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The human pharmacokinetics of oral ingestion of glucosamine and chondroitin sulfate taken separately or in combination

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Summary

Objective: As part of the National Institutes of Health (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 20 μg/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 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.

Key words: Osteoarthritis, Chondroitin sulfate, Pharmacokinetics, Glucosamine, Therapeutics.

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, National Institutes of Health (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

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report the single-dose and steady-state (multiple-dose) pharmacokinetics (PK) 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 h thereafter. The demographic characteristics for this group are shown in Table I. 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 (six capsules containing 250 mg each; eight subjects), 1200 mg of CS (six 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 III. 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 36 h interval following single dosage of GlcN or CS, which was taken following 3 months of daily ingestion of either GlcN (500 mg, three times per day, nine subjects), CS (400 mg, three times per day, nine 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 h following ingestion. The demographics for this group are shown in Table V.

ANALYTICAL METHODS FOR QUANTITATION OF PLASMA GLCN AND CS

The concentration of GlcN in all phases of this study was determined by a fluorophore-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 (GlcN) in the circulation (limit of detection about 10 ng/ml), others have reported endogenous concentrations up to about 50 ng/ml in both humans[10] and horses[11]. For CS, blood was collected into heparinized tubes and plasma samples (1 ml 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 [High resolution (HR)30/30] at 0.5 ml/min in 100 mM ammonium bicarbonate. Fractions collected at the total volume of each run were analyzed 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 0.1M ammonium acetate, pH 7.3 and digested with Chondroitinase ABC (0.025 units) for 16 h at 37°. Dried samples were fluoro-tagged with 5 μl of 2-aminoacridone (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 0.01 s, 0.1 s, 0.25 s, 0.5 s, 0.75 s and 1.5 s exposures on a Ultra-violet Products (UVP) High Performance Ultra violet Transilluminator. Band intensities were converted into concentrations based on fluoro-tagged 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 $P \leq 0.05$.

Table I
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

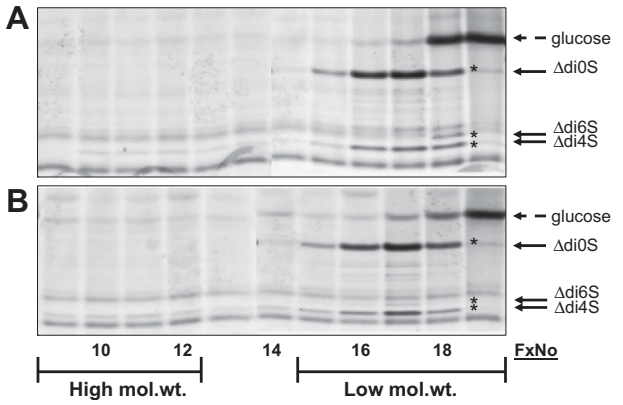

Fig. 1. FACE analyses of the CS in fractions from Superose 6 chromatography. Disaccharide analysis is shown for Superose fractions (9–19) derived from 1 ml of proteinase K-digested plasma from a typical naive patient (A) and a normal patient after a single dosage of CS alone (B). The naive patient was from the Phase 1 study and the single CS dosage from Phase 2.

Results

ANALYSIS OF CS IN HUMAN PLASMA

The concentration of CS in mammalian plasmas, including human, have been widely reported at 5–20 μg/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. 1(A)] and a normal patient, 3 h after a single 1200 mg dose of CS alone [Fig. 1(B)]. 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 s exposure) of each disaccharide in fractions 8–19 from the naive patient [Fig. 2(A)], and the single CS dosage patient [Fig. 2(B)], is given along with the same fractionation and FACE analysis of the CS used in the oral dosing [Fig. 2(C)]. This shows that a single CS dose resulted in no detectable change in either the hydrodynamic size or disaccharide composition of the plasma CS, 3 h after dosing. Indeed the same result was found for plasma samples taken at all time periods after dosing from 0.25 h to 36 h.

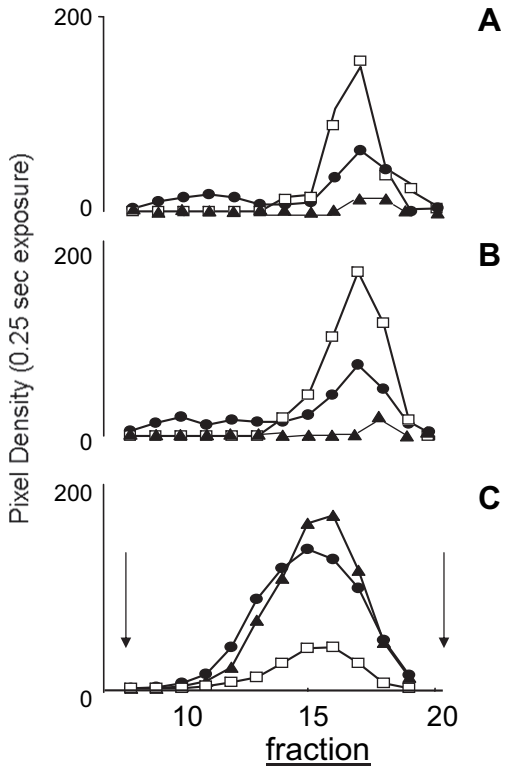

Fig. 2. Quantitation of CS disaccharides in Superose 6 fractions abundance of CS (as disaccharides) in the Superose fractions (8–19) derived from 1 ml of proteinase K-digested plasma from a typical naive patient (A) and a normal patient after a single dosage of CS alone (B). The naive patient was from 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. Ddi4S (closed circles), Ddi6S (closed triangles), Ddi0S (open squares).

Moreover, the hydrodynamic size profile and the disaccharide composition (~45% Δdi4S, ~45% Δdi6S, ~10% Δdi0S) of the CS used for dosing [Fig. 2(C)] were both distinct from the CS chains recovered in the post-hepatic circulation under all oral dosing regimes employed in this study.

PK 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 II). GlcN was undetectable at every time point whereas the level of CS was approximately 19 μg/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

Table II
Baseline plasma levels of GlcN and CS in Phase 1 subjects (Mean ± SD)

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

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

Dosing regimen	GlcN	CS	GlcN + CS
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

obtained (Fig. 3). This revealed no major effect of combined dosing. The overall PK data [Mean ± standard deviation (SD)] for 8 individuals taking GlcN alone and eleven individuals taking the combination dosage is provided in Table IV. 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 pre-dosing schedules (Fig. 4). The overall PK data (Mean ± SD) for a single dose of GlcN after 3 months pre-dosing with GlcN or GlcN + CS is provided in Table VI. It was found that the Area under Curve (AUC) for GlcN was significantly lower for the combination predosage relative to pre-dosing 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 24 h 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 1200 mg dose of CS was also examined (Fig. 6). While there appeared to be a trend towards higher CS

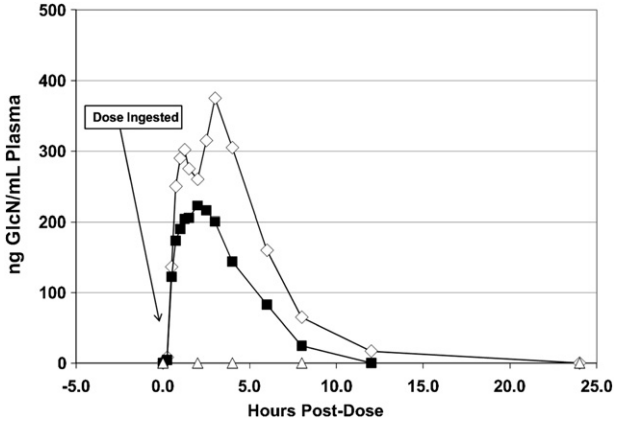

Fig. 3. The effect of combined dosing on the concentration profile for plasma GlcN in a normal individual. The plasma concentration of GlcN following a single dose of GlcN (1500 mg) taken alone (open diamonds) or in combination with 1200 mg CS (closed squares) is shown. Without dosing the plasma GlcN was undetectable (open triangles).

Table IV
PK data (Mean ± SD) for single-dose GlcN when taken alone or with CS in Phase 2

Parameter	GlcN	GlcN + CS
AUC∞ (ng hr/mL)*	2380 ± 935	1860 ± 892
Cmax (ng/mL)†	492 ± 161	311 ± 103*
Tmax (h)	2.31 ± 1.19	2.05 ± 1.33
T_{lag}(h)	0.29 ± 0.23	0.26 ± 0.18
t, abs (h)	0.86 ± 0.56	0.77 ± 0.85
$t_{,el}$(h)	2.51 ± 1.84	2.90 ± 2.50

$P < 0.05$.
*AUC determined by trapezoidal rule with extrapolation to infinity.
†Cmax and Tmax taken directly from data.

concentrations following pre-dosing with GlcN + CS, these values were not significantly different from the other groups assayed.

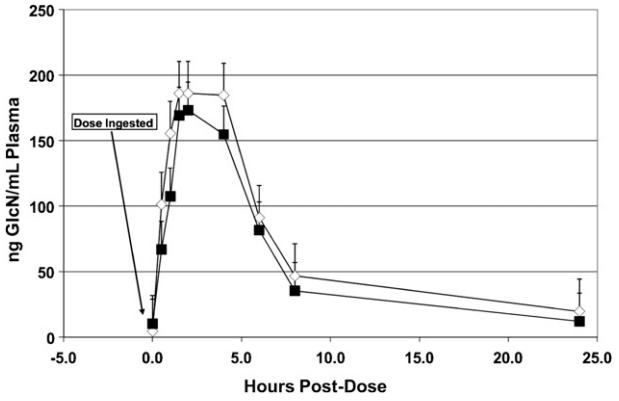

Fig. 4. The effect of pre-dosing on the concentration profile for a single dose of GlcN The plasma concentration of GlcN following a single dose of GlcN (1500 mg) after 3 months of pre-dosing with GlcN (open diamonds) or GlcN + CS (closed squares).

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, PK 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 PK 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 24 h 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 200 ng/ml change in total CS since, in keeping with others[13,18] we found the endogenous CS concentration to be about 20 μg/ml and this baseline can vary in an individual by about 5 μg/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 11 μg/ml in human plasma[19]. Indeed, our finding that the disaccharide composition of CS in baseline human plasma samples was largely unsulfated disaccharide of chondroitin sulfate (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.

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

Dosing regimen	GlcN	CS	GlcN + CS
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

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 (1870 ± 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 IV). 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 sodium-dependent glucose transporter (SGLT)1 and glucose transporter

Table VI
PK 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	GlcN	GlcN + CS
AUC∞ (ng hr/mL)*	1,870 ± 638	1,099 ± 466*
Cmax (ng/mL)†	211 ± 93.1	217 ± 72.8
Tmax (h)	2.25 ± 0.98	2.80 ± 1.3
T_{lag}(h)	0.29 ± 0.23	0.260 ± 0.180
$t_{,el}$(h)	3.94 ± 2.45	2.42 ± 1.82

*$P < 0.05$.
*AUC determined by trapezoidal rule with extrapolation to infinity.
†Cmax and Tmax taken directly from data.

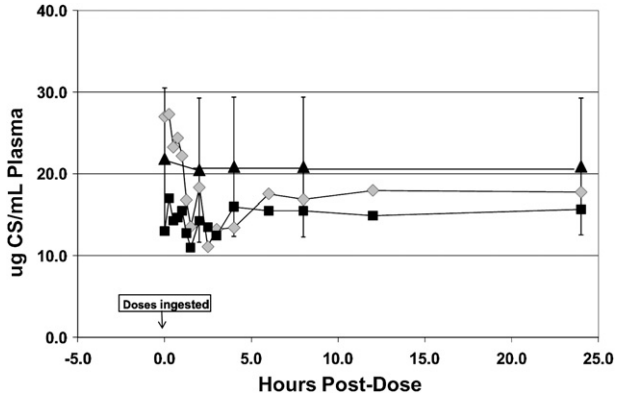

Fig. 5. The effect of combined dosing on the concentration profile for plasma CS. The plasma concentration of CS following a single dose of CS (1200 mg) taken alone (filled diamonds) or in combination with 1500 mg of GlcN (closed square) is shown. Also shown is control data without CS dosing (closed triangles).

(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].

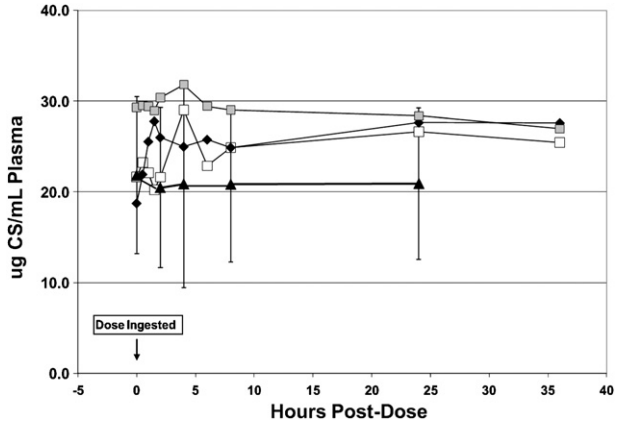

Fig. 6. The effect of pre-dosing on the concentration profile for a single dose of CS. The plasma concentration of CS following a single dose of CS (1200 mg) after 3 months of pre-dosing with GlcN (open squares), CS (closed diamonds) or GlcN + CS (closed squares). Also shown (closed triangles) is the control data without CS dosing.

Conflict of interest

No authors have a conflict in association with this manuscript.

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References

1. Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, *et al*. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum 1998;41:778–99.
2. McNair PJ, Simmonds MA, Boocock MG, Larmer PJ. Exercise therapy for the management of osteoarthritis of the hip joint: a systematic review. Arthritis Res Ther 2009;11:R98.
3. Reichmann WM, Katz JN, Kessler CL, Jordan JM, Losina E. Determinants of self-reported health status in a population-based sample of persons with radiographic knee osteoarthritis. Arthritis Rheum 2009; 61:1046–53.
4. Corsinovi L, Martinelli E, Fonte G, Astengo M, Sona A, Gatti A, *et al*. Efficacy of oxycodone/acetaminophen and codeine/acetaminophen vs conventional therapy in elderly women with persistent, moderate to severe osteoarthritis-related pain. Arch Gerontol Geriatr 2009.
5. Hochberg MC. Nutritional supplements for knee osteoarthritis–still no resolution. N Engl J Med 2006;354:858–60.
6. Clegg DO, Reda 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.
7. Laverty S, Sandy JD, Celeste C, Vachon P, Marier JF, Plaas AH. Synovial fluid levels and serum pharmacokinetics in a large animal model following treatment with oral glucosamine at clinically relevant doses. Arthritis Rheum 2005;52:181–91.
8. Plaas AH, West L, Midura RJ, Hascall VC. Disaccharide composition of hyaluronan and chondroitin/dermatan sulfate. Analysis with fluorophore-assisted carbohydrate electrophoresis. Methods Mol Biol 2001;171:117–28.
9. Zhong S, Zhong D, Chen X. Improved and simplified liquid chromatography/electrospray ionization mass spectrometry method for the analysis of underivatized glucosamine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2007;854:291–8.
10. Persiani S, Rotini R, Trisolino G, Rovati LC, Locatelli M, Paganini D, *et al*. Synovial and plasma glucosamine concentrations in osteoarthritic patients following oral crystalline glucosamine sulphate at therapeutic dose. Osteoarthritis Cartilage 2007;15:764–72.
11. Meulyzer M, Vachon P, Beaudry F, Vinardell T, Richard H, Beauchamp G, *et al*. Comparison of pharmacokinetics of glucosamine and synovial fluid levels following administration of glucosamine sulphate or glucosamine hydrochloride. Osteoarthritis Cartilage 2008; 16:973–9.
12. Myers AL, Upreti VV, Khurana M, Eddington ND. Characterization of total plasma glycosaminoglycan levels in healthy volunteers following oral administration of a novel antithrombotic odiparcil with aspirin or enoxaparin. J Clin Pharmacol 2008;48:1158–70.
13. Zinellu A, Pisanu S, Zinellu E, Lepedda AJ, Cherchi GM, Sotgia S, *et al*. A novel LIF-CE method for the separation of hyaluronan- and chondroitin sulfate-derived disaccharides: application to structural and quantitative analyses of human plasma low- and high-charged chondroitin sulfate isomers. Electrophoresis 2007;28: 2439–47.
14. Fox BA, Stephens MM. Glucosamine/chondroitin/primorine combination therapy for osteoarthritis. Drugs Today (Barc) 2009;45:21–31.
15. Vangsness Jr CT, Spiker W, Erickson J. A review of evidence-based medicine for glucosamine and chondroitin sulfate use in knee osteoarthritis. Arthroscopy 2009;25:86–94.
16. Tat SK, Pelletier JP, Verges J, Lajeunesse D, Montell E, Fahmi H, *et al*. Chondroitin and glucosamine sulfate in combination decrease the pro-resorptive properties of human osteoarthritis subchondral bone osteoblasts: a basic science study. Arthritis Res Ther 2007; 9:R117.
17. Theodosakis J. A randomized, double blind, placebo controlled trial of a topical cream containing glucosamine sulfate, chondroitin sulfate,

and camphor for osteoarthritis of the knee. J Rheumatol 2004;31:826. author reply 826–827.
18. Volpi N. HPLC and on-line MS detection for the analysis of chondroitin sulfates/hyaluronan disaccharides derivatized with 2-aminoacridone. Anal Biochem 2009.
19. Matsuzaki H, Kobayashi H, Yagyu T, Wakahara K, Kondo T, Kurita N, *et al.* Plasma bikunin as a favorable prognostic factor in ovarian cancer. J Clin Oncol 2005;23:1463–72.
20. Zhuo L, Salustri A, Kimata K. A physiological function of serum proteoglycan bikunin: the chondroitin sulfate moiety plays a central role. Glycoconj J 2002;19:241–7.
21. Boudry G, Cheeseman CI, Perdue MH. Psychological stress impairs Na+-dependent glucose absorption and increases GLUT2 expression in the rat jejunal brush-border membrane. Am J Physiol Regul Integr Comp Physiol 2007;292:R862–867.
22. 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.
23. 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.