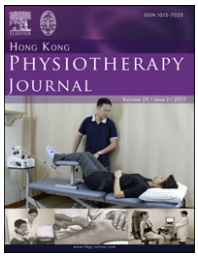

LITERATURE REVIEW

A review on the effects of glucosamine for knee osteoarthritis based on human and animal studies

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KEYWORDS
experimental osteoarthritis;
glucosamine;
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Abstract Glucosamine (GlcN) is a popular nutritional supplement/prescription for relieving symptoms of osteoarthritis (OA), particularly for the knee joint. Although there are certain studies reporting the positive effects of GlcN for OA, its use remains controversial and the mechanism behind is unclear. This article critically reviewed published papers on the effects of GlcN in human clinical trials and animal studies. Twelve human clinical studies were reviewed and half of the studies reported positive effects of GlcN for OA or regular knee pain. Eight animal studies were reviewed and most of them had involved histological examination of cartilage, glycosaminoglycan content, subchondral bone, and synovium. Besides, nociceptive behaviour, biochemical markers, and immunohistochemistry of the joints were also examined. There is some evidence showing the beneficial effects of GlcN on joint structural repair in animals, but further research is needed to confirm the applicability of these models in human.

Introduction

Osteoarthritis

Osteoarthritis (OA) is the most common joint disorder affecting elderly people. According to the report from the World Health Organisation, OA is the 6th leading cause of nonfatal burden in the world in the new millennium. The most frequently affected joints are the hands, knees, and hips [1]. Most studies of OA have focused on the change of cartilage, but OA is not solely a disorder of the articular cartilage, other components of the joint such as the subchondral bone [2,3], synovial lining [4], ligaments, and periarticular muscles [5] are also affected. The symptoms are often associated with inflammation; which include pain, stiffness, and loss of mobility thus resulting in functional impairment [6]. The condition is not reversible and has few effective medical remedies [1].

Glucosamine

Glucosamine (GlcN) has been the focus of research for relieving symptoms of OA for the last two decades with most of the studies focussing on the knee joint. Glucosamine (2-amino-2-deoxyalpha-D-glucose) is a naturally occurring amino monosaccharide comprising a glucose

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molecule attached to an amino group and present in the matrix of all connective tissues. The human body can naturally synthesize GlcN by means of the hexosamine pathway by combining glutamine with fructose [7]. GlcN is one of the principal substrates used in the biosynthesis of hyaluronan, chondroitin, dermatan, keratin, glycosaminoglycans, and proteoglycans and all of which are fundamental components of the extracellular matrix of articular cartilage [7]. Therefore, the notion of GlcN in relieving the symptoms of OA is based on the assumptions that abundant administration of the precursors of extracellular matrix components would help chondrocytes to proliferate and replace the degenerated cartilage.

The half-lives of the metabolic turnover of cartilage proteoglycans were measured in days in younger animals and months in elder animals [8], thus cartilage is believed to be continually rebuilding itself [9,10]. Oral consumption of GlcN is thought to augment the endogenous production of GlcN as well as proteoglycan and hence maintain a normal turnover of the cartilage [11]. Furthermore, recent studies have proposed that the efficacy of GlcN may be because of its anti-inflammatory and anti-catabolic properties [12,13].

The Osteoarthritis Research Society International (OARSI) has recommended GlcN as a symptom-relieving and structure-modifying agent for knee OA [14]. Oral GlcN is widely used for modulating pain associated with OA. It can come in combination with other supplements such as chondroitin or by itself in the form of GlcN hydrochloride or GlcN sulphate. The recommended dosage is 1500 mg/d (20 mg/kg in a 75 kg subject) or 500 mg three times a day, which can ensure plasma concentrations of 10μM of GlcN [15], but without recognizable pattern of adverse effects [16—19].

Although GlcN is a popular nutritional supplement/prescription around the world, its efficacy is uncertain. There are three meta-analyses critiquing the efficacy of GlcN and advocating the benefits of GlcN on OA [20—22]. However, cautions should be paid on the interpretation of the results as some studies had suffered from methodological problems such as inadequate allocation concealment and absence of intent-to-treat approaches, which might have overestimated the actual benefits of GlcN [20]. Towheed et al [22] showed that GlcN prepared by Rotta Pharmaceuticals (Eatontown, NJ, USA) was superior to placebo in the treatment of pain and functional impairment resulting from OA. Contrary to those reports, a recent meta-analysis has concluded that GlcN could neither reduce joint pain nor increase joint space [23]. The use of GlcN to treat OA remains controversial and the mechanism behind is unclear. Therefore, this article aimed to critically review articles of GlcN on OA, with focus in the knee joint, in both human clinical trials and animal studies.

Human clinical studies

A Medline search was performed in February 2011 for the years 2000—2010 using the key words "glucosamine", "human clinical trials", "OA", and "knee" to screen all citations that involved GlcN in the management of knee OA or knee pain. Studies that compared GlcN-only preparations with placebo and double-blind, placebo controlled randomized clinical trials were deemed appropriate. With the above criteria, 12 studies were identified to fit in the criteria and were included for this review (Tables 1—4).

Baseline characteristics of participants

To have meaningful comparisons between treatment groups, the patient characteristics should be similar at baseline. Nevertheless, the baseline characteristics of patients were significantly different between the groups in two of the reports [24,25]. In McAlindon's [25] study, the placebo group had more female participants, used more nonsteroidal anti-inflammatory drug, and had higher body mass index than the GlcN group at baseline. Higher body mass index has been reported to associate with increase in physician- and patient-assessed levels of pain [26], which might affect the outcome measure. In Cibere's [24] study, more female participants and less severity in OA were found in the placebo group.

Gender difference among the participants

In most of the studies, women had accounted for more than 50% of the subjects ranging from 56.5% to 87.4%. The exceptions were Braham's [10] and Rindon's [27] studies in which the proportion of women was 28.3% and 5.1% correspondingly (Table 1). Women, particularly, those aged 55 years or above have a higher risk of developing knee OA, and more functional disabilities than men [28]. The gender difference of coping with knee OA should also be considered. Men and women adopt different gait strategies to reduce pain and to cope with the loads acting on the affected joints [29]. Women having OA reported more severe pain and physical disability [30]. Because the common clinical outcome measures of OA are self-reported pain and functional scales, it is possible that gender difference in perception of pain may affect the scores in the outcome measures.

Medication used during the experimental period

Because nonsteroidal anti-inflammatory drugs and analgesics would provide significantly greater pain relief than placebo [31], continuous use of these mediations during the experimental period may mask the efficacy of GlcN. Two of the studies reviewed did not encourage the use of rescue medication [10,32] and hence less than 9% of participants in these studies had consumed rescue medication (Table 2). For the study of Braham et al [10], it also set a washout period of 1 week before the assessment date.

In the study of McAlindon et al [25], the amount of rescue medication use was a secondary outcome measure, but this outcome measure is questionable because significant difference between groups was found at baseline measurement. In the study of Herrero-Beaumont et al [33], there were significantly less subjects completing the study using rescue medication in the GlcN group. Other studies which demonstrated GlcN to be more effective over placebo had set washout periods ranging from 1 week to 2

Table 1 Baseline characteristics of participants

Study	No. of subjects (groups)	% of female participants	Mean age	Mean BMI	History of OA/knee pain (yr)	Severity of OA/knee pain	Duration of treatment
Braham et al [10]	46 (2)	28	43	Not shown	median >10	Pain section of KOOS score 55	12 wk
Sawitzke et al [19]	662 (5)	68	57	~50% of subjects had BMI >30	10	KL score 2—3	24 mo
Cibere et al [24]	137 (2)	57 (placebo *vs.* GlcN, $p < 0.05$)	64	27.5	3	KL score 2—4 (placebo *vs.* GlcN, $p < 0.05$)	6 mo
McAlindon, T [25]	186 (2)	64 (placebo *vs.* GlcN, $p = 0.04$)	45—95, median 55—64	31 in GLcN, 34.1 in placebo, $p = 0.01$	Not shown	84% of participants with severe OA (total joint space loss)	12 wk
Rindone et al [27]	98 (2)	5	64	Not shown	13	KL score 1—4	2 mo
Petersen et al [32]	36 (3)	60	62.4	27.8	Not shown	KL score 1—4	12 wk
Herrero-Beaumont [33]	318 (3)	88	64	27.7	7	KL score 2—3	6 mo
Reginster et al [34]	212 (2)	76	66	27.4	7.9	KL score 2—3	3 yr
Usha & Naidu [35]	118 (4)	64	51	Not shown	3	Lequesne index 8—18	12 wk
Clegg et al [36]	1583 (5)	64	59	31.7	9.5—10.4	KL score 2—3	24 wk
Hughes & Carr [37]	80 (2)	68	62	Not shown	7.62	KL score 1—4	6 mo
Pavelka et al [38]	202 (2)	78	62.4	25.7	10.5	KL score 2—3	3 yr

KOOS, consists of 5 sections namely pain, symptoms, activities of daily living, sport/recreation, and knee related quality of life. It ranges from 0 to 80 with higher scores representing lower pain levels KL, ranges from 0 to 4, with 0 being normal and 4 being severe OA Leguesne index: OA specific questionnaire addressing pain, function limitation and ability to walk. It ranges from 0 to 24 with higher score indicating more severe of OA.

BMI = body mass index; GlcN = Glucosamine; KL = Kellgren and Lawrence radiographic grading scale; KOOS = Knee injury and osteoarthritis outcome score; OA = osteoarthritis.

Table 2 Medication used

Study	% of subjects taking rescue medications at baseline	% of subjects taking medication during the study period	Mean quantity of medication used	Washout period before assessment	Difference between groups	
					At baseline	During treatment
Braham et al [10]	Not shown	8.7% (6.5% in placebo, 2.2% in GlcN)	Not shown	1 wk	Not shown	
Sawitzke et al [19]	Washout period before baseline	Not shown	570 mg of paracetamol daily	24 hr	No	Not shown
Cibere et al [24]	68% in GlcN, 73%in placebo (acetaminophen and NSAID)	72% in GlcN, 76% in placebo	Not shown	No	No	No
McAlindon, T [25]	74% in GlcN, 87% in placebo, $p = 0.03$ (NSAID)	Not shown	Not shown	Not described	Significantly less subjects used NSAID in GlcN	No
Rindone et al [27]	28% (NSAID, acetaminophen, hydrocodone)	Not shown	Not shown	Not described	No	Not shown
Petersen et al [32]	33.3% (NSAID)	8.6% (5.7% in placebo, 2.9% in ibuprofen)	50 mg of tramadol for less than 5 d	Not described	Not shown	
Herrero-Beaumont [33]	Washout period before baseline	91% in placebo, 78% in GlcN	0.2—0.26 tablets of 400 mg ibuprofen daily	1 wk	No	Significantly less subjects used ibuprofen in GlcN
Reginster et al [34]	49% (NSAID, analgesics, corticosteroids)	50%	Less than 1 dose of rescue drug every 6 d	5 half-lives	No	No
Usha & Naidu [35]	Washout period before baseline	Not shown	30—95 tablets of 500 mg paracetamol	2 wk	No	Significantly less subjects used paracetamol in combination of GlcN and MSM groups
Clegg et al [36]	Not shown	Not shown	1.6—1.9 tablets of 400 mg acetaminophen daily	24 hr	No	No
Hughes & Carr [37]	22.5% analgesics, 46% NSAIDs	Not shown	43 tablets of paracetamol in GlcN, 45 in placebo	Not described	No	No
Pavelka et al [38]	Not shown	30—40% in both groups	500 mg Acetaminophen every 3 d	Not described	No	No

GlcN = Glucosamine; MSM = Methylsulfonylmethane; NSAID = nonsteroidal anti-inflammatory drug.

Table 3 Form of glucosamine

Study	Dosage (mg/d)	Form of GlcN	Supplier of GlcN	Selection criteria of GlcN use at baseline
Braham et al [10]	2000	HCl	Not shown	Not shown
Sawitzke et al [19]	1500	HCl	Not shown	Not shown
Cibere et al [24]	Equivalent to the dosage of GlcN intake before, max: 1500	SO_4	Vita health (dietary supplement)	Present daily-user for >1 mo
McAlindon, [25]	1500	SO_4	Physiologics (initial supplier) Rotta Pharmaceuticals (prescription drug) (Subsequent supplier)	Present users excluded
Rindone et al [27]	1500	SO_4	Applehart Laboratories	Past and present users excluded
Petersen et al [32]	1500	SO_4	Ferrosan	Washout period: 1 mo
Herrero-Beaumont [33]	1500	SO_4	Rotta Pharmaceuticals (prescription drug)	Washout period: 6 mo
Reginster et al [34]	1500	SO_4	Rotta Pharmaceuticals (prescription drug)	Not shown
Usha & Naidu [35]	1500	SO_4	Healers Limited	Not shown
Clegg et al [36]	1500	HCl	Ferro Pfanstiehl Laboratories	Not shown
Hughes & Carr [37]	1500	SO_4	Health Perception (dietary supplement)	Not shown
Pavelka et al [38]	1500	SO_4	Rotta Pharmaceuticals (prescription drug)	Not shown

weeks or 5 half-lives of the drug before symptom assessment [10,33–35].

History of glucosamine used

Among the articles reviewed, seven did not mention whether the participants had used GlcN before baseline measurement [10,19,34–38] (Table 3). Two had excluded present users of GlcN [25,27] and two others had set washout period for subjects with histories of GlcN consumption [32,33]. Based on the data from a long-term study [38], the change of Lequesne index after GlcN consumption was greater in the first year. Because GlcN has become popular and easily available, it is difficult to recruit subjects who have never consumed this substance before. Furthermore, there may also be carryover effect with GlcN consumption [39], which is not well reported and it should be further investigated.

Quality control of glucosamine preparation

The classification of GlcN is different among countries. Although it is a prescription in all European Union countries, it is classified as a dietary supplement in the USA [40]. Substances in the class of dietary supplement are usually under less stringent control when compared with prescriptions [41]. Among the 12 studies, 4 had used registered drug [25,32–34,38], 2 had used dietary supplement [24,37] and the others had used unknown sources of GlcN (Table 3). Most of the studies did not describe the quality control on the composition of GlcN. Based on a study assessing the content of active ingredient in over-the-counter GlcN sulphate preparations, it has revealed that the amount of active ingredient varied from 41% to 108% when compared with the content stated on the label [42]. The large discrepancy in the claimed compositions and actual content of the GlcN preparations would affect the analysis of the efficacy of GlcN.

Towheed et al [22] and Vlad et al [43] suggested that GlcN prepared by Rotta Pharmaceuticals was superior to placebo in the treatment of pain and functional impairment resulting from symptomatic OA. The effect size for trials using the Rotta pharmaceuticals preparation was much higher than that of other preparations (ES: 0.55 *vs.* 0.11) [43]. Although the GlcN manufactured by Rotta Pharmaceuticals is under the category of pharmaceuticals/prescription drug, this might explain the superiority of this GlcN preparation to the placebo group [33,34,38].

Forms of glucosamine

The common form of GlcN includes GlcN sulphate and GlcN hydrochloride. Only three of the reviewed studies had used the hydrochloride form (Table 3). One of them showed pain relief and improvement in knee related quality of life [10], whereas another showed decreased in OA-related pain level [Western Ontario and McMaster Universities (WOMAC) OA pain score 301–400] when GlcN was consumed with chondroitin sulphate [36]. Early hypothesis suggested that a component of the activity of GlcN sulphate was related to the sulphate residues in the compound as sulphur is an essential nutrient for stabilizing the extracellular matrices of connective tissue [7]. Vlad et al [43] also concluded that GlcN hydrochloride had no effect on pain as the effect size of GlcN hydrochloride was much smaller than that of GlcN sulphate. However, Verbruggen [44] found that the preparation used in many studies were two single molecules of GlcN and sulphate instead of a GlcN sulphate ester, therefore the active ingredient should be GlcN. Block et al [15] also rejected the hypothesis that increasing sulfate anion supply could boost the synthesis of the tissue matrix, because the concentration of serum sulfate after consumption of

Table 4 Outcome measures of human trials

Study	Efficacy	WOMAC pain, stiffness and function	Lequesne index	OMERACT/ OARSI	Joint space narrowing	Others
Braham et al [10]	Yes	N/A	N/A	N/A	N/A	(Knee Pain Scale, $p = 0.004$; knee related quality of life, $p = 0.038$)[a]
Sawitzke et al [19]	No	NS (pain, $p = 0.97$, function, $p = 0.56$)	N/A	NS ($p > 0.05$)	N/A	
Cibere et al [24]	No	NS (total score, $p = 0.96$)	N/A	N/A	N/A	NS (EQ-5D questionnaire and disease flare, $p > 0.05$)
McAlindon, T [25]	No	NS (total score, $p = 0.81$)	N/A	N/A	N/A	NS (use of rescue medications, $p = 0.12$)
Rindone et al [27]	No	N/A	N/A	N/A	N/A	NS (Visual analogue scale of pain at rest and during walking, $p = 0.66$—0.90, NS)
Petersen et al [32]	Yes	N/A	N/A	N/A	N/A	(Serum COMP, $p = 0.0378$), NS (Urinary CTX-II, $p = 0.1$)[a]
Herrero-Beaumont [33]	Yes	($p = 0.018$)[a]	($p = 0.01$)[a]	N/A	N/A	(Use of rescue medications, $p = 0.027$)[a]
Reginster et al [34]	Yes	($p = 0.016$)[a]	N/A	N/A	($p = 0.038$)[a]	NS (use of rescue medications, $p > 0.05$)
Usha & Naidu [35]	Yes	N/A	($p < 0.001$)[a]	N/A	N/A	(Pain index, $p < 0.001$; swelling index, walking time and join mobility index, $p < 0.05$)[a]
Clegg et al [36]	No	NS (Pain, $p = 0.73$; stiffness, $p = 0.68$)	N/A	NS ($p = 0.35$)	N/A	NS (use of rescue medications, joint swelling etc, $p > 0.05$
Hughes & Carr [37]	No	NS ($p = 0.54$ —0.77)	N/A	N/A	N/A	NS (Global pain, McGill sensory etc, $p > 0.05$)
Pavelka et al [38]	Yes	($p = 0.01$)[a]	($p = 0.002$)[a]	N/A	($p = 0.01$)[a]	NS (use of rescue medications, $p > 0.05$)

[a] significant difference between GlcN group and control group.
Efficacy: treatment of GlcN was significantly superior to the placebo in improving the outcome measure(s) stated.
COMP = serum cartilage oligomeric matrix protein, marker of aggrecan cataboism; CTX-II = c-telopeptide of Type II collagen, marker of type II collagen catabolism; EQ-5D = European Quality of Life Questionnaire; GlcN = Glucosamine; N/A = not applicable; NS = no significant difference between GlcN group and control group; OMERACT/OARSI = Outcome Measure in Rheumatology Clinical Trials/ Osteoarthritis Research Society International; WOMAC = Western Ontario and McMaster Universities Osteoarthritis.

glucosamine sulfate is far below the concentration required for effective uptake of the cell.

Efficacy of glucosamine on OA

The outcome measures of OA have generally been focused on symptomatic, structural, and biochemical changes. GlcN is said to be "efficacious" towards OA if GlcN treatment was significantly superior to placebo in improving the outcome measure(s) (Table 4). Among the articles reviewed, 6 showed that GlcN was effective towards OA or regular knee pain which included improving the symptoms [10,33—35,38], preventing joint structural change, [34,38] and altering cartilage turnover in the subjects [32] when compared with placebo treatment. The durations of treatment ranged from 6 weeks to 3 years. For those reporting positive effects of GlcN on OA, the treatment would last for more than 12 weeks. Nonetheless, the action of GlcN on OA-related symptoms could be detected as early as 2 weeks [21]. It may imply that the present outcome measures may not be stable and sensitive enough to detect the changes.

Most of the studies have adopted the WOMAC OA index as the primary or secondary outcome measures (Table 4). The WOMAC scale is a questionnaire that measures dysfunction and pain associated with OA of the lower extremities and it consists of 3 subscales on pain, stiffness, and physical functioning [45]. This instrument has been well studied and found to be reliable and valid [46,47]. However, as patients with OA often take analgesics, the effects of analgesics may cloud the action of other treatment preparation. Therefore, it is not sufficient to only measure pain without some objective measures.

Table 5 Outcome measures of animal studies

Study	Induced OA model	Mankin score/OOCHAS	Macroscopic cartilage assessment	GAG content	Others
Omegma et al [11]	Injection of protease	(More lesion in protease/GlcN, $p < 0.05$)[a]	N/A	(Higher GAG content in protease/GlcN, $p < 0.01$)[a]	(Higher expression of biglycan in protease/low GlcN, $p < 0.05$)[a]
Lippiello et al [53]	ACLT	(Less lesion in ACLT/combination treatment trial of CS, GlcN, & manganese ascorbate; $p < 0.05$)[a]	N/A	NS	N/A
Tiraloche et al [54]	ACLT	(Less lesion in ACLT/GlcN, $p = 0.049$)[a]	(Lower rate of disease in the ACLT/GlcN trial at LTP, $p = 0.046$)[a]	(Higher GAG content in ACLT/GlcN, $p < 0.05$)[a]	N/A
Wang et al [55]	ACLT	N/A	N/A	N/A	(Higher osteoid volume in ACLT/GlcN, $p = 0.041$)[a] (Higher trabecular bone volume in ACLT/GlcN, $p = 0.033$)[a]
Chen et al [56]	ACLT	(Less lesion in ACLT/GlcN, $p < 0.05$)[a]	N/A	N/A	(Higher expression of TGF-β in ACLT/GlcN, $p < 0.05$)[a] (Lower expression of IL-1-β in ACLT/GlcN, $p < 0.05$)[a]
Naito et al [57]	ACLT	NS	N/A	N/A	(Lower CTX-II level in ACLT/GLcN, $p < 0.001$)[a] (Increased CPII level in ACLT/GlcN, $p < 0.001$)[a]
Silva et al [58]	ACLT	(Less cartilage damage in combination of ACLT/combination treatment of GlcN and CS, $p < 0.05$)[a]	N/A	N/A	(Lower pain value in ACLT/combination of GlcN and CS, $p < 0.05$)[a]
Wen et al [59]	ACLT	(Less lesion in ACLT/GlcN, $p < 0.001$)[a]	(Lower macroscopic score in ACLT/GlcN, $p = 0.005$)[a]	N/A	(Lower synovitis score in ACLT/GlcN, $p < 0.001$)[a] (Improved nociceptive behavior in ACLT/GlcN, $p < 0.001$)[a] (Lower p38 kinase in ACLT/GlcN, $p = 0.002$)[a] (Lower JNK in ACLT/GlcN, $p = 0.006$)[a]

[a] significant difference compared with ACLT or protease/control.

ACLT = anterior cruciate ligament transaction; CP-II = carboxy propeptide of Type II collagen, marker of Type II collagen synthesis; CTX-II = Type II collagen C-telopeptide, marker of Type II collagen catabolism; GAG = Glycosaminoglycan; GlcN = Glucosamine; IL-1-β = interleukin-1- beta; JNK = c-Jun N-terminal kinase, activated in response to inflammation cytokines; LTP = Lateral tibial plateau; N/A = not applicable; NS = no significant difference compared with ACLT or enzyme induced/control; OA = osteoarthritis; OOCHAS = Osteoarthritis Research Society International (OARSI) Cartilage Histopathology Assessment System; p38 kinase = belongs to the family of mitogen-activated protein kinase. It is activated in response to inflammation cytokines; TGF-β = transforming growth factor beta.

Presently, measurement for the width of joint space is commonly used as an indirect measure of cartilage repair. However, the measurement is not sensitive enough as it often takes 1—2 years before there is reliable information on the preventive effect of the treatment preparation [48] and it also has large precision errors [49]. Biochemical markers could be a more specific outcome measure for improvement of joint disease. There was a study using biochemical markers, namely, serum cartilage oligomeric matrix protein and urine c-telopeptide of Type II collagen (CTX-II) as indicators of catabolism of aggrecan and Type II collagen, respectively, and found significant changes in the serum cartilage oligomeric matrix protein level [32]. Nevertheless, cautions should be paid when interpreting the data because it is not sure whether the markers level in blood/urine truly represents the change in joint structure [48]. Further research is needed to test the reliability of specific markers and develop more sensitive indicators for monitoring the disease.

Animal studies

Owing to the slow progress and unclear pathogenesis of OA [50], human clinical trials have mainly focused on symptomatic relief. The poor sensitivity of the diagnostic tools and the difficult access to disease tissues also hinder the research of OA in human [51]. Animal models can therefore provide an alternative for studying potential antiarthritis agents.

A Medline search was performed for the years 2000—2010 using the key words "glucosamine", "*in vivo*", "OA", and "experimental OA" to screen all citations that involved oral consumption of GlcN in the management of experimental OA. Studies that compared GlcN-only preparations with placebo (induced OA control) were included. There were 8 studies meeting the criteria above (Tables 5 and 6).

Models adopted

The design of animal models on prediction of the effectiveness of a drug should be based on the track record of predictability of drug induced modification of the disease progression. Although there are no such agents proven to modify disease progression of OA [52], most of the models adopted in testing GlcN were based on the histopathological similarities to human disease [50]. For example, anterior cruciate ligament transaction (ACLT) of rabbits [53—56] and rats [57—59] and enzyme-induced model [11] are commonly used to study OA. The advantages of these models include rapid development and reproducible damage relevant to the traumatic forms of OA [51]. On the other hand, spontaneous models are better in simulating the slow progress of the human disease but it usually takes a long time before observable changes can be recorded [52]. As yet, there is no consensus on which model and species are the most relevant for human OA.

Efficacy of glucosamine on change of joint structures

Histologic/Histochemical Grading System (HHGS, Mankin score) and the OARSI Cartilage Histopathology Assessment System are two common methods for analyzing the progression of cartilage lesions. Both the HHGS and OARSI Cartilage Histopathology Assessment System have excellent

Table 6 Glucosamine treatment in animal studies

Study	Model	Form of GlcN	Dosage of GlcN	Determination of dosage
Omegma et al [11]	Injection of CP to the middle of the patellar tendon	GlcN HCl	20 mg/kg/d for low GlcN diet, 100 mg/kg/d for high GlcN diet	Based on the recommended dosage for human (1.5 g/70 kg/d = 20 mg/kg/d approx.)
Lippiello et al [53]	ACLT, PCLT and medial meniscus removed NZ white rabbits	GlcN HCl	2% by weight of 500 mg GlcN daily	Not shown
Tiraloche et al [54]	ACLT, NZ white rabbits	GlcN HCl	100 mg/d	Based on the recommended dosage for human (1.5 g/70 kg/d = 20 mg/kg/d approx.)
Wang et al [55]	ACLT NZ white rabbits	GlcN HCl	100 mg/d	Not shown
Chen et al [56]	ACLT, NZ white rabbits	GlcN HCl	150 mg/kg/d	Not shown
Naito et al [57]	ACLT, Sprague-Dawley rats	GlcN HCl	1000 mg/kg/d	Based on a pharmacokinetics study of rat [64]
Silva et al [58]	ACLT male Wistar rats	GlcN SO_4	500 mg/kg/d	Based on preliminary studies
Wen et al [59]	ACLT, Wistar rats	GlcN SO_4	250 mg/kg/d	Not shown

ACLT = anterior cruciate ligament transaction; CP = protease-chymopapain; GlcN = Glucosamine; NZ = New Zealand; PCLT = posterior cruciate ligament transaction.

intra- and inter-observer reproducibility. The correlation between the scores is good [60]. (Table 5).

A number of studies have reported that the positive treatment effect of GlcN would decrease the ACLT induced cartilage lesion as compared with the ACLT control [54,56,59]. On the other hand, two of the studies reviewed showed that combination of GlcN and CS was superior to GlcN-only treatment in reducing cartilage damage [53,58].

In the study by Tiraloche et al [54], the GlcN group had a significantly lower rate of disease in the lateral tibial plateau compartment compared with that of the placebo group. The less severe cartilage lesion and erosion in tibial plateau compartment but not the other compartments implied the site-specificity of GlcN. As suggested by Handley [61], chondrocytes are sensitive to their biomechanical environment and therefore the metabolic characteristics are different for weight-bearing and non-weight bearing regions of articular cartilage.

Although the HHGS was based on study of specimens with very advanced OA [62], it is valid for normal and severe OA cartilage, but it does not show a linear relationship with the change of articular cartilage in mild and moderate OA [63]. Therefore, caution should be paid on Oegema's [11] study, as it used a slightly lower dose of enzyme to induce a mild OA condition. Contrary to the studies discussed above [54,56,59], the Mankin score was significantly higher in GlcN group compared with control, which indicated more severe damage in the GlcN group.

In studies that measured the cartilage glycosaminoglycan (GAG) content, controversies were reported. Some researchers found that the GlcN-treated group had significantly preserved the GAG content when compared with the placebo group [11,54], whereas Lippiello et al [53] could not find differences in the GAG content between the ACLT/GlcN and the ACLT/placebo group.

Apart from cartilage, a study had reported significant reduction in the osteoid volume of the ACLT/GlcN group compared with that of the ACLT/placebo group [55]. This finding implied that GlcN treatment has reduced the subchondral bone changes. Furthermore, based on the histological assessment, synovial inflammation was less severe in the ACLT/GlcN group compared with that of ACLT/placebo group [59].

Efficacy of glucosamine on pain relief

Pain is a parameter commonly measured in clinical studies but not with animal studies because of the question of validity of measuring pain in animals [51]. In the study by Silva et al [58], pain was assessed using an articular incapacitation method of the paw elevation time when the sole was placed on a rotating cylinder to induce painful stimulation to the paw. They found that a combination of GlcN and CS had significantly reduced the paw elevation time, but not with the GlcN treatment alone. Wen et al [59] investigated the nociceptive behaviour of OA rats with mechanical allodynia and weight-bearing distribution test. The force required for paw withdrawal in the mechanical allodynia and the amount of weight shifted to the non-injured limb were indicators of nociceptive behaviour. They found that GlcN treatment did reduce pain in the rats.

Effect of glucosamine on changes of biochemical markers

There is substantial interest on the use of biochemical markers to assess the progress of diseases. It is important to use biochemical markers in the clinical monitoring for patients with OA because these markers usually response rapidly to treatment [48]. Articular cartilage is rich in Type II collagen and according to the study of Naito et al [57] the treatment of GlcN would suppress Type II collagen degradation and enhance Type II collagen synthesis in rats with the ACLT.

Dosage of glucosamine

Most *in vivo* studies did not determine the Cmax (peak concentration of a drug observed after its administration) of GlcN, which may affect the therapeutic relevance of these studies for human OA [15]. Some studies determined the dosage based on the recommendation for human of 1.5 g/70 kg BW/d i.e., about 20 mg/kg/d [11,54] (Table 6). Although GlcN is consumed orally, the absorption efficiency and metabolism of GlcN may be different among species.

The prediction method used in animal studies could be used to estimate the effective dosage for a particular animal model, but the result may not be extrapolated to human OA. Naito et al [57] had used the dosage of oral GlcN that achieved comparable serum level with human according to a pharmacokinetics study of rat. This determination method was close to the suggestion from Block et al [15] and it showed better relevance for Human OA.

Conclusion

Based on the results of human clinical trials, half of the studies reported beneficial effects of GlcN for OA or regular knee pain. The conflicting results may be because of the different study design and control of confounding variables.

Treatment of GlcN for experimental OA has been found to facilitate cartilage healing, increase cartilage GAG content, reduce subchondral structural changes, relieve nociceptive behaviour, and synovitis. However, because there are large variations in the animal models, GlcN administration, and outcome measures, the efficacy of GlcN on different animal models is hard to compare. Further research is needed to study the validity of these animal models and the applicability of these models for human.

References

[1] Bijlsma JWJ, Knahr K. Strategies for the prevention and management of osteoarthritis of the hip and knee. Best Pract Res Clin Rheumatol 2007;21:59—76.
[2] Buckland-Wright C. Subchondral bone changes in hand and knee osteoarthritis detected by radiography. Osteoarthritis Cartilage 2004;12:10—9.
[3] Burr DB, Schaffler MB. The involvement of subchondral mineralized tissues in osteoarthrosis: quantitative microscopic evidence. Microsc Res Tech 1997;37:343—57.

[4] Hill CL, Gale DG, Chaisson CE, Skinner K, Kazis L, Gale ME, et al. Knee effusions, popliteal cysts, and synovial thickening: association with knee pain in osteoarthritis. J Rheumatol 2001;28:1330—7.
[5] Pelletier J, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. Arthritis Rheum 2001;44:1237—47.
[6] Goldring S, Goldring M. Clinical aspects, pathology and pathophysiology of osteoarthritis. J Musculoskelet Neuronal Interact 2006;6:376—8.
[7] Kelly GS. The role of glucosamine sulfate and chondroitin sulfates in the treatment of degenerative joint disease. Altern Med Rev 1998;3:27—39.
[8] Sweeney C, Mackintosh D, Mason R. UDP-sugar metabolism in Swarm rat chondrosarcoma chondrocytes. Biochem J 1993; 290:563—70.
[9] da Camara C, Dowless G. Glucosamine sulfate for osteoarthritis. Ann Pharmacother 1998;32:580—7.
[10] Braham R, Dawson B, Goodman C. The effect of glucosamine supplementation on people experiencing regular knee pain. Br J Sports Med 2003;37:45—9.
[11] Oegema Jr TR, Deloria LB, Sandy JD, Hart DA. Effect of oral glucosamine on cartilage and meniscus in normal and chymopapain-injected knees of young rabbits. Arthritis Rheum 2002;46:2495—503.
[12] Herrero-Beaumont G, Rovati LC, Castaneda S, Alvarez-Soria MA, Largo R. The reverse glucosamine sulfate pathway: application in knee osteoarthritis. Expert Opin Pharmacother 2007;8:215—25.
[13] Nagaoka I, Igarashi M, Hua J, Ju Y, Yomogida S, Sakamoto K. Recent aspects of the anti-inflammatory actions of glucosamine. Carbohydr Polym 2011;84:825—30.
[14] Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, et al. OARSI recommendations for the management of hip and knee osteoarthritis. Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008;16:137—62.
[15] Block JA, Oegema TR, Sandy JD, Plaas A. The effects of oral glucosamine on joint health: is a change in research approach needed? Osteoarthritis Cartilage 2010;18:5—11.
[16] AbdelFattah W, Hammad T. Chondroitin sulfate and glucosamine: a review of their safety profile. JANA 2001;3:16—23.
[17] Anderson JW, Nicolosi RJ, Borzelleca JF. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food Chem Toxicol 2005;43:187—201.
[18] Hathcock JN, Shao A. Risk assessment for glucosamine and chondroitin sulfate. Regul Toxicol Pharmacol 2007;47:78—83.
[19] Sawitzke AD, Shi H, Finco MF, Dunlop DD, Harris CL, Singer NG, et al. Clinical efficacy and safety of glucosamine, chondroitin sulphate, their combination, celecoxib or placebo taken to treat osteoarthritis of the knee: 2-year results from GAIT. Ann Rheum Dis 2010;69:1459—64.
[20] McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. J Am Med Assoc. 2000; 283:1469—75.
[21] Richy F, Bruyere O, Ethgen O, Cucherat M, Henrotin Y, Reginster J-Y. Structural and symptomatic efficacy of glucosamine and chondroitin in knee osteoarthritis: a comprehensive meta-analysis. Arch Intern Med 2003;163:1514—22.
[22] Towheed T, Maxwell L, Anastassiades Tassos P, Shea B, Houpt JB, Welch V, et al. Glucosamine therapy for treating osteoarthritis. Cochrane Database Syst Rev 2005; Issue 2. Art No.: CD002946.
[23] Wandel S, Juni P, Tendal B, Nuesch E, Villiger PM, Welton NJ, et al. Effects of glucosamine, chondroitin, or placebo in patients with osteoarthritis of hip or knee: network meta-analysis. Br Med J 2010;341:4675—83.
[24] Cibere J, Kopec JA, Thorne A, Singer J, Canvin J, Robinson DB, et al. Randomized, double-blind, placebo-controlled glucosamine discontinuation trial in knee osteoarthritis. Arthritis Rheum 2004;51:738—45.
[25] McAlindon T, Formica M, LaValley M, Lehmer M, Kabbara K. Effectiveness of glucosamine for symptoms of knee osteoarthritis: results from an internet-based randomized double-blind controlled trial. Am J Med 2004;117:643—9.
[26] Lubbeke A, Duc S, Garavaglia G, Finckh A, Hoffmeyer P. BMI and severity of clinical and radiographic signs of hip osteoarthritis. Obesity 2009;17:1414—9.
[27] Rindone JP, Hiller D, Collacott E, Nordhaugen N, Arriola G. Randomized, controlled trial of glucosamine for treating osteoarthritis of the knee. West J Med 2000;172:91—4.
[28] Srikanth VK, Fryer JL, Zhai G, Winzenberg TM, Hosmer D, Jones G. A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. Osteoarthritis Cartilage 2005;13:769—81.
[29] Debi R, Mor A, Segal O, Segal G, Debbi E, Agar G, et al. Differences in gait patterns, pain, function and quality of life between males and females with knee osteoarthritis: a clinical trial. BMC Musculoskelet Disord 2009;10:127.
[30] Keefe FJ, Lefebvre JC, Egert JR, Affleck G, Sullivan MJ, Caldwell DS. The relationship of gender to pain, pain behavior, and disability in osteoarthritis patients: the role of catastrophizing. Pain 2000;87:325—34.
[31] Towheed TE, Hochberg MC. A systematic review of randomized controlled trials of pharmacological therapy in osteoarthritis of the knee, with an emphasis on trial methodology. Semin Arthritis Rheum 1997;26:755—70.
[32] Petersen SG, Saxne T, Heinegard D, Hansen M, Holm L, Koskinen S, et al. Glucosamine but not ibuprofen alters cartilage turnover in osteoarthritis patients in response to physical training. Osteoarthritis Cartilage 2010;18:34—40.
[33] Herrero-Beaumont G, Ivorra JAR, del Carmen Trabado M, Blanco FJ, Benito P, Martín-Mola E, et al. Glucosamine sulfate in the treatment of knee osteoarthritis symptoms: a randomized, double-blind, placebo-controlled study using acetaminophen as a side comparator. Arthritis Rheum 2007; 56:555—67.
[34] Reginster JY, Deroisy R, Rovati LC, Lee RL, Lejeune E, Bruyere O, et al. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. Lancet 2001;357:251—6.
[35] Usha PR, Naidu MUR. Randomised, double-blind, parallel, placebo-controlled study of oral glucosamine, methylsulfonylmethane and their combination in osteoarthritis. Clin Drug Investig 2004;24:353—63.
[36] Clegg DO, Rega DJ, Harris CL, Klein MA, O'Dell JR, Hooper MM, et al. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. N Engl J Med 2006;354:795—808.
[37] Hughes R, Carr A. A randomized, double-blind, placebo-controlled trial of glucosamine sulphate as an analgesic in osteoarthritis of the knee. Rheumatology 2002;41:279—84.
[38] Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacovelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. Arch Intern Med 2002; 162:2113—23.
[39] Thie NM, Prasad NG, Major PW. Evaluation of glucosamine sulfate compared to ibuprofen for the treatment of temporomandibular joint osteoarthritis: a randomized double blind controlled 3-month clinical trial. J Rheumatol 2001;28: 1347—55.
[40] Reginster J. The efficacy of glucosamine sulfate in osteoarthritis: financial and nonfinancial conflict of interest. Arthritis Rheum 2007;56:2105—10.

[41] McAlindon T. Why are clinical trials of glucosamine no longer uniformly positive? Rheum Dis Clin North Am. 2003;29:789—801.
[42] Russell A, Aghazadeh-Habashi A, Jamali F. Active ingredient consistency of commencially available glucosamine sulfate products. J Rheumatol 2002;29:2407—9.
[43] Vlad SC, LaValley MP, McAlindon TE, Felson DT. Glucosamine for pain in osteoarthritis: why do trial results differ? Arthritis Rheum 2007;56:2267—77.
[44] Verbruggen G. Chondroprotective drugs in degenerative joint diseases. Rheumatology 2006;45:129—38.
[45] Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 1988;15:1833—40.
[46] Bellamy N, Campbell J, Stevens J, Pilch L, Stewart C, Mahmood Z. Validation study of a computerized version of the Western Ontario and McMaster Universities VA3.0 Osteoarthritis index. J Rheumatol 1997;24:13—5.
[47] Roos EM, Klässbo M, Lohmander LS. WOMAC osteoarthritis index. Reliability, validity, and responsiveness in patients with arthroscopically assessed osteoarthritis. Western Ontario and MacMaster Universities. Scand J Rheumatol 1999;28:210—5.
[48] Garnero P. Osteoarthritis: biological markers for the future? Joint Bone Spine 2002;69:525—30.
[49] Garnero P, Rousseau JC, Delmas PD. Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. Arthritis Rheum 2000;43:953—68.
[50] Bendele A. Animal models of osteoarthritis. J Musculoskelet Neuronal Interact 2001;1:363—76.
[51] Ameye LG, Young MF. Animal models of osteoarthritis: lessons learned while seeking the 'Holy Grail'. Curr Opin Rheumatol 2006;18:537—47.
[52] Bendele A, Mccomb J, Gould T, Mcabee T, Sennello G, Chlipala E, et al. Animal models of arthritis: relevance to human disease. Toxicol Pathol 1999;27:134—42.
[53] Lippiello L, Woodward J, Karpman R, Hammad TA. In Vivo chondroprotection and metabolic synergy of glucosamine and chondroitin sulfate. Clin Orthop 2000;381:229—40.
[54] Tiraloche G, Girard C, Chouinard L, Sampalis J, Moquin L, Ionescu M, et al. Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. Arthritis Rheum 2005;52:1118—28.
[55] Wang SX, Laverty S, Dumitriu M, Plaas A, Grynpas MD. The effects of glucosamine hydrochloride on subchondral bone changes in an animal model of osteoarthritis. Arthritis Rheum 2007;56:1537—48.
[56] Chen D, Zhang Z, Cao J, Zhange D, Jia B. Effect of glucosamine hydrochloride capsules on articular cartilage of rabbit knee joint in osteoarthritis. Chin J Reparative Reconstr Surg 2010;24:287—91.
[57] Naito K, Watari T, Furuhata A, Yomogida S, Sakamoto K, Kurosawa H, et al. Evaluation of the effect of glucosamine on an experimental rat osteoarthritis model. Life Sci 2010;86: 538—43.
[58] Silva F, Yoshinari N, Castro R, Girão V, Pompeu M, de Andrade Feitosa J, et al. Combined glucosamine and chondroitin sulfate provides functional and structural benefit in the anterior cruciate ligament transection model. Clin Rheumatol 2009;28:109—17.
[59] Wen ZH, Tang CC, Chang YC, Huang SY, Hsieh SP, Lee CH, et al. Glucosamine sulfate reduces experimental osteoarthritis and nociception in rats: association with changes of mitogen-activated protein kinase in chondrocytes. Osteoarthritis Cartilage 2010;18:1192—202.
[60] Custers RJH, Creemers LB, Verbout AJ, van Rijen MHP, Dhert WJA, Saris DBF. Reliability, reproducibility and variability of the traditional Histologic/Histochemical Grading System vs the new OARSI Osteoarthritis Cartilage Histopathology Assessment System. Osteoarthritis Cartilage 2007;15: 1241—8.
[61] Handley C. Institute of bone and joint research symposium regeneration and repair of connective tissues: fact or fiction? Int J Rheum Dis 2002;5:A1—2.
[62] Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips: II. Correlation of morphology with biochemical and metabolic date. J Bone Joint Surg Am 1971; 53:523—37.
[63] Ostergaard K, Andersen CB, Petersen J, Bendtzen K, Salter DM. Validity of histopathological grading of articular cartilage from osteoarthritic knee joints. Ann Rheum Dis 1999; 58:208—13.
[64] Aghazadeh-Habashi A, Sattari S, Pasutto FM, Jamali F. Single dose pharmacokinetics and bioavailability of glucosamine in the rat. J Pharm Pharm Sci 2002;5:181—4.