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The Bioavailability and Pharmacokinetics of Glucosamine Hydrochloride and Low Molecular Weight Chondroitin Sulfate After Single and Multiple Doses to Beagle Dogs

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ABSTRACT: *Objective*—The purpose of this study was to determine the oral bioavailability and pharmacokinetics of a glucosamine (GL) and the disaccharides of chondroitin sulfate (CS) after single and multiple-dosing of a GL/CS combination (Cosamin^{R}*, Cosequin^{R}*). *Methods*—Male beagle dogs ($n = 8$, 12 kg) received the following treatments: (1) IV GL (500 mg)/CS (400 mg), (2) p.o. GL (1500 mg)/CS (1200 mg), (3) p.o. GL (2000 mg)/CS (1600 mg), (4) p.o. GL (1500 mg)/CS (1200 mg) QD for days 1–7 and p.o. GL (3000 mg)/CS (2400 mg) from days 8 to 14. Blood samples were collected over 24 h and glucosamine and the disaccharides of chondroitin sulfate were determined. Pharmacokinetic analysis was performed on glucosamine and total chondroitin sulfate disaccharides and parameters were compared across treatments using ANOVA with post hoc analysis. *Result*—After the IV administration, glucosamine declined rapidly in a bi-exponential fashion with a mean ($\pm$ S.D.) elimination $t_{1/2}$ of 0.52 (0.25) h. GL absorption was relatively fast ($C_{max} = 8.95\,\mu g/ml$, and T_{max} 1.5 h after 1500 mg dose) and the mean bioavailability of glucosamine after single dosing was approximately 12%. The extent of absorption of chondroitin sulfate as indicated by the mean C_{max} (21.5 μg/ml) and mean AUC (187 μg/ml h) of total disaccharides after dosing (1600 mg) provides evidence that chondroitin sulfate is absorbed orally. The bioavailability of CS ranged from 4.8 to 5.0% after single dosing and 200–278% upon multiple dosing. *Conclusion*—The results of this study show that both glucosamine and chondroitin sulfate (measured as total disaccharides) are bioavailable after oral dosing. In addition, the low molecular weight chondroitin sulfate used in this study displays significant accumulation upon multiple dosing. Copyright © 2002 John Wiley & Sons, Ltd.

Key words: glucosamine; chondroitin sulfate; bioavailability; disaccharides

Introduction

Degenerative joint disease (DJD) or osteoarthritis (OA) continues to pose major therapeutic problems in the humans and canines. Treatment with non-steroidal anti-inflammatory agents (NSAIDs) are designed to reduce pain and inflammation associated with the disease. Long-term use of NSAIDs has been associated with adverse effects including gastrointestinal ulceration, hepatic toxicity, hemorrhage [1] and some having negative effects on chondrocytes and cartilage-matrix formation [2–3]. These adverse events have led many researchers to search for safer alternatives to NSAIDs to manage OA, such

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as glucosamine and chondroitin sulfate. Originally, these compounds were considered to only serve as building blocks for cartilage and exogenous sources of cartilage matrix components. Recently research has shown that low molecular weight chondroitin sulfate (LMWCS) and glucosamine when used in combination can up-regulate chondrocytes and reduce the extent of cartilage degredation [4–5]. Both glucosamine and LMWCS have been shown to be efficacious in the management of OA in humans and animals [6–8], but recent work has indicated that the combination may enhance this efficacy [4,9]. Studies in the literature suggest that both chondroitin sulfate and glucosamine are bioavailable but the results are variable.

The pharmacokinetics of glucosamine is difficult to investigate because it is an endogenous substance which is rapidly utilized by the body for the biosynthesis of other normal constituents. Also the assay methods presently available for glucosamine have insufficient sensitivity to detect the small quantities of glucosamine occurring in the body fluids after administration of therapeutic doses [10]. The pharmacokinetics of glucosamine has been studied in rats, dogs and man [10–12] using [^{14}C] uniformly labeled glucosamine hydrochloride. [^{14}C] Glucosamine rapidly diffuses in most tissues and organs and has a tropism for the articular tissues and bone.

Chondroitin sulfate, a much larger molecule than glucosamine, is a glycosaminoglycan made up of glucuronic acid and sulfated *N*-acetyl galactosamine. Like glucosamine it has also been subjected to radiolabled studies indicating bioavailablilty and shows tropisim for articular cartilage and synovial fluid [13]. Administration of the same LMWCS used in the present study to humans showed absorption of intact molecules detected by agarose-gel electrophoresis [14]. Presently it is unknown if intact, fragmented or disaccharides are the efficacious component(s) of exogenously administered chondroitin sulfate [15]. Certainly *in vitro* work where LMWCS was administered intact and not exposed to GAG-degrading enzymes has shown efficacy in both stimulating chondrocytes and protecting them from degradation [4]. Conceivably, after oral dosing, some of the chondroitin sulfate molecule may be partially digested in the gastrointestinal tract or be subjected to a large first pass effect as occurs with other glycosaminoglycans [16].

The pharmacokinetic studies of chondroitin sulfate and glucosamine in the dog when administered concomitantly are lacking. It should be noted that the combination of chondroitin sulfate and glucosamine are used extensively in dogs for the treatment of degenerative joint disease. One of the major challenges associated with assessing the bioavailability and pharmacokinetics of these nutraceutical compounds has been the lack of sensitive analytical methods that can quantify these agents in biological matrices. To overcome this problem, we developed an ultraviolet—high-performance liquid chromatography (UV-HPLC) method using pre-column derivatization for glucosamine [17]. This method was found to be specific, accurate, sensitive and the interday and intraday precision was less than 11%. In addition, assays have been developed that detect disaccharides formed from chondroitin sulfate after treatment with chondroitinase ABC [18]. The resultant disaccharides formed after enzymatic treatment are detected using ultraviolet or fluorescent methods. Therefore, this approach has the potential of assessing the oral absorption of chondroitin sulfate by completely converting this compound to disaccharides. The chondroitin sulfate molecule is degraded to three primary unsaturated disaccharides (Figure 1): (1) 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose [ΔDi-OS], (2) 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulpho-D-glactose [ΔDi-4S] and (3) 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulf-D-glactose [ΔDi-6S] [19,20]. A definitive determination of chondroitin sulfate absorption could be the detection of its breakdown products (ΔDi-OS, ΔDi-4S, and ΔDi-6S), the unsaturated disaccharides in plasma after oral administration [19,20].

We propose that the oral bioavailability and pharmacokinetics of glucosamine and chondroitin sulfate plays an important role in optimizing DJD therapy because of the high variability of sources of glucosamine and chondroitin sulfate in relation to purity, molecular weight and physiochemical properties [14,21]. We chose to test materials that have been shown efficacious

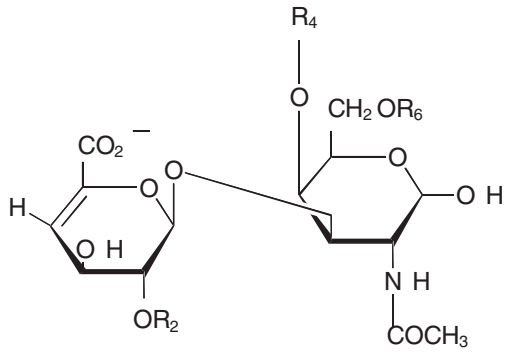

Figure 1. Chemical structures of disaccharides of chondroitin sulfate as indicated in the following table

Disaccharide (all are sodium salts)	R_2	R_4	R_6
ΔDi-OS, ΔHexA-GalNAc	H	H	H
Δ Di-6S, ΔHexA-GalNAc (6-OS0$_3$)	H	H	SO_3^-
Δ Di-4S, ΔHexA-GalNAc (4-OS0$_3$)	H	SO_3^-	H

[4,5,7,8] and also shown to meet label claim [21]. The purpose of this study was to determine the bioavailability and pharmacokinetic properties of glucosamine and the disaccharides of chondroitin sulfate after single and multiple dosing in dogs.

Materials and Methods

Materials and reagents

Glucosamine HCl (FCHG49^{TM}*) and low molecular weight chondroitin sulfate (TRH122^{TM}*) were donated by Nutramax Laboratories®, Inc., Edgewood MD. Cosequin®/Cosamin® capsules used in the oral dosing which contains glucosamine HCl (FCHG49^{TM}*) and low molecular weight chondroitin sulfate (TRH122^{TM}*) with manganese ascorbate was also donated by Nutramax Laboratories® Inc. Methanol, acetonitrile, phenylisothiocyanate (PITC), sodium phosphate and acetic acid were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). D(+)-glucosamine (2-amino-2-deoxy-D-glucose) hydrochloride was purchased from Sigma Chemical Co. (St Louis, MO). All chemicals and solvents were ACS analytical grade or HPLC grade. Deiorized water was prepared by an ultrapure water system Pyrosystem Plus® (Hydro, Research Triangle Park, NC).

Animals: Beagle dogs ($n = 8$), obtained from Marshall Farms, North Rose, NY) 6 months of age and weighing approximately 9 kg were used in the study. The animals were housed individually, and maintained in an AAALAC-accredited animal facility operated on a 12-h light-dark cycle at a room temperature of $72 \pm 2^{\circ}$F. Animals received food and water *ad libitum* except on the evening prior to dosing when all food was removed and withheld until 8 h after dosing. Care and use of the animals followed the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication no. 86-23)'.

Study design: In order to characterize the bioavailability and pharmacokinetics of chondroitin sulfate and glucosamine, the following two studies were conducted: (1) single dose bioavailability and dose proportionality study and (2) multiple dose pharmacokinetic study.

Single dose bioavailability and dose proportionality study

Eight normal beagle dogs were used for this study. The single dose study was conducted as a randomized three-way crossover study with a 1-week washout period between treatments. Animals were randomly assigned to receive each of the following treatments: (A) I.V. solution of 500 mg glucosamine HCl and 400 mg of LMWCS, (B) 1500 mg of glucosamine HCl and 1200 mg of LMWCS p.o. (equivalent to three double strength Cosequin®/Cosamin® capsules), (C) 2000 mg of glucosamine HCl and 1600 mg of LMWCS p.o.(equivalent to four double strength Cosequin®/Cosequin® capsules). Blood samples (2 ml s) were collected via the jugular vein into plastic tubes containing EDTA. A typical blood sampling scheme was as follows: 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 10, 12, 14, and 24 h after drug administration. Separation of plasma was by centrifuging at 3000 rpm for 5 min. The plasma was then stored at -85°C until analyzed by validated assay methods described below.

Multiple dose pharmacokinetic study

Eight normal beagle dogs were used in this study. The multiple dose study was conducted as

an open study. Animals received the following two treatments daily: (D) 1500 mg of glucosamine HCl and 1200 mg of LMWCS p.o. (equivalent to three double strength Cosequin®/Cosamin® capsules) from days 1 to 7 and (E) 3000 mg of glucosamine HCl and 2400 mg of LMWCS p.o. (equivalent to six double strength Cosamin®/Cosequin® capsules) from days 8 to 14. Blood samples (2 ml) were collected via the jugular vein into plastic tubes containing EDTA on days 7 and 14 to determine steady-state concentrations of chondroitin sulfate and glucosamine. A typical blood sampling scheme was as follows: 0 (predose), 0.5, 1, 2, 4, 6, 8, 10, 12, 14, and 24 h after drug administration. Separation of plasma was by centrifuging for 5 min at 3000 rpm.The plasma was then stored at −85°C until analyzed and analyzed by a validated HPLC method described below.

Glucosamine sample analysis

A previously described selective and specific high performance liquid chromatography method was used to quantitative glucosamine hydrochloride in plasma [17]. Dog plasma was used to prepare standard curves in the concentration range of 1.25–20 μg/ml. Precipitation of plasma proteins was accomplished with acetonitrile to separate interfering endogenous products from the compounds of interest. The supernatant was derivatized using phenylisocyanate in phosphate buffer (pH = 8.3) at 42°C and subsequently evaporated to dryness under a nitrogen stream at 50°C. The residue was dissolved in 250 μl mobile phase and injected onto the chromatographic system. The assay was linear in concentration ranges of 1.25–20 μg/ml (r >0.999). Intra- and inter-day precision was ⩽5.23 and 5.65%, respectively, and the intra- and inter-day accuracy, indicated by relative error, ranged from −8.6 to 10.35%.

Chondroitin sulfate sample analysis:

A validated HPLC method using pre-column derivatization and flurometric detection (excitation wavelength of 350 nm and emission at 530 nm) was used to quantify the disaccharides (ΔDi-OS, ΔDi-4S, ΔDi-6S) derived from chondroitin sulfate in plasma [22]. Standards of ΔDi-OS, ΔDi-4S, ΔDi-6S (1–20 μg/ml in 90% methanol) were prepared from a stock solution (100 μg/ml in 90% methanol). Prior to the addition of 0.5 ml of dog plasma, the standards were evaporated under nitrogen gas (40°C, 5 min). Plasma (0.5 ml) was added to achieve standards concentrations of 1, 2, 5, 10, and 20 μg/ml. Plasma samples (0.5 ml) were treated with 50 mU of chondroitinase ABC in 50 μl of 1 mM sodium phosphate buffer (pH 7.0) at 37°C for 6 h. The reaction was blocked by boiling the sample for 1 min. ΔDi-2S (internal standard, 2 μg/ml) was added to 0.5 ml of plasma standard or digested plasma sample (0.5 ml). A 300 μl of 25% trifluoroacetic acid in ethanol was added, vortexed and centrifuged. The upper layer was poured into a 10 ml tube and evaporated under nitrogen at 50°C for 20 min. Residue was dissolved in 25 μl of 90% methanol, 25 μl of 0.75% trichloroacetic acid, and 25 μl of 1% dansylhydrazine in ethanol. Mixture reacted at 40°C for 3 h, after which it was diluted with 225 μl water and stored at −20°C or applied to HPLC. The volume of solution injected onto the HPLC was 50 μl.

The chromatographic conditions consisted of a μ-Bondapack NH_2 column, mobile phase of acetonitrile: 100mM acetate buffer pH 5.6 (90:20) and a flow rate of 2.0 ml/min. A separations module Waters 2690 liquid chromatograph with fluorescence detection (excitation at 350 nm and emission at 530 nm) was utilized to quantitate the eluate. A μ-Bondapack NH_2 column was used and the mobile phase consisted of acetonitrile: 100mM acetate buffer pH 5.6 (76:20). The calibration curves were found to be linear ($r \geqslant 0.99$) in the range of 1.0–20.0 μg/ml. Intra-run precision's were in all in the range of 90%. The absolute recovery of analytes in dog plasma samples was ⩾90%.

Pharmacokinetic data analysis

The pharmacokinetics of glucosamine and chondroitin sulfate was determined using non-compartmental analysis and estimated using Winnonlin™. The following pharmacokinetic parameters were estimated: area under the plasma concentration time curve (AUC), maximum plasma concentration (C_{max}), time of

maximum plasma concentration (T_{max}), elimination half-life ($t_{1/2}$), clearance (Cl/F), volume of distribution (Vd/F), bioavailability (F) and *apparent* bioavailability (F_a). The F_a for the disaccharides of chondroitin sulfate was defined as: [$AUC_{inf[total\ disaccharides]p.o.}/AUC_{inf\ [total\ disaccharides]i.v.}$]*100. The F and F_a for glucosamine and the total disaccharides of chondroitin sulfate, respectively, were also determined on days 7 and 14 after multiple dosing The AUC_{inf} after the single IV dose was assumed to be equivalent to the $AUC_{0-24\,h}$ if the IV doses were administered in a multiple dosing fashion. Based on this assumption, the AUC_{inf} after the IV doses were used to determine the F and F_a on days 7 and 14 for both glucosamine and the total disaccharides of chondroitin sulfate.

Statistical analysis

The estimated pharmacokinetic parameters from each treatment were statistically compared using one way ANOVA model and *post hoc* analysis was performed with Dunnett's test. Statistical significance was assessed at a level of $p < 0.05$.

Results and Discussion

Glucosamine single and multiple dosing studies

Mean pharmacokinetic parameters after the administration of glucosamine hydrochloride to beagle dogs are summarized in Table 1. Figure 2A presents the plasma glucosamine concentration vs time profile after a 500 mg i.v. dose in a representative dog (# 6). After the IV administration, glucosamine declined rapidly in a bi-exponential fashion with a mean elimination half-life of 0.52 h. Previous studies have reported that glucosamine is mainly excreted in the urine [10–12]. The elimination half-life in dogs was found to be 118 min in dogs which approximates the half-life found in our studies.

The volume of distribution (Table 1) was found to be 6160 ml or 616 ml/kg. After I.V. and I.M.

Table 1. Mean (± S.D.) pharmacokinetic parameters for glucosamine hydrochloride and total chondroitin sulfate disaccharides after oral and intravenous administration to beagle dogs ($n = 8$). Disaccharide concentrations were determined after treatment of plasma samples with chondroitinase ABC prior to HPLC analysis

Treatment	C_{max} (μg/ml)	T_{max} (h)	AUC (μg/ml h)	$T_{1/2}$ (h)	CL[1] (ml/h)	Vd[a] (ml)	F_a(%)
Glucosamine							
i.v. 500 mg	—	—	67.8 (43.3)	0.52 (0.25)	9730 (4.93)	6160 (1.8)	—
p.o. 1500 mg	8.95 (4.07)	1.5 (0.53)	17.8 (10.6)	1.52 (0.57)	—	—	12.7
p.o. 2000 mg	12.4 (5.8)	1.63 (0.52)	27.9 (12.2)	2.4 (1.7)	—	—	12.1
p.o. 1500 mg[b]	7.1 (4.3)	1.1 (0.53)	15.0 (5.7)	1.92 (0.65)	—	—	9.7
p.o. 3000 mg[c]	12.1 (6.8)	1.4 (0.5)	32.0 (12.8)	1.99 (1.5)	—	—	10.6
Total Disaccharides							
i.v. 400 mg	—	—	967 (2.7)	1.56 (0.002)	447 (1.47)	989 (2.6)	—
p.o. 1200 mg	19.0 (0.06)	1.54 (0.01)	144 (1.3)	9.35 (0.09)	—	—	5.0
p.o. 1600 mg	21.5 (0.06)	2.7 (0.013)	187 (1.12)	12.1 (0.12)	—	—	4.8
p.o. 1200 mg[b]	96.3	1.0	1416	—	—	—	200[d]
p.o. 2400 mg[c]	208	1.5	2088	—	—	—	278[d]

[a] Only determined after the intravenous dose.
[b] Day 7 after multiple dosing.
[c] Day 14 after multiple dosing.
[d] AUC_{inf} (i.v. 400 mg) = AUC_{0-24hr} (i.v. 400 mg QDx 14 days).

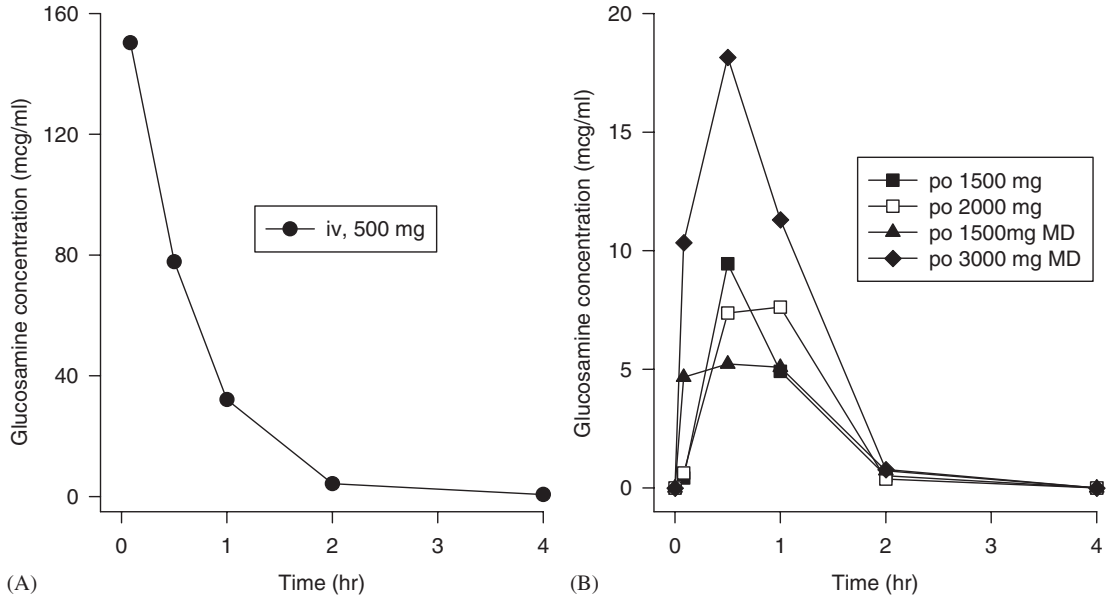

Figure 2. Plasma glucosamine concentration vs time profiles in a representative beagle dog. (A) Glucosamine plasma concentration after single 500 mg I.V. dose and (B) Glucosamine plasma concentration after single 1500 mg p.o., 2000 mg p.o. and multiple doses of 1500 mg p.o. QD on day 7 and 3000 mg p.o. QD on day 14

administration to the dog and man, the volume of distribution of glucosamine was 105 and 71 ml/kg, respectively [10–12]. The higher volume of distribution observed for the study reported herein (616 ml/kg) may be due to analytical differences used in each of the pharmacokinetic studies. The aforementioned study used radiolabeled glucosamine whereas our study did not. Nonetheless, glucosamine has been reported to be non-protein bound form which very rapidly transported into most tissues and organs [11]. Several organs and tissues also showed a capacity to concentrate glucosamine from plasma which tends to increase its volume of distribution [11].

The glucosamine concentration profile after single oral doses of 1500 and 2000 mg to dog # 6 are illustrated in Figure 2B. In addition, days 7 and 14 glucosamine concentration profile after 1500 and 2000 mg daily dosing, respectively, in dog #6 are presented in Figure 2B. The absorption of glucosamine was relatively fast with a T_{max} range of 1.1–1.6 h after the oral treatments. The C_{max} values ranged from 7.1 to 12.1 mcg/ml. The rapid absorption of labeled glucosamine in the dog has been previously reported after oral administration of uniformly labeled drug [11].

The mean bioavailability (Table 1) of glucosamine after single (12.1–12.7%) and multiple dosing (9.7–10.6%) was found not to be significantly different. Studies in dogs with radiolabeled glucosamine report an AUC after oral administration which was 26% of that after I.V. administration [11]. As previously stated, the use of radiolabeled glucosamine to determine total radioactivity in biological fluids fails to detect presystemic metabolism in the gut or liver during absorption since drug and metabolites are not differentiated. Consequently systemic availability will be overestimated when radioactivity is used and may explain the difference found between the present study and the previous studies.

After multiple dosing of 1500 mg of glucosamine for 7 days followed by 3000 mg for 7 days, no significant differences were found between the single and multiple dose pharmacokinetics.

Chondroitin sulfate single and multiple dosing studies

Following oral or intravenous administration of chondroitin sulfate and treatment of the plasma with chondroitinase ABC, each of the three

disaccharides were identified. In general, higher levels of either the ΔDi-4S and ΔDi-6S disaccharide were found after oral and iv dosing with lower levels of the ΔDi-OS. This correlates with the LMWCS used in this study as it contains approximately 60% chondroitin 4-sulfate and 40% 6-sulfate. These disaccharides are normally found circulating in human serum, with higher concentrations of both the ΔDi-4S and ΔDi-OS and trace levels of ΔDi-6S [19,20]. Figure 3 presents the plasma concentrations vs time profile for each disaccharide and total disaccharide after the 500 mg intravenous dose (Figure 3A), 1200 mg oral dose (Figure 3B) and 1600 mg oral dose (Figure 3C) to a representative beagle dog (#6). The pharmacokinetic parameters for total disaccharide after oral and I.V. dosing of chondroitin sulfate are presented in Table 1. After intravenous dosing, the mean elimination half-life of total disaccharides was found to be significantly lower (1.56 h) as compared to this parameter after the 1200 mg (9.35 h) and 1600 mg (12.1 h) oral dose.

The absorption of chondroitin sulfate appears to be relatively rapid after oral administration with a mean C_{max} and mean T_{max} (across two doses) range of 19.0–21.5 μg/ml and 1.54–2.7 h, respectively. The extent of absorption of chondroitin sulfate as indicated by the C_{max} and AUC of total disaccharides after dosing provides evidence that orally administered chondroitin sulfate was absorbed. The bioavailability of chondroitin sulfate as total disaccharides ranged from 4.8 to 5.0% after the 1200 and 1600 mg dose. Several studies which have examined the oral and parenteral absorption of GAGs including chondoritin sulfate with inconsistent results. Radiolabled absorption studies have indicated a higher bioavaliability than found in our trial [13]. However as discussed earlier radiolabled studies can overestimate actual values. Nonetheless, This study provides the first determination of the bioavailability of chondroitin sulfate in combination with glucosamine.

The oral absorption of chondroitin sulfate after multiple daily dosing was also determined. Figure 4A illustrates individual and total disaccharide concentration vs time profile on day 7 after daily dosing with 1200 mg of chondroitin sulfate. Individual and total disaccharide concentration vs time profile on day 14 after 7 days additional daily doses of 2400 mg of chondroitin sulfate (Figure 4B). Table 1 summarizes the pharmacokinetic parameters after multiple dosing with chondroitin sulfate. Unlike the multiple dosing observed with glucosamine, total disac-

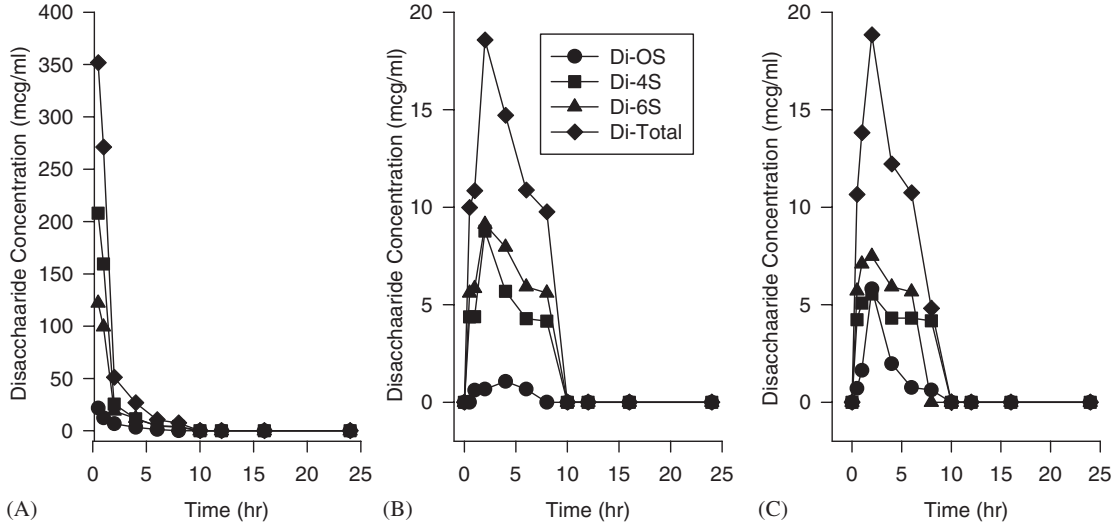

Figure 3. Individual and total plasma disaccharide concentration vs time profiles in a representative beagle dog after single doses of CS. (A) Plasma ΔDi-OS, ΔDi-4S and ΔDi-6S and total disaccharide concentration vs time profile after a 400 mg i.v. CS dose. (B) Plasma ΔDi-OS, ΔDi-4S and ΔDi-6S and total disaccharide concentration vs time profile after a 1200 mg p.o. CS dose and (C) Plasma ΔDi-OS, ΔDi-4S and ΔDi-6S and total disaccharide concentration vs time profile after a 1600 mg p.o. CS dose

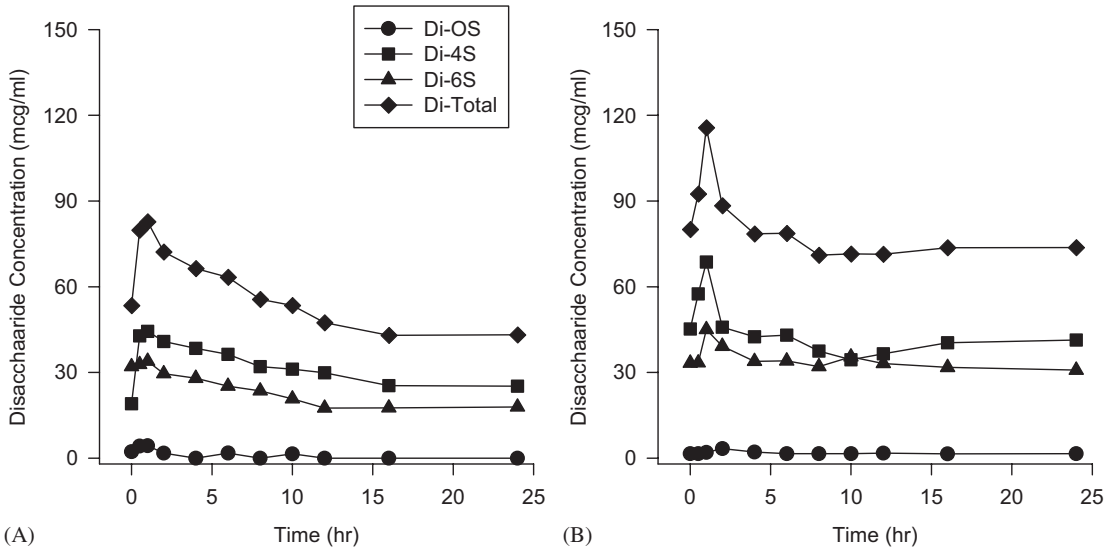

Figure 4. Individual and total plasma disaccharide concentration vs time profiles in a representative beagle dog after multiple doses of CS. (A) Plasma ΔDi-OS, ΔDi-4S and ΔDi-6S and total disaccharide concentration vs time profile for day 7 after daily 1200 mg p.o. CS doses, and (B) plasma ΔDi-OS, ΔDi-4S and ΔDi-6S and total disaccharide concentration vs time profile for day 14 after daily 2400 mg p.o. CS doses

charides accumulated in the plasma after multiple dosing (Figure 4, Table 1). This may be why chondroitin sulfate has been reported to have a significant carry-over effect in clinical trials [23–25]. The mean C_{max} was 96.3 and 208 μg/ml; and the mean AUCss was 1416 and 2088 μg h/ml after the 1200 and 2400 mg doses. Significant differences were observed for dose normalized parameters (C_{max}ss, AUCss) after multiple dosing as compared to single dose. The bioavailability of total disaccharides after multiple dosing with 1200 and 2400 mg was 200 and 278%. This assumes that the AUC_{inf} after the 400 mg I.V. dose is equivalent to the $AUC_{0-24\,h}$ at steady state. It should be noted that the intravenous dose was only administered as a single dose and it is not known if there is significant accumulation with I.V. dosing.

In summary, this report is the first that evaluates the absorption and pharmacokinetics of glucosamine and a low molecular weight chondroitin sulfate (measured as total disaccharides) after intravenous and oral dosing in dogs, and also the first to document bioaccumulation of LMWCS following multiple day dosing. Both agents were determined to be bioavailable, and the chondroitin sulfate used in this study was found to accumulate significantly after multiple dosing.

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