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THE HUMAN PHARMACOKINETICS OF ORAL INGESTION OF GLUCOSAMINE AND CHONDROITIN SULFATE TAKEN SEPARATELY OR IN COMBINATION

Christopher G. Jackson, M.D[#,1], **Anna H. Plaas, Ph.D**[$,1], **John D. Sandy, Ph.D**[$], **Crystal Hua, MS**[@], **Samantha Kim-Rolands, M.D**[@], **Jamie G. Barnhill, Ph.D**[*,%], **Crystal L. Harris, Pharm.D**[*], and **Daniel O. Clegg, MD**[#]

[#] University of Utah School of Medicine, Salt Lake City, UT

[$] Rush University Medical Center, Chicago IL

[@] Dept. of Internal Medicine, College of Medicine, University of South Florida, Tampa, FL

[*] Albuquerque VA Cooperative Studies Program Clinical Research Pharmacy Coordinating Center, Albuquerque, NM

[%] University of New Mexico College of Pharmacy, Albuquerque, NM

Abstract

Objective—As part of the NIH-sponsored Glucosamine/Chondroitin Sulfate Arthritis Intervention Trial (GAIT) our objective here was to examine 1) the pharmacokinetics (PK) of glucosamine (GlcN) and chondroitin sulfate (CS) when taken separately or in combination as a single dose in normal individuals (n=29) and 2) the PK of GlcN and CS when taken as a single dose after 3 months daily dosing with GlcN, CS or GlcN+CS, in patients with symptomatic knee pain (n=28).

Methods—The concentration of GlcN in the circulation was determined by established fluorophore-assisted carbohydrate electrophoresis (FACE) methods. The hydrodynamic size and disaccharide composition of CS chains in the circulation and dosage samples was determined by Superose 6 chromatography and FACE.

Results—We show that circulating levels of CS in human plasma are about 20ug/ml. Most significantly, the endogenous concentration and CS disaccharide composition were not detectably altered by ingestion of CS, when the CS was taken alone or in combination with GlcN. On the other hand, the Cmax (single dose study) and AUC values (multiple dose study) for ingested GlcN were significantly reduced by combination dosing with CS, relative to GlcN dosing alone.

Conclusions—We conclude that pain relief perceived following ingestion of CS probably does not depend on simultaneous or prior intake of GlcN. Further, such effects on joint pain, if present, probably do not result from ingested CS reaching the joint space but may result from changes in cellular activities in the gut lining or in the liver, where concentrations of ingested CS, or its breakdown products, could be substantially elevated following oral ingestion. Moreover, since

Corresponding Author: John D. Sandy.
[1]Drs. Christopher Jackson and Anna Plaas contributed equally to this study.

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combined dosing of GlcN with CS was found to reduce the plasma levels seen with GlcN dosing alone, any improved pain relief by combination dosing cannot be explained by higher circulating concentrations of GlcN.

INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis in the United States and is projected to double in prevalence within the next two decades[1]. Its pathogenesis remains unknown but is currently thought to be a complex interaction of biologic (inflammation, fibrosis) and mechanical processes resulting in failure of the articular cartilage[2]. The potential for true disease modification is uncertain and limits the present rationale for pharmacologic intervention in the management of OA to relief of symptoms[3]. The use of acetaminophen, alone or in combination with a nonsteroidal anti-inflammatory drug, is recommended as initial therapy but the utility of these traditional agents is limited due to marginal efficacy and/or toxicity[4]. There is considerable interest in GlcN and CS in treating OA but no consensus exists as to the proper role of these nutriceuticals as existing clinical studies have yielded disparate results, perhaps due in part to the use of different formulations of these agents[5]. Further, the daily dosages and dosing regimens employed have been largely empiric because of scant pharmacologic information. The recently completed Glucosamine/chondroitin Arthritis Intervention Trial (GAIT) was a 24-week, NIH-sponsored, double blind, placebo controlled parallel trial comparing GlcN.HCl, 500 mg (3 per day) with CS, 400 mg (3 per day) alone and in combination, with celecoxib 200 mg daily and placebo as positive and negative controls, respectively. We found that no regimen (GlcN alone, CS alone or the two combined) was superior to placebo in pain relief but benefit from the combination was suggested in a pre-specified subset having more severe knee pain. Radiographic evidence of disease modification was not observed[6]. Herein we report the single-dose and steady-state (multiple-dose) pharmacokinetics of the orally-administered capsule dosage forms containing GlcN.HCl, CS, and capsules containing both agents which were utilized in the GAIT.

METHODS

Patient populations and study designs

This investigation was conducted in three phases as follows: In Phase 1, the presence and diurnal variation of endogenous plasma levels of GlcN and CS were determined by obtaining blood samples from 14 naïve subjects. Following an overnight fast, samples were obtained at 0800 and 2, 4, 8, and 24 hours thereafter. The demographic characteristics for this group are shown in Table 1. In Phase 2, the single-dose PK of GlcN and CS were determined from concentration-time data obtained from 29 normal human subjects who were randomized to receive either 1500 mg of GlcN.HCl (6 capsules containing 250 mg each;8 subjects), 1200 mg of CS (6 capsules containing 200 mg each;10 subjects), or the combination of GlcN and CS (11 subjects). Following an overnight fast, the study medication was ingested at 08:00 and blood samples were obtained at 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, and 36 h following ingestion. The demographics for this group are shown in Table 3. In Phase 3, the PK of GlcN and CS were determined from concentration-time data in 28 subjects, age 40 and older with symptomatic knee pain. The plasma samples were obtained over a 36h interval following single dosage of GlcN or CS, which was taken following 3 months of daily ingestion of either GlcN (500 mg, 3 times per day,9 subjects), CS (400 mg, 3 times per day, 9 subjects) or the combination (10 subjects). At the end of the 3 months and following an overnight fast, the medication (either GlcN or CS) was ingested at 08:00 and blood samples were obtained at 0, 1, 1.5, 2, 4, 6, 8, 12, 24, and 36 hours following ingestion. The demographics for this group are shown in Table 5.

Analytical methods for quantitation of plasma GlcN and CS

The concentration of GlcN in all phases of this study was determined by a fluorescence-assisted carbohydrate electrophoresis (FACE) method used previously to measure GlcN in horse serum [7]. While our method[7,8] and a mass spectrometric method [9] were unable to detect endogenous glucosamine in the circulation (limit of detection about 10ng/ml), others have reported endogenous concentrations up to about 50ng/ml in both humans[10] and horses[11]. For CS, blood was collected into heparinized tubes and plasma samples (1ml portions) were delipidated with LiposorbTM (Calbiochem) and deproteinized by digestion with proteinase K. After boiling for 5 min, insoluble material was removed by centrifugation. Clarified supernatants (containing all CS components) were mixed with 10,000 cpm 3H glucose, prior to fractionation on Superose 6 (HR30/30) at 0.5ml/min in 100mM ammonium bicarbonate. Fractions collected at the total volume of each run were analysed for 3H-glucose content and the precise % recovery (generally 75–80%) was taken as the recovery of all CS components and was incorporated into the final calculation of plasma contents. The CS assay was also validated by "spiking" normal plasma samples with CS (in the form used in oral dosages) and determining the percent recovery through the assay procedure. This was routinely greater than 75%.

For total CS quantitation, samples eluted in fractions 9–19 (see Fig. 1 for analysis of individual fractions by FACE) were pooled, speedvac dried, residues washed twice by resuspension in water and drying. Final residues were digested in 0.5 ml of 0.1 M ammonium acetate, pH 7.3 and digested with Chondroitinase ABC (0.025 units) for 16h at 37 degrees. Dried samples were fluoro-tagged with 5ul of AMAC, separated on acrylamide gels (29.3% separating and 5% stacking) and the fluorescent products were imaged using a Kodak 1D Scientific Imaging System at 0.01s, 0.1s, 0.25s, 0.5s, 0.75s and 1.5s exposures on a UVP High Performance UV Transilluminator. Band intensities were converted into concentrations based on fluorotagged disaccharide standards as published[8]. The plasma concentration – time data were analyzed by standard methods to determine the human PK of GlcN [7]. Statistical comparisons were made between groups within Phase 2 and 3 using Student's t-test with statistical significance determined at $p \leq 0.05$.

RESULTS

Analysis of CS in human plasma

The concentration of CS in mammalian plasmas, including human, have been widely reported at 5–20 ug/ml [12,13]. Because of this, determining the plasma concentration of ingested CS required analysis of both the structure (polymer size, disaccharide isomer composition) and the total concentration of CS (as disaccharides) in the pre-dose and post-dose plasma samples. For this purpose, we developed a protocol (see Methods for detail) which includes size fractionation (Superose 6) of the CS in proteinase K-digested plasma, followed by FACE determination of the CS disaccharide composition (Δdi0S,Δdi4S, Δdi6S) and abundance. Importantly, the Superose 6 step removes essentially all the plasma glucose which at about 4 mg/ml interferes with the FACE quantitation of the low abundance CS disaccharides.

FACE analyses is shown for the plasma CS in the Superose fractions (9–19) derived from a typical naive patient (Fig. 1A) and a normal patient, 3h after a single 1200mg dose of CS alone (Fig. 1B). For both individuals the Superose 6 resolved the CS chains present in proteinase K-digested plasma samples into two populations. A very minor high molecular weight CS chain population, composed of Δdi4S only, eluted between fractions 10–13; the majority of plasma CS however was in a lower molecular weight form (fractions 15–20), and these chains were composed of ~60% Δdi0S, ~30% Δdi4S and ~ 10% Δdi6S. The relative abundance (pixel density per 0.25 sec exposure) of each disaccharide in fractions 8–19 from the naive patient

(Fig. 2A), and the single CS dosage patient (Fig. 2B), is given along with the same fractionation and FACE analysis of the CS used in the oral dosing (Fig 2C). This shows that a single CS dose resulted in no detectable change in either the hydrodynamic size or disaccharide composition of the plasma CS, 3h after dosing. Indeed the same result was found for plasma samples taken at all time periods after dosing from 0.25h to 36h. Moreover, the hydrodynamic size profile and the disaccharide composition (~ 45% Δdi4S, ~ 45% Δdi6S, ~10% Δdi0S) of the CS used for dosing (Fig. 2C) were both distinct from the CS chains recovered in the post-hepatic circulation under all oral dosing regimes employed in this study.

Pharmacokinetic data for plasma GlcN and CS in subjects from each dosing study

Baseline plasma levels of GlcN and CS were determined at five times throughout a single day (Table 2). GlcN was undetectable at every time point whereas the level of CS was approximately 19ug/ml with no apparent diurnal fluctuation. To examine the possible effect of combined dosing on the PK for GlcN, the time-concentration profile for a single dose of GlcN taken alone or in combination with CS was obtained (Fig. 3). This revealed no major effect of combined dosing. The overall PK data (mean +/− SD) for eight individuals taking GlcN alone and eleven individuals taking the combination dosage is provided in Table 4. The Cmax for GlcN when dosed alone (about 490 ng/ml), was significantly ($p<0.05$) higher than that observed with combined dosing (about 310 ng/ml), however there was no significant difference in any of the other PK parameters determined. The possible cumulative effect of 3 months of pre-dosing with GlcN or GlcN+CS, on the PK of GlcN was also examined. It was found that the concentration profile for plasma GlcN was essentially identical for the two predosing schedules (Fig. 4). The overall PK data (mean +/− SD) for a single dose of GlcN after 3 months predosing with GlcN or GlcN+ CS is provided in Table 6. It was found that the AUC for GlcN was significantly lower for the combination predosage relative to predosing with GlcN alone, however there were no significant differences in the other PK parameters determined for these two groups.

The possible effect of combined dosing on the concentration profile for plasma CS was studied next (Fig. 5). It was found that the plasma concentration of CS for 24h following a single dose of CS (1200 mg), taken alone or in combination with 1500 mg of GlcN, was not affected by the combined dosing. In both cases the CS concentration was not detectably different from baseline levels. Finally, the possible cumulative effect of 3 months of pre-dosing with GlcN,CS or GlcN+CS, on the plasma levels after a single 1200mg dose of CS was also examined (Fig 6). While there appeared to be a trend toward higher CS concentrations following predosing with GlcN +CS, these values were not significantly different from the other groups assayed.

DISCUSSION

It has been suggested, without extensive data support, that the effectiveness of oral GlcN or CS in providing pain relief from OA might depend on combined and/or long-term dosing [6, 14–17]. This idea implies that there is likely to be a synergistic effect on absorption, pharmacokinetics or cell biological activity for these two agents when taken orally over extended periods. In this study with human patients from the GAIT study, we have examined the possibility that combined short or long-term dosing might alter the pharmacokinetic profile for these two agents.

We found no evidence for absorption of oral CS into the circulation under any dosing regimen

We have been unable to detect any of the dietary CS in the circulation under any dosing condition used; these conditions involved both long-term (3 months) and acute dosing, both alone and in combination with GlcN. The sensitivity of the FACE analysis used for CS disaccharide analysis is sufficient to detect product at about 10 ng/ml. Therefore, if the

absorption of CS into the circulation was similar to that which has been established for the same dose of GlcN, the Cmax for plasma CS (or its disaccharide and larger breakdown products) would be at about 200 ng/ml. However, our compositional analysis of the plasma at multiple time points up to 24h after dosing, showed no change in the content of C6S disaccharide, which represents about 45% of the dosage compound but less than 10% of the endogenous CS (see Fig. 1). We therefore conclude that little, if any, of the ingested CS reaches the circulation in a form which is unchanged or composed of disaccharides or larger fragments. On the other hand, our quantitative determination would likely not detect a 200ng/ml change in total CS since, in keeping with others[13,18] we found the endogenous CS concentration to be about 20ug/ml and this baseline can vary in an individual by about 5ug/ml throughout the day (see Fig. 6). In this regard, it is likely that the major source for plasma CS is the circulating serine proteinase inhibitor, bikunin-CS, which has been measured at about 11ug/ml in human plasma[19]. Indeed, our finding that the disaccharide composition of CS in baseline human plasma samples was largely OS and 4S disaccharides is consistent with a major contribution from bikunin-CS[20]. Other potential sources of plasma CS sulfate are fibroblast and/or chondrocyte CS-proteoglycans, such as versican and aggrecan, released from the tissue into the plasma as part of normal turnover processes

The Cmax for GlcN was higher when the GlcN was taken alone compared to combination dosing with CS

In the present study the Cmax values for GlcN taken alone (492 ± 160 ng/ml) were significantly higher than when the GlcN was taken with CS (311 ± 103 ng/ml). Further, when GlcN was taken alone in multiple doses, the AUC values for a single dose (1,870 ± 638 ng·hr/mL) were significantly higher than when the GlcN was taken in multiple doses with CS (1,099.0 ±466.0 ng·hr/mL) (Table 4). These findings suggest that including CS with GlcN ingestion interferes with GlcN absorption into the circulation, which occurs via the glucose transporter system and involves both SGLT1 and GLUT-2.[21] Such an inhibitory effect supports the notion that dietary CS impacts the metabolism of gut lining cells and in this regard it would be interesting to determine whether it can inhibit either of the glucose transporters responsible for G absorption.

Summary

The data provided suggest that the variable pain relief apparently experienced by OA patients following ingestion of GlcN, CS or the two in combination cannot be readily explained by synergistic effects of the two agents on intestinal absorption. This follows from the finding that absorption of dietary CS is undetectable whether it is taken alone or with GlcN, and absorption of GlcN appears to be inhibited by combined dosing with CS. Further research directed towards understanding the possible indirect effects of these agents[22] on joint health appears to represent the most productive way forward at present[23].

Acknowledgments

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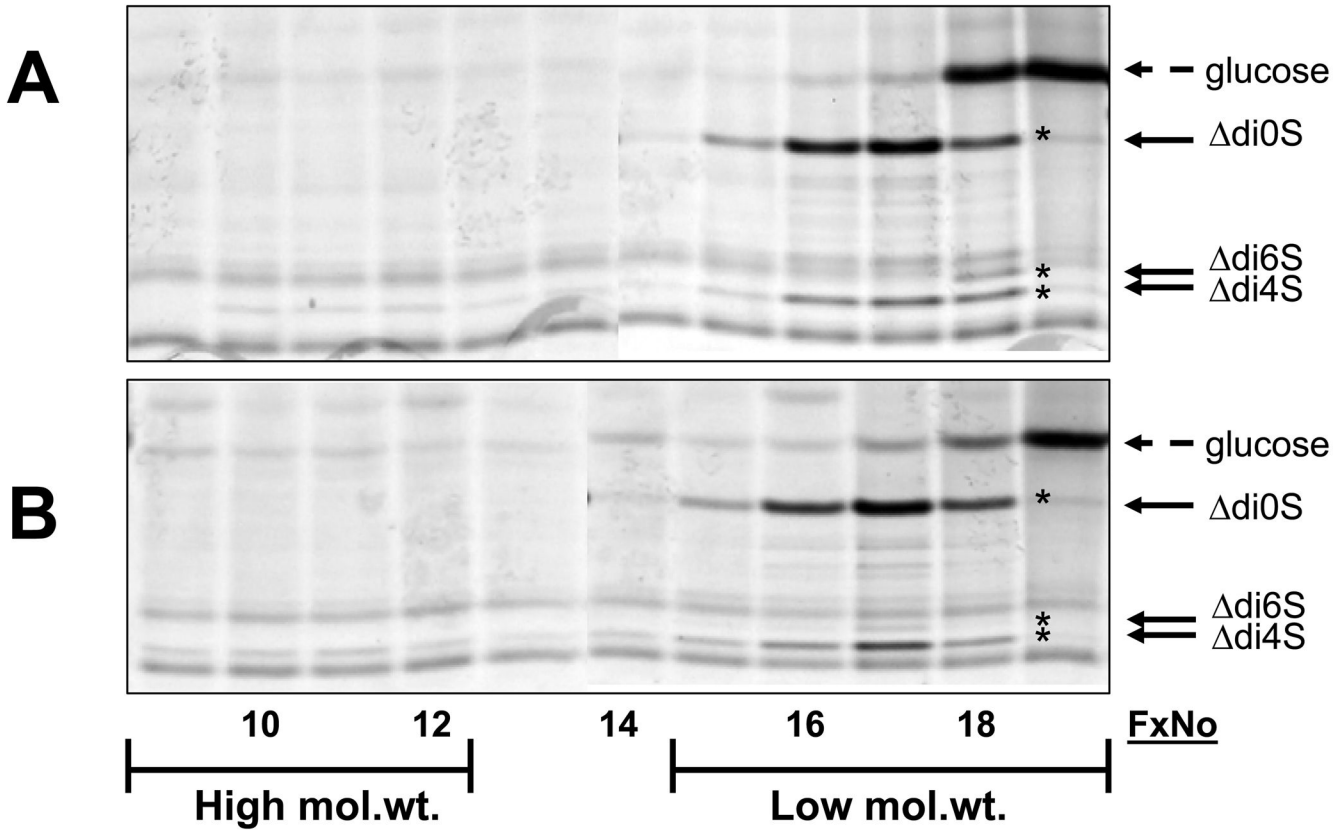

Figure 1. FACE analyses of the CS in fractions from Superose 6 chromatography

Disaccharide analysis is shown for Superose fractions (9–19) derived from 1 ml of proteinase K-digested plasma from a typical naive patient (A) and a normal patient after a single dosage of CS alone (B). The naive patient was from the Phase 1 study and the single CS dosage from Phase 2.

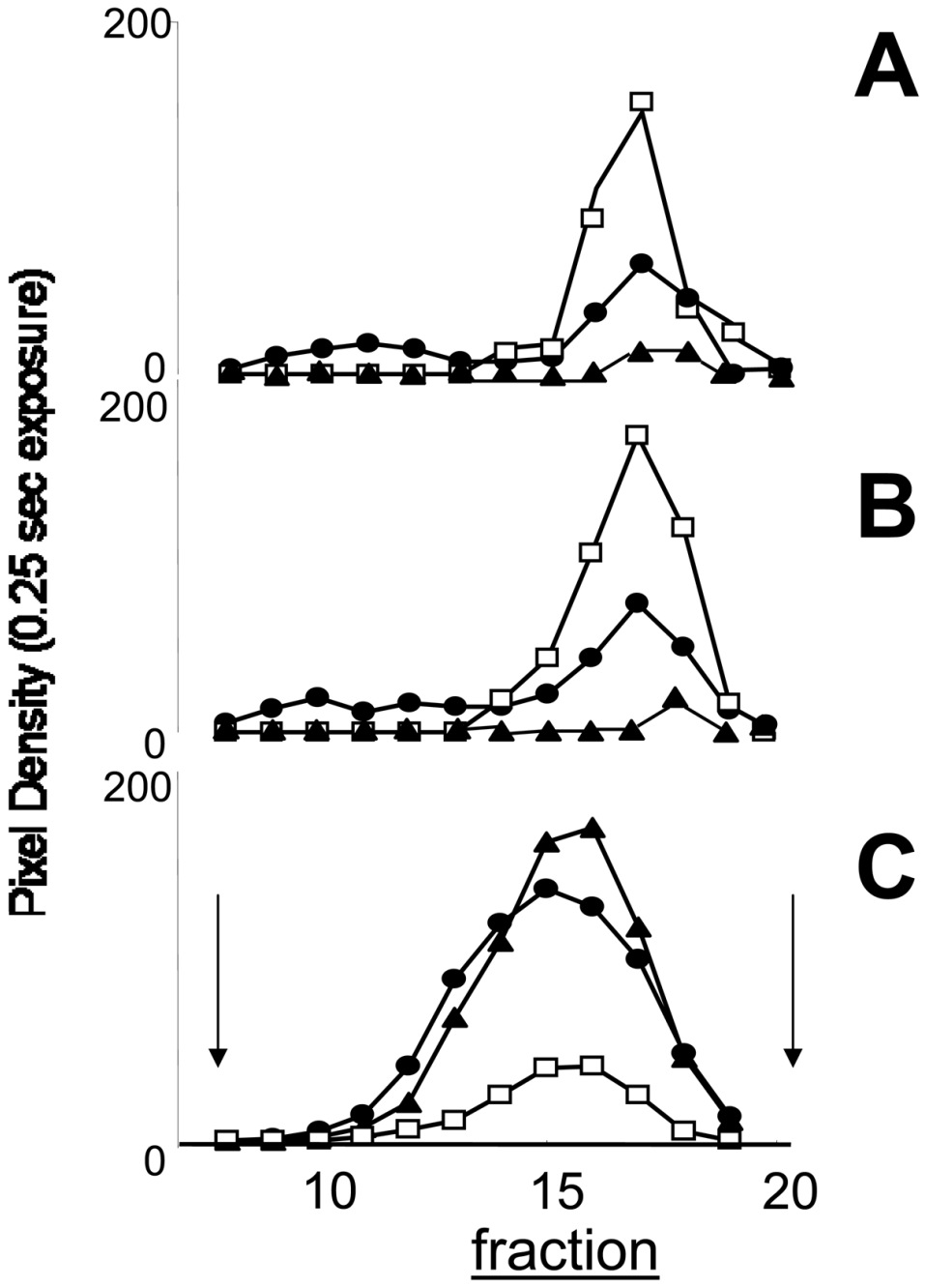

Figure 2. Quantitation of CS disaccharides in Superose 6 fractions

Abundance of CS (as disaccharides) in the Superose fractions (8–19) derived from 1 ml of proteinase K-digested plasma from a typical naive patient (A) and a normal patient after a single dosage of CS alone (B). The naive patient was from the Phase 1 study and the single CS dosage from Phase 2. Also shown (C) is the same analysis of the CS used for oral dosing.

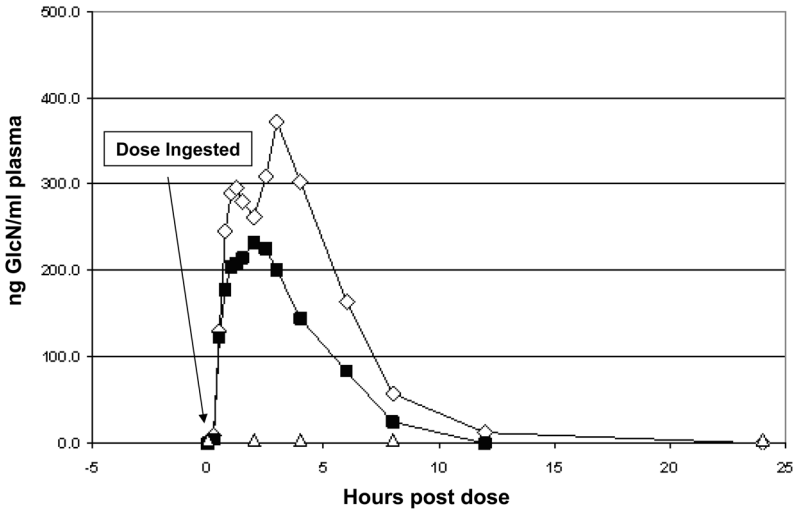

Figure 3. The effect of combined dosing on the concentration profile for plasma glucosamine in a normal individual

The plasma concentration of glucosamine following a single dose of GlcN (1500 mg) taken alone (open diamonds) or in combination with 1200mg CS (closed squares) is shown. Without dosing the plasma GlcN was undetectable (open triangles).

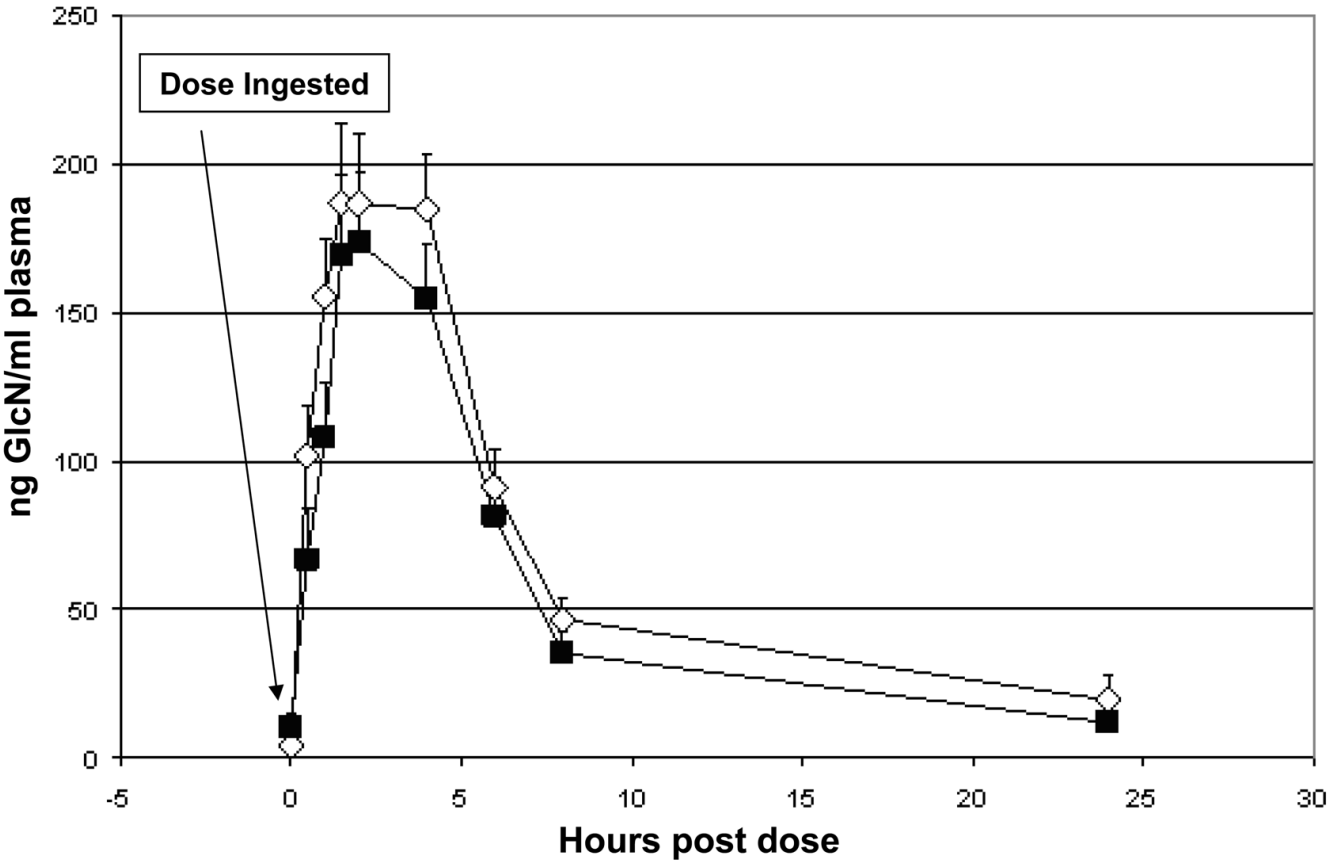

Figure 4. The effect of predosing on the concentration profile for a single dose of GlcN

The plasma concentration of GlcN following a single dose of GlcN (1500mg) after three months of predosing with GlcN (open diamonds) or GlcN+CS (closed squares).

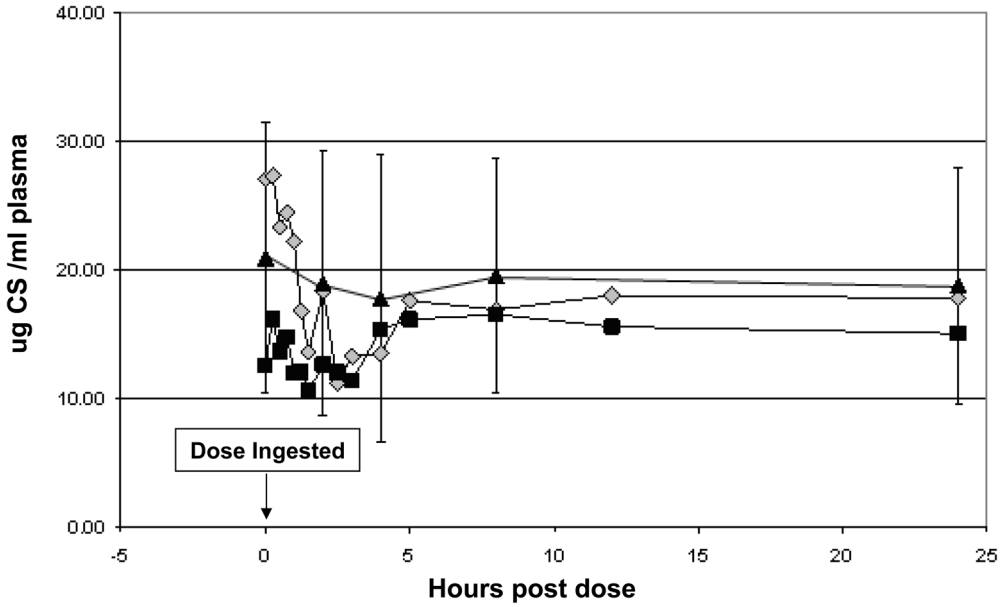

Figure 5. The effect of combined dosing on the concentration profile for plasma CS

The plasma concentration of CS following a single dose of CS (1200 mg) taken alone (filled diamonds) or in combination with 1500 mg of GlcN (closed square) is shown. Also shown is control data without CS dosing (closed triangles).

Figure 6. The effect of predosing on the concentration profile for a single dose of CS

The plasma concentration of CS following a single dose of CS (1200mg) after three months of predosing with GlcN (open squares), CS (closed diamonds) or GlcN+CS (closed squares). Also shown (closed triangles) is the control data without CS dosing.

Table 1

Demographic characteristics of Phase 1 subjects (Mean±SD).

Number of Subjects	14
Weight (kg)	84.2 ± 20.6
Height (cm)	172 ± 17.2
Age (years)	41.4 ± 17.9
Gender (male/female)	7/7

Table 2

Baseline plasma levels of glucosamine and chondroitin sulfate in Phase 1 subjects (Mean±SD).

Time	Glucosamine (ng/mL)	Chondroitin Sulfate (μg/mL)
0 hrs (0800)	< limit of detection	20.8 ± 10.5
2 hours	< limit of detection	18.9 ± 10.3
4 hrs	< limit of detection	17.7 ± 11.2
8 hrs	< limit of detection	19.5 ± 9.11
24 hrs	< limit of detection	18.7 ± 9.15

Table 3

Demographics for the 29 normal subjects in Phase 2 (Mean±SD).

Dosing Regimen	Glucosamine	Chondroitin Sulfate	Glucosamine + Chondroitin Sulfate
Number of Subjects	8	10	11
Weight (kg)	76.8 ± 13.2	76.2 ± 22.4	83.9 ± 23.4
Height (cm)	178. ± 8.1	169 ± 9.4	173 ± 6.9
Age (years)	32.3 ± 12.0	37.8 ± 18.3	34.2 ± 10.5
Gender (male/female)	6/2	2/8	4/7

Table 4

Pharmacokinetic data (mean +/−SD) for single-dose GlcN when taken alone or with chondroitin sulfate in Phase 2.

Parameter	Glucosamine	Glucosamine + Chondroitin Sulfate
AUC∞ (ng·hr/mL)[1]	2,380 ± 935	1,860 ± 892
Cmax (ng/mL)[2]	492 ± 161	311 ± 103.*
Tmax (hr)	2.31 ±1.19	2.05 ± 1.33
T_{lag} (hr)	0.29 ± 0.23	0.26 ± 0.18
$t_{½}$, abs (hr)	0.86 ± 0.56	0.77 ± 0.85
$t_{½}$, el (hr)	2.51 ± 1.84	2.90 ± 2.50

[1] AUC determined by trapezoidal rule with extrapolation to infinity.

[2] Cmax and Tmax taken directly from data.

* $p < 0.05$

Table 5

Demographics for the 28 subjects with knee pain in Phase 3 (Mean±SD).

Dosing Regimen	Glucosamine	Chondroitin Sulfate	Glucosamine + Chondroitin Sulfate
Number of Subjects	10	9	9
Weight (kg)	100. ± 27.1	97.7 ± 20.9	89.6 ± 27.3
Height (cm)	172 ± 11.0	168.0 ± 3.7	173 ± 2.7
Age (years)	56.3 ± 8.60	55.2 ± 9.2	58.0 ± 9.9
Gender (male/female)	5/5	3/6	3/6

Table 6

Pharmacokinetic data (mean +/−SD) for single-dose GlcN when taken after 3 months of multiple dosing of either GlcN or GlcN plus CS (Phase 3).

Parameter	Glucosamine	Glucosamine + Chondroitin Sulfate
AUC∞ (ng·hr/mL)[1]	1,870 ± 638	1,099 ± 466 *
Cmax (ng/mL)[2]	211 ± 93.1	217 ± 72.8
Tmax (hr)	2.25 ± 0.98	2.80 ± 1.3
T_{lag} (hr)	0.29 ± 0.23	0.260 ± 0.180
$t_{½}$ el (hr)	3.94 ± 2.45	2.42 ± 1.82

[1] AUC determined by trapezoidal rule with extrapolation to infinity.

[2] Cmax and Tmax taken directly from data.

* $p < 0.05$