Editorial

Glucosamine Therapy for Osteoarthritis: An Update

Since our 1999 editorial in *The Journal* on the interesting and evolving field of glucosamine (GlcN) therapy for osteoarthritis (OA)[1], a number of important developments have taken place. We will highlight the most significant recent developments.

We recently updated our Cochrane Review of the efficacy and toxicity of GlcN therapy in OA[2]. The 2005 update includes a systematic review and metaanalysis of 20 randomized controlled trials (RCT). Collectively, the 20 RCT found GlcN to be superior to placebo, with a 28% (change from baseline) improvement in pain and a 21% (change from baseline) improvement in function using the Lequesne Index. The standardized mean differences were equivalent to 0.61 for pain and 0.51 for function. In the 4 RCT in which the Rotta preparation of GlcN was compared with a nonsteroidal antiinflammatory drug, GlcN was superior in 2 and equivalent in 2.

It is importance to recognize, however, that the results from the 20 RCT were not uniformly positive, and that the reasons for this discrepancy remain largely unexplained[3,4]. Five RCT failed to show that GlcN was more effective than placebo in OA[5-9]. In addition, the Cochrane Review reported that, in patients administered GlcN, the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) outcomes of pain, function, and stiffness did not reach statistical significance. Analysis restricted to 8 RCT with adequate allocation concealment also failed to show a benefit of GlcN for pain and WOMAC function. Therefore, the impressive degree of improvement with GlcN that was noted in the earlier Cochrane Review in 1999 was found to be significantly less in the updated Cochrane Review in 2005. The recently published trial by Clegg, *et al*[10] also failed to show any overall efficacy of GlcN.HCl in subjects with OA of the knee.

A discrepancy was also observed when subgroup analyses were performed comparing the Rotta preparation of glucosamine sulfate (GlcN.S) versus other GlcN preparations. In the 10 RCT in which the Rotta preparation of GlcN was compared to placebo, GlcN was found to be superior in terms of pain and functional impairment resulting from symptomatic OA. Pooled results from studies using a non-Rotta preparation failed to show a benefit for both pain and function.

Why are the favorable results from the published RCT no longer uniformly positive[2,3]? Several explanatory factors might help account for this discrepancy, including differences in the subjects enrolled and the outcomes evaluated, the methodological quality of the trials, and the degree of cointervention that was allowed in the RCT.

Also, there is a possibility that the GlcN.S preparation utilized could contain small amounts of a contaminant(s) with biological activity. Such a possibility could account for differences between studies performed with GlcN.HCl versus GlcN.S, or even between different GlcN.S preparations (e.g., Rotta vs non-Rotta). However, that an additional molecule is contained within such preparations has not been shown.

Another important limitation with extrapolating the generally favorable results from the GlcN RCT lies in the fact that most of the studies (65%) in the updated Cochrane Review evaluated exclusively the prescription medicine made by the Rotta Pharmaceutical Company — a GlcN.S preparation that is approved as a prescription drug for OA in the European Union. In North America, GlcN is not considered a conventional prescription drug, rather it is considered as a dietary supplement, which is widely available as an over the counter preparation. Since the content and purity of the various over the counter preparations is known to vary markedly, the relative efficacy and safety of the various preparations may also vary markedly[11-14]. It is apparent that if GlcN is to be used as a therapeutic agent in OA, it is important that GlcN products conform to a standard in their description.

It is important to define in chemical terms, at the outset, what is meant by "glucosamine" (GlcN) and by "glucosamine sulfate" (GlcN.S). The latter term can be confusing as it is not clear if it means that the compound administered contains GlcN as an ionically-bound sulfate salt, or if the molecule of GlcN is covalently bound to sulfate, at one or more positions around the sugar ring. The type of chemical binding of GlcN to the sulfate could greatly affect the biological and chemical properties of the compound being tested. We suggest that "glucosamine sulfate salt" should be abbreviated as GlcN.S, to mean GlcN and an ionically bound sulfate salt. Correspondingly, "glucosamine hydrochloride salt" should be abbreviated as GlcN.HCl. For "glucosamine sulfate," where a SO_4 molecule(s) is covalently bound to a specific carbon of the GlcN molecule, standard biochemical notation should be employed, as for example GlcN-3-SO_4 or GlcN-6-SO_4. In this

editorial, we comment only on studies where GlcN.HCl and GlcN.S have been used. From a biochemical perspective, one would expect that after ingesting equimolar amounts of GlcN.HCl or of GlcN.S the same number of molecules of GlcN would enter the circulation and be delivered to the tissues and metabolized.

If there is an effect of oral GlcN on OA symptoms or restoration of cartilage function, the mechanism of action(s) is not known. The term "building block" has been used for GlcN with respect to the function of GlcN and the glycosaminoglycans (GAG) in articular cartilage. However, the most abundant GAG in cartilage, the chondroitin sulfate in aggrecan molecules, contains N-acetylgalactosamine, derived from the enzymatic epimerization of UDP-GlcNAc. GlcN is found in hyaluronic acid and keratan sulfate as well as in matrix glycoproteins, where it is invariably N-acetylated (GlcNAc). The reason why GlcN and other hexosamines found in mammalian complex carbohydrates are, in general, N-acetylated is not clear. However, substituting the N group of GlcN with an acetyl group, or with any acyl moiety, greatly changes its biological properties[15,16]. The carbon source for the GlcN synthetic pathway is physiologically from glucose (through glucose-6-P), not GlcN. As pointed out below, pharmacokinetic studies suggest that there are very low levels of GlcN in the serum, even after substantial amounts of ingested GlcN.

A large number of publications[15-20] have appeared describing *in vitro* studies, mostly with chondrocyte cultures, utilizing either GlcN.S or GlcN.HCl. These studies attempt to demonstrate mechanisms of action of GlcN in cartilage. Such demonstrations would provide a correlation with animal models and positive human OA studies, keeping in mind that the positive RCT used mostly pain and function as outcomes. The reported mechanisms of action of GlcN have included increased proteoglycan (PG) synthesis and decreased degradation through interleukin 1ß (IL-1ß)-induced nuclear factor-κB activation of chondrocytes. However, it may be difficult to differentiate between effects on new PG synthesis, PG release, or inhibition of degradation of presynthesized PG, depending on protocols utilized and the culture system utilized. Also, GlcN.HCl (100 µg/ml) suppressed prostaglandin E_2 production from OA chondrocytes and matrix metalloproteinases from normal but not OA chondrocytes[21]. The importance of defining cells and conditions in these types of experiments and in matrix synthesis has been emphasized[21,22]. In general, anchorage-dependent (AD), also referred to as "2-dimensional," culture systems (chondrocytes grown on plastic) provide uniform access of the test substances (e.g., added GlcN) to the cells. However, AD systems have a strong tendency to exhibit dedifferentiation of the chondrocyte phenotype on subculture. Anchorage-independent (AI) systems, also referred to as "3-dimensional," tend to retain the chondrocyte phenotype better, but pose problems with uniform accessibility of the test substance and interpretation of amounts of matrix proteins synthesized on a per-cell (e.g., DNA) basis. Indeed, there is no agreement on what cell culture system would be the best model for the damaged cartilage of human OA of weight-bearing joints where, if repair does occur, it is presumably by ("dedifferentiated") fibrocartilage.

GlcN has many inhibitory effects in biological systems. It is toxic to some rodent tumors[23], possibly by competing with glucose utilization, and the addition of GlcN to cultured adipocytes induces insulin resistance[24]. In cartilage explant systems, aminosugars in general, added in high concentrations (maximal inhibition for mannosamine at 1.35 mM and up to 10-fold greater concentrations for GlcN), inhibited aggrecanase activity[25]. The addition of GlcN and also mannosamine, in millimolar concentrations, inhibited degradation of aggrecan in cartilage explants, probably through inhibition of aggrecanase[26]. GlcN added to equine cartilage explants in high concentrations (25 mg/ml) inhibited PG and metalloproteinase release in the media[27]. Also, GlcN results in apoptosis of cartilage *in situ* and in chondrocyte cultures[28]. Thus, the effects of the addition of GlcN to cartilage explant cultures, at high concentrations, appear to be largely mediated by inhibition of the degradation of PG, rather than stimulation of synthesis. This interpretation is also supported by recent gene expression studies, suggesting that enzymatic breakdown of the extracellular matrix is reduced by the addition of GlcN (5 mM), and that restoration of already damaged cartilage is not to be expected, because gene expression of anabolic genes is also downregulated[29].

Given the relatively low serum concentrations after oral ingestion of GLcN.S, it appears unlikely that the high concentrations (> 100 µM) of GLcN used in the *in vitro* studies can be replicated in the human plasma or synovial fluid of the osteoarthritic joint[30-32].

Three recent studies, using horse and human models, have quantitated the serum levels of free GlcN after administration of clinically relevant dosages of GlcN[30-33]. Biggee, *et al*[30] used high performance liquid chromatography with a high sensitivity Metrohm-Peak instrument for pulsed amperometric measurement of human serum GlcN. The detection limit of 0.5 µM at 1:10 serum dilution is the lowest reported detection limit to date in any published study. The maximum concentration achieved after oral ingestion of 1500 mg of the Rotta preparation of GlcN.S was only 11.5 µM (range 1.9 to 11.5 µM). There was no correlation with age, weight, and body mass index. Previous studies by these same authors have shown that, at this maximum concentration of 11.5 µM, one would expect that no more than 2% of the galactosamine incorporated into chondroitin sulfate would be derived from incubations of GlcN with cultured human chondrocytes[34]. The majority of the galactosamine production results by preferentially utilizing endogenous glucose. The authors concluded that insignificant trace amounts of GlcN enter human serum after oral ingestion and that this amount is far below any amount that might contribute directly to chondroitin synthesis. Therefore, based on the results of this study, GlcN probably

does not act in OA simply by acting as a "building block" for the synthesis of glycosoaminoglycans in the articular cartilage.

Laverty, *et al*[31] reported the first published animal data confirming that free GlcN can be detected in the synovial fluid after administration. They measured free GlcN concentrations both in the serum and in the synovial fluid of the radiocarpal joints in horses using a sensitive fluorophore-assisted carbohydrate electrophoresis. Two methods of administration of GlcN were evaluated (via nasogastric tube and via intravenous administration). Following nasogastric dosing (20 mg/kg), the maximal serum concentration of GlcN was only 6.1 μM (range 4.4 to 7.6 μM). This is a result similar to that obtained by Biggee, *et al*[30]. Following intravenous (IV) dosing (20 mg/kg), the maximum concentration of GlcN in serum reached 300 μM. Synovial fluid concentrations reached 9–15 μM with IV dosing and 0.3 to 0.7 μM with nasogastric dosing. Synovial fluid levels of GlcN after both IV and nasogastric dosing were found to be < 10% of those in serum collected at the same timepoint. The mean bioavailability of GlcN was only 5.9% and the mean elimination half-life was only 2.8 h. The authors concluded that clinically relevant dosing of GlcN.HCl in the horse results in serum and synovial fluid concentrations that are at least 500-fold lower than those reported to modify chondrocyte activities in tissue and cell culture experiments.

Persiani, *et al*[32] used a liquid chromatography method with mass spectrometry detection for the determination of GlcN in the plasma of humans who ingested clinically relevant dosages of GlcN.S. Maximum serum concentrations were again found to be only in the 10 μM range. Preliminary data from the same group have also been reported in abstract form from the 2005 EULAR meeting[33]. For the first time, free GlcN was detected in the knee synovial fluid in 2 patients with OA who were assayed 24 h post oral ingestion of a 15 day treatment course (1500 mg/day of GlcN.S) in concentrations of 497 and 1978 ng/ml[33]. In these 2 patients, there was a good correlation between the plasma and synovial fluid levels.

We are now left with several puzzling questions. How could GlcN actually work in OA if very little actually gets to the joint? Are there other mechanisms behind its action in OA, such as, for example, does it have other therapeutic properties? Does GlcN act in OA by affecting tissues other than the synovial joint? Can the relatively small amount of GlcN reaching the joint affect other biological pathways in OA (i.e., apart from a direct action on cartilage metabolism) that have therapeutic benefit in terms of improved pain and functional status in OA?

In the study by Laverty, *et al*[31] it is of interest to note that the synovial fluid concentrations of GlcN remained elevated in most animals even at 12 h after dosing (concentrations ranged from 0.1 to 0.7 μM). This is in contrast to the fate of GlcN observed in serum since there was nearly complete clearance of GlcN by 6 h post administration. Unfortunately, the authors did not obtain synovial fluid samples beyond 12 h post administration of the single dose of GlcN.

Based on the preliminary work by Laverty, *et al*[31] and Persiani, *et al*[32,33], one cannot exclude the possibility that the observed low sustained GlcN concentrations in the synovial joint may have biological properties that are therapeutic in OA (e.g., antiinflammatory effects and/or inhibition of IL-1 related effects)[15,20]. This possibility may be even more plausible if repeated dose administration of GlcN is considered as an approach to treatment of OA. It is obvious that further studies are needed, not only to corroborate the work of Persiani, *et al*[32,33], but also to establish the clinical relevance of the observed GlcN concentrations in the synovial joint in light of the possible mechanisms of action of GlcN in OA.

The study by Clegg, *et al*[10] (GAIT) also highlighted the relatively high placebo responses that can be observed in RCT evaluating pharmacological therapies in OA. In the GAIT study, "placebo" effectiveness was seen in up to 60% of patients as measured by the WOMAC scores. It can be argued that it may be difficult to see responses attributed to drug therapy above these scores. Although the elements that contribute to the high placebo responses have not been fully identified, it must be remembered that in blinded RCT, the full response of a true placebo is probably never seen. Historically in medicine, a true placebo would have been administered if an influential and trusted physician, who believed that the administered substance had no effect, would say to an impressionable patient something along the lines: "take this, madam, and it shall cure your pain!" Ethics committees insist that clinical trial participants must be informed of the probability of taking a "placebo" and presumably the global expectation of improvement during the RCT is accordingly reduced. However, it might still be possible to identify part of the effect of a true placebo response for a well publicized therapy that is available in the market. In an open design, one would anticipate a greater improvement in pain scores from baseline compared to well blinded and unbiased RCT. Selection of subjects for such an open design would be subject to significant bias, and one would have to begin by using large observational, randomly sampled population studies to identify GlcN use[35].

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