The Glucosamine Controversy; A Pharmacokinetic Issue

Ali Aghazadeh-Habashi, Fakhreddin Jamali

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.

ABSTRACT- Glucosamine (GlcN) is a naturally occurring aminosugar that is widely used to treat osteoarthritis despite controversial clinical trial results. Animal studies, on the other hand, unequivocally suggest anti-inflammatory and disease modifying effects for GlcN. Many explanations have been offered as to the root of the controversy. They include superiority of a crystalline sulphate salt over HCl, industry bias, insensitive assessment metrics and poor methodology. Herein, we rule out a difference in bioequivalence between GlcN salts and that of chemically equivalent doses and suggest additional factors; i.e., inconsistency in the chemical potency of some products used, under-dosing of patients as well as variable and erratic bioavailability indices for the lack of GlcN efficacy observed in some studies. Clinical trials using higher doses of pharmaceutical grade GlcN or formulations with greater bioavailability should yield positive results.

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Glucosamine (GlcN), a naturally occurring aminosugar, is widely used to treat osteoarthritis (OA). Regardless of continuous debate on its effectiveness, it is popular among patients as its global sale of >$2 billion in 2009 attests (1). Several studies suggest that GlcN modifies the symptom of OA and halts the disease progression with a favourable safety profile (2,3). Similarly, animal studies strongly suggest disease-modifying effects and anti-inflammatory properties (Table 1). However, the compound remains, perhaps, the most misunderstood therapeutic agent in use. While some reports suggest beneficial effects, other clinical trials and subsequent meta-analyses are inconclusive with their results ranging from strongly effective to negligible or no benefit to patient (4-12).

This article is presented in an attempt to shed new light onto the reasons for the controversy surrounding the issue of beneficial effects of GlcN. Since two thoughtful reviews on the topic have appeared since 2009 (13,14), we will briefly mention the source of controversy and will focus on new issues.

Evidence for and against the beneficial effects of glucosamine

In general, animal and in vitro studies have focused on the effect of GlcN on damaged joints and particularly on the site-specific beneficial effects (Table 1). Although results of such studies are often difficult to extrapolate to beneficial effects in humans, it appears that, indeed, GlcN does positively influence the biology of damaged issues. These studies ascribe anti-inflammatory properties to GlcN through inhibition of various pro-inflammatory mediators such as nitric oxide, cyclooxygenase-2 (COX-2), matrix metalloproteinases (MMP) but mainly in the context of OA (15-27). In addition to these mainly site specific studied, Hua et al (28), have reported the inhibitory effect of GlcN on the emergence of adjuvant arthritis (AA) in the rat (29). They have shown that daily administration of GlcN commencing on the day of adjuvant injection to induce AA, inhibits the emergence of the disease. AA is a type of severe arthritis that influences all joints and is associated with various systemic signs and symptoms. AA is often considered as a model for rheumatoid arthritis (RA).

Corresponding author: F. Jamali, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8; fjamali@pharmacy.ualberta.ca

Table 1. Selected studies on the effectiveness of GlcN on experimental osteoarthritis (OA) and adjuvant arthritis (AA)

Animal	Salt	Dose, mg/kg/day	Duration, weeks	Outcome	Reference
Rabbit (OA)	HCl	27	8	Detectable, site-specific, partial disease-modifying effect	(30)
Rabbit (OA)	HCl	20 and 100	8	Dose-dependent increase of glycosaminoglycan content in contralateral knee	(31)
Rabbit (OA)	HCl	100	8	Improved subchondral bone turnover, structure, and mineralization	(32)
Rat (OA)	Sulfate	250	10	Attenuates the development of OA; reduces nociception; modulates chondrocyte metabolism;	(33)
Rabbit (OA)	Sulfate or HCl	800-1000	8	Chondroprotective effect; reduced MMP-1	(34)
Rat (OA)	HCl	1000	8	Chondroprotective by inhibiting degradation and enhancing synthesis of type II collagen	(35)
Rat (AA)	HCl	300	3	Suppress progression of adjuvant arthritis	(28)

Despite the overwhelming evidence generated using experimental animals in favour of beneficial effects for GlcN in the treatment of arthritis, randomized human clinical trials are not conclusive as some have observed benefit for both pain and joint function (e.g., 2,36) and others have seen no or negligible positive effects (e.g., 37,38). Similarly, subsequent meta-analysis and systemic reviews that included the original reports were not quite in agreement (39). To the best of our knowledge, the latest meta-analysis is that of Wandel et al that has pulled together data from both GlcN alone and various combinations of GlcN and chondroitin (6). The authors did not find any beneficial effect for the treatments. They were later criticised for the study design and criteria used by two groups of authors who had conducted industry-sponsored clinical trials on GlcN with positive results (40,41).

Among the reported clinical trials a few have attracted considerable attention. They include the studies that were typically sponsored by the European producer of glucosamine crystalline sulfate (GlcN-S, Rottapharm, S.p.A., Monza Italy) that typically demonstrated positive results (e.g., 42-45). The other highly publicized trial is the independent Glucosamine/chondroitin Arthritis Intervention Trial (GAIT) (37). This study tested the effectiveness of GlcN HCl alone and in combination with chondroitin and concluded that none of the treatments were superior to placebo in pain relief but suggested beneficial effects of the combination is a pre-specified group with severe knee pain. Previously a Cochrane Collaboration review of clinical trials conducted on GlcN (39) had also concluded that "Pooled results from studies using a non-Rotta preparation or adequate allocation concealment failed to show benefit in pain and WOMAC function while those studies evaluating the Rotta preparation showed that glucosamine was superior to placebo in the treatment of pain and functional impairment resulting from symptomatic OA." WOMAC is a self-administered knee and hip osteoarthritis index.To make the matter even more complicated 2 two-year follow-up reports of a cohort of patients enlisted in GAIT have detected no significant difference between the groups treated with GlcN HCl, celecoxib or placebo (8,46). This is when other studies have suggested effectiveness for celecoxib in the treatment of OA (47). The observed lack of superiority over placebo of two years of treatment with GlcN and celecoxib is attributed to a low baseline level of pain that can render the treatment effect difficult to assess, and also as the placebo group demonstrated improvement, to the very high expectation bias on the part of 2 years of treatment (46). The authors suggest re-evaluation of the assessment factors involved is designing future OA trials. Interestingly, despite the publicity around the negative results of the GAIT study, GlcN has maintained its popularity among OA patients (1,48).

Potential Sources of controversy

Inconsistency of commercial products and its consequence on clinical trial outcomes

We have previously shown that, at least for the Canadian products, 13 out of 14 tested formulations contained substantially lower than label claims of the active ingredient (Table 2) (49). This is mainly due to the physical instability of GlcN crystals that is

overcome with co-crystallization with KCl. The crystals of the active ingredient, therefore, are diluted with KCl. Except for the European Union, where GlcN is regulated as a pharmaceutical, the quality control of the commercially available products of GlcN has been a prerogative of the manufacturer, hence, it might have used the diluted crystals without allowance given for the co-crystalization.

A typical clinical trial report has either no mention of the validity of the label-claim of the product used or contains only a statement conveying the manufacturer's claim with no assurance indicating an actual dose-potency measurement. While the formulation used in the GAIT study (37) is reported to be tested for its active ingredient content, for the studies included in the meta-analysis of Wandel et al (6) assurances from the manufacturers appear to be deemed sufficient as a measure of the products chemical potency (e.g., 50,51). It is, therefore, reasonable to suggest that the patients in some of the reported clinical trials may have been under-dosed.

It is important to note that the results of some of the GlcN clinical trials with negative outcomes do not totally rule out potential benefits or trend toward efficacy of the treatment in some patients (8,37,46,51). This, coupled with the possibility of under-dosing with the less than claimed 1500 mg/day regimens, highlight the need for clinical trials using higher pharmaceutical grade GlcN doses or formulations that yield greater plasma concentrations.

Dose-effect relationship and bioequivalence

Almost all of the human clinical trials are carried out with a dosage regimen of 1500/day. Interestingly, at certain stage, investigators should have known that such a dosage regimen was going to yield concentration substantially lower than those used in in vitro or in animal studies (13,27,52). Also, the positive trend toward GlcN's beneficial effect reported by several authors (8,37,46, 51) should have alarmed them of the possibility of under-dosing. Nevertheless, to the best of our knowledge, no attempt was made to test a higher dosage regimen even by the investigators involved in the GAIT study who used a pharmaceutical grade formulation. The physical size of glucosamine products (e.g., 1.4 g for a 500 mg tablet) might have been, at least in part, a deterrent for using higher dose levels.

It is well-known that most reported animal data on the pharmacological properties of GlcN are generated following high doses. However, the minimum effective dose, hence, minimum effective concentration of GlcN in animals has remained unknown. Consequently, the gap between plasma GlcN concentration in human and that associated with effectiveness in experimental animals is unknown. The available human pharmacokinetic data demonstrate great inter-study variations (Table 3). The reported peak concentration following a 1500 mg dose ranges from 0.492 to 3.36 mg/L; i.e., a 6-fold

Table 2. Content of glucosamine (mg/capsule or tablet) in commercially available products in Canada

Preparation	Capsule or Tablet	Labeled Content, mg	Sulfate or HCl	GlcN, mg	GlcN, mg Sulfate equivalent	% of Stated Amount (as base)
1	T	500	S + C	542	688	108
2	C	500	S	409	519	82
3	C	500	S	277	351	55
4	C	500	S	325	445	65
5	C	500	S	330	419	66
6	C	500	S	248	315	50
7	T	1500	S	634	804	42
8	C	500	S	233	295	41
9	C	500	S	298	378	60
10	C	500	S	231	293	46
11	C	500	S	274	348	55
12	C	500	S	238	302	48
13	C	300	S	169	214	56
14	C	500	S	262	332	52

Adopted from Ref (49).

Table 3. GlcN pharmacokinetic data generated from humans

Dose, mg	Cmax		AUC_{0-t}	$AUC_{0-\infty}$	Reference
	mg/L	μM	mg.h/L		
1500	3.11±2.2	17.4±11.96	9.85±4.1 (8 h)[f]	10.3±4.1	(53)
500[a]	1.11±0.51	6.2±2.85	5.25±2.16 (14 h)[f]	5.31±2.16	(54)
500[a]	3.36[b]	19.0[b]	NR	19.7[b]	(55)
1500	1.60±0.42	8.95±2.37	20.22±5.02 (48 h)[f]	14.6±4.14	(56)
1500[c]	0.492±0.16	2.75±0.9	NR	2.38±0.94	(57)
1500	0.90±0.43[d]	5.04±2.4[d]	NR	NR	(52)

a, indices were calculated for 1500 mg doses; b, geometric means, hence, no SD; all sulphate except 'c'; all value at steady state except 'd'; f, the value in bracket indicate the time for the last measured concentration; NR, not reported.

difference between the highest (GlcN crystalline sulphate) and the lowest (GlcN HCl as used in GAIT). This brings up an important question: Is the source of the discrepancy between the outcomes of various clinical trials a difference in GlcN bioavailability from different formulations?

Although the values listed in Table 3 are from different studies, hence, are not generated according to a cross-over or simultaneous parallel fashion, the fact that the human exposure to GlcN following ingestion of the HCL salt used in the GAIT study (57) is so much lower than that of the sulphate salt (Table 3) raises a good question that we cannot address. It is intuitively accepted that following ingestion and subsequent dissolution in the gut, both sulfate and HCl salts of GlcN are immediately ionized to glucosamine, hence, the nature of the salt becomes irrelevant. If so, the differences between the two products should be at the level of formulation and not the nature of the salt.

To assess the effect of the salt nature on the bioavailability of GlcN, we present a preliminary set of data generated following a random cross-over oral gavage to the of equal doses (equivalent to 100 mg/kg GlcN base grinded and suspended in PEG 400) of HCl (Sigma-Aldrich Canada, LTD, Oakville, ON) as used in the GIAT trial [Personal communication with J.D. Sandy(57)] or the crystalline sulfate (Dona, Lot No. PR 24080004, RottaPhram, Monza, Italy, purchased from a community pharmacy in Florence, Italy). We have previously reported the dosing and sampling methods (58,59) as well as the assay (59) used.

No significant difference in bioavailability indices was observed between the two formulations (Fig 1, Table 4). This cross-over assessment unequivocally proves that the nature of the salt does not influence the bioavailability of GlcN administered orally.

The rat data presented herein confirm a previous observation that, in horses, GlcN HCl and sulfate are bioequivalent (60) but does not rule out the effect of formulation *per se*. We therefore, designed a brief open-label cross-over bioavailability study in healthy volunteers to directly compare GlcN-HCl as used in the GAIT study with a Rottapharm product. We used the urinary excretion data for comparison as, despite the limited excretion of intact GlcN, it has been found to be a reliable and less variable measure of the pharmacokinetic indices of GlcN (61).
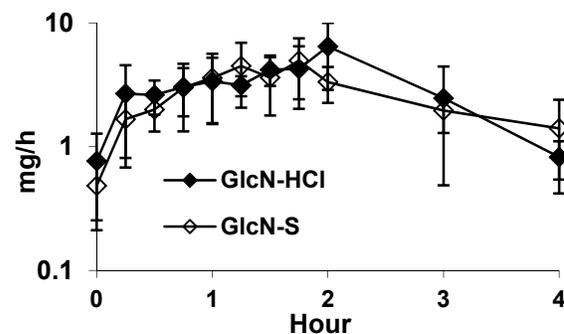

Figure 1. Glucosamine plasma concentration vs time after oral administration of 100 mg/kg as HCl or sulfate salts into the rat. GlcN plasma concentrations were measured according to a previously described method (59). Formulations were grinded and suspended in polyethylene glycol 400 before administration. Their potency measured by HPLC (62) was 99.9% for the HCl and 95.2% for the sulfate. Concentrations in zero h samples demonstrate endogenous GlcN. Male Sprague-Dawley rats (300-360 g; n=5/group) were dosed through a gastric gavage after an overnight food deprivation but free access to water. Blood samples were collected over a 4 h post-dose period, plasma separated and stored at -20° C until analyzed for GlcN.

Table 4. GlcN Pharmacokinetic indices following cross-over oral administration of single 100 mg doses of the compound as HCl or crystalline sulfate to 5 rats.

Parameters	GlcN-HCl	GlcN-S
t_{max}, h	1.50±0.73	1.30±0.33
C_{max}, mg/L	7.49±2.76	7.92±1.84
90% Confident interval of geometric means, %		94.9 (80.9%-109.0)
AUC_t mg.h/.L	13.59±3.64	10.12±2.54
90% Confident interval of geometric means, %		112.4 (100.4%-124.4)
Data are presented as mean±SD (n=5/group).		

Four healthy volunteers (1female and 3 males, 47±12.5 yr, 81.8 ±11.6 kg, 177±7 cm) took 1500 mg GlcN crystalline sulphate (Dona, 250 mg tablets Lot No. PR 24080004, RottaPhram, Monza, Italy) or its equivalent of GlcN HCl (Sigma-Aldrich Canada, LTD, Oakville, ON) dispensed in capsules as used in GIAT. They took the formulations after an overnight fast with 250 mL water in a random fashion with a 2 week washout period. GlcN was measured in total urine output and the total and rate of excretion measured for 13 h post-dose using HPLC (59). No significant differences were found between the two products in either urinary excretion rate plots (Figure 2) or in the total amount excreted (Table 5). This cross-over study suggests that the formulations used in the GAIT study and those reported for GlcN crystalline sulfate are bioequivalent and rules out the effect the effect of formulation differences between the two products.

Our data are preliminary and, perhaps, need to be confirmed with a more details study. Nevertheless, the rat and human data on the bioequivalence of GlcN salts and formulations strongly suggest that the HCl formulation used in GAIT and the commercially available Rottapharm tablets, indeed, yield equal body exposure to the compound. The discrepancy in the reported pharmacokinetic indices in general and the relatively low concentration of the formulation used in the GAIT study in particular, needs further attention.

It is known that inflammatory conditions may inhibit clearance of drugs that efficiently undergo hepatic metabolism (i.e., first pass effect) (64-66). There are two reasons to rule out this possibility for the present case: i) there is no evidence of efficient hepatic metabolism for GlcN as its low bioavailability appears to be due to its loss in the gut (58) and ii) except for one (52), all studies have been carried out in normal volunteers indicating a great variation in healthy state.
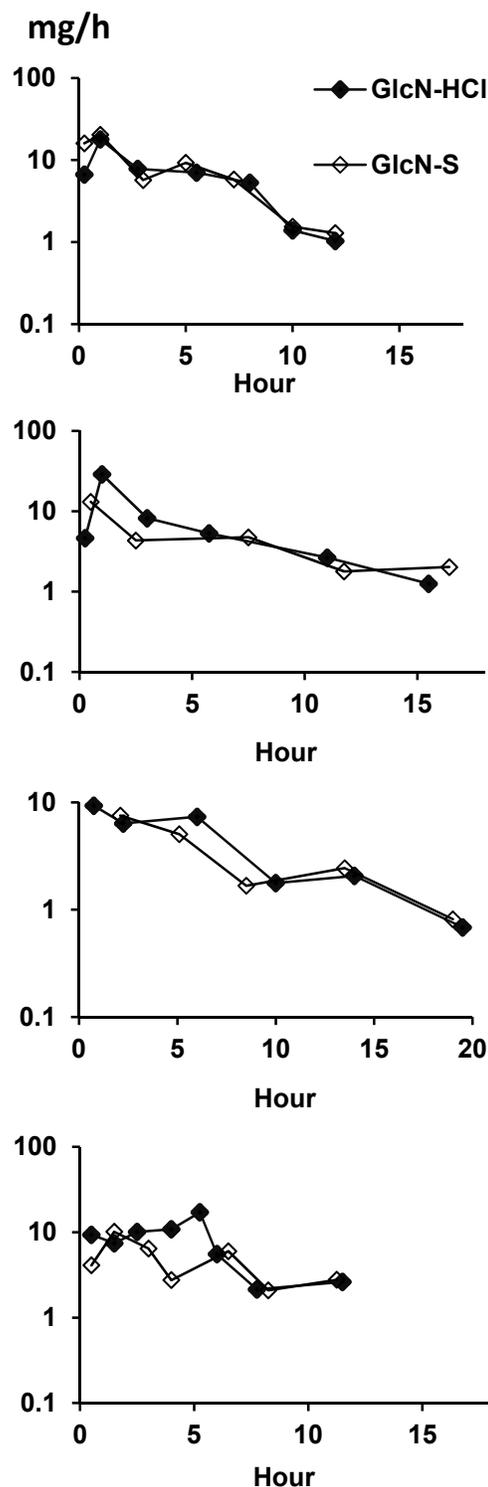

Figure 2. Glucosamine urinary excretion rate vs mid time point of urine collection period following single oral dose of 1500 mg GlcN crystalline sulfate or its equivalent HCl salt to humans. Each graph represents one individual.

Table 5. Individual subject pharmacokinetic indices of GlcN in urine after an oral dose of 1500 mg GlcN crystalline sulfate or its equivalent HCl salt in human

	GlcN excreted in 13 h					
Compound:	GlcN-HCl			GlcN sulfate		
Subjects	$t_{1/2}$, h	mg/13 h	% dose[a]	$t_{1/2}$, h	mg/13 h	% dose[a]
1	1.69	37.2	3.10	2.12	38.8	3.23
2	4.71	40.9	3.41	7.37	30.3	2.53
3	5.87	36.0	3.00	8.35	25.3	2.11
4	6.54	37.7	3.14	5.17	22.2	1.85
Mean	4.70	38.0	3.16	5.75	29.2	2.43
SD	2.14	2.12	0.18	2.76	7.23	0.60
90% Confident interval of geometric means, %				108.7 (103.1-114.3)		

a, calculated based on 26% absolute bioavailability of radiolabelled GlcN (63)

Some authors have suggested industry bias as one of the sources of the differences between the two sides of the debate (12). Such a notion, however, does not address the issue of plasma concentration differences between the two formulations. Among the sets of data reported thus far, those of Jackson et al (i.e., the HCl formulation used in GAIT) stands out for its substantially lower peak plasma concentration (0.49 mg/L). Since other studies with peak concentrations range of 0.9 to 3.36 mg/L are not all sponsored by a single industry source, it is reasonable to rule out the possibility of an industry bias for the reported pharmacokinetic data. Hence, the question remains as to the reason for such a low bioavailability for the GAIT formulation and the possibility of a link between the negative beneficial effects of the latter. This is particularly important since several authors have suggested a concentration dependent-effect for GlcN (13,27). For example, it has been suggested that for the long-term protection of cartilage to stimulated aggrecan loss in osteoarthritis continual presence of GlcN in plasma is required (67).

Based on in vitro and animal data, several investigators have pointed out the need for higher concentration in human studies to reach therapeutic levels (13,14,27,68). Repeated 300 mg/kg dose of GlcN to the rat completely inhibit emergence of adjuvant arthritis (28). Such a regimen should yield a peak plasma concentration of approximately 16 mg/L [Aghazedeh et al (58) reported a peak concentration of 18.8 mg/L following a single 350 mg/kg dose] which is much greater than those reported following 1500 mg/kg doses to humans (Table 3). The 300 mg/kg regimen, however, is not necessarily the minimum effect dose (Table 1). This should prompt investigators to assess GlcN efficacy following higher doses of GlcN or formulations with improved bioavailability.

Therapeutic outcome measurements

In assessing the effectiveness of GlcN in OA, double blind placebo-controlled methods are used. The outcome measurement includes various measures such as WOMAC arthritis index which is a self-administered knee and hip assessment, various pain scales, radiographic techniques and joint space narrowing. The sensitivity of these methods to differentiate between treatments may be questioned. Indeed, some investigators who were following GlcN effects in a sub group of patients enrolled in the GAIT study have not even been able to differentiate between a non-controversial treatment (celecoxib) (46) and placebo so that they suggested a re-evaluation of the assessment factors involved is designing future OA trials. Interestingly, these authors attribute the observed lack of superiority over placebo of the two year treatment with GlcN and celecoxib to the possibility of a low baseline level of pain and a placebo effect due to a very high expectation bias on the part of 2 years of treatment (46). If so, the same must be applied to other clinical trials as well and, indeed, gives more credence to the issue of heterogeneity across studies (12). With such assessment difficulties in place, the possibility of detecting moderate beneficial effects on mild to moderate OA is expected to be remote. This is, perhaps, why patients ignore the results of the scientific studies and continue using GlcN. It may be that some but not all patients do benefit from the treatment.

CONCLUSION

GlcN has anti-inflammatory properties that are evident only upon administration to experimental animals of high doses or, perhaps, after dosing with formulations with high bioavailability. The discrepancy between the reported human clinical data is not due the nature of the salt or formulation properties. Regardless of the formulation used, following the commonly used 1500 mg/day doses, no or marginal beneficial effects may be observed because of under-dosing which stems from low GlcN bioavailability and inconsistency in chemical potency of some commercially available products. Limited and erratic bioavailability of GlcN may also contribute to the problem. In addition, insensitive clinical outcomes and inclusion of patients with low baseline pain might have contributed to the unsatisfactory treatment outcome.

The source of the controversy in the efficacy of GlcN seems to be pharmacokinetic in nature as it is generally agreed that the available GlcN formulations yield sub-therapeutic plasma concentrations. At this stage there is an obvious need to determine the minimum effective GlcN dose and/or concentration and conduct clinical trials following higher doses of GlcN or formulations with improved bioavailability.

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