BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **25**: 109–116 (2004)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/bdd.392

The Bioavailability and Pharmacokinetics of Glucosamine Hydrochloride and Chondroitin Sulfate after Oral and Intravenous Single Dose Administration in the Horse

Jianping Du[a], Nathaniel White[b] and Natalie D. Eddington[a,*]
[a] *Pharmacokinetics-Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, 100 Penn Street, AHB 540C, Baltimore, MD 21201, USA*
[b] *Marion duPont Scott Equine Center, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, P.O. Box 1938, Leesburg, VA 22075, USA*

ABSTRACT:
Objective—The purpose of this study was to determine if glucosamine (GL) hydrochloride (FCHG49®) and low molecular weight (LMW) chondroitin sulfate (CS) (TRH122™) are absorbed after oral administration to horses. The bioavailability of LMWCS was evaluated by quantifying the total disaccharides found in the plasma following chondroitinase ABC digestion.
Methods—Two separate studies were conducted. In study 1, ten adult horses received the following four treatments in a randomized crossover fashion: (1) i.v. LMWCS (3 g of 8 kDa), (2) p.o. LMWCS (3 g of 8 kDa), (3) i.v. LMWCS (3 g of 16.9 kDa) and (4) p.o. LMWCS (3 g of 16.9 kDa). Each group received 9 g GL with LMWCS. In a second study, each horse ($n = 2$) was randomly assigned to receive either i.v. administration of GL HCl (9 g) or p.o. administration of GL HCl (125 mg/kg). Blood samples were collected, assayed and pharmacokinetic parameters were determined.
Results—GL was absorbed after oral dosing with a mean C_{max} of 10.6 (6.9) μg/ml and a mean T_{max} of 2.0 (0.7) h. The extent of absorption of LMWCS after dosing with both the 8.0 and 16.9 kDa provides evidence that LMWCS is absorbed orally. C_{max} and *AUC* were higher ($p < 0.05$) for the 16.9 kDa material compared with 8.0 kDa. However, the 16.9 kDa bioavailability was less than 8.0 kDa, but this difference was not significant.
Conclusions—This study provides the first report of the bioavailability of orally administered GL and LMWCS in the horse. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: glucosamine; chondroitin sulfate; bioavailability; pharmacokinetics

Introduction

The management of degenerative joint disease (DJD) continues to pose major therapeutic problems in the equine. Degenerative forms of arthritis are common in horses, constituting approximately 33% of all equine lameness, and represent one of the most important maladies [1]. Treatment with nonsteroidal antiinflammatory agents (NSAIDs) is designed to reduce pain and inflammation associated with the disease. Long term use of NSAIDs has been associated with adverse effects including gastrointestinal ulceration [2–4] and negative effects on chondrocytes and cartilage matrix formation [5–7]. Efforts have been made to find safe agents that would block or slow down the destruction the of cartilage matrix during DJD.

*Correspondence to: Univ. of Maryland School of Pharmacy, Dept of Pharmaceutical Sciences, 100 Penn St AHB, Baltimore, MD 21201-6808, USA.
E-mail: neddingt@rx.umaryland.edu, nwhite@mediasoft.net

Received 12 June 2003
Revised 31 August 2003
Accepted 9 September 2003

The first such agents used in the treatment of equine DJD were injectable glycosaminoglycan-based compounds (GAGs). The two most common compounds are polysulfated GAGs and hyaluronic acid (HA), which is a non-sulfated GAG. Recently there has been a surge of orally administered GAG products; glucosamine hydrochloride (GL) and low molecular weight chondroitin sulfate (LMWCS) have been shown to be safe and efficacious in adequate and well-controlled equine studies [8–10]. Other compounds, including other salts of GL, higher molecular weight CS and other GAGs or GAG sources have not been adequately evaluated for efficacy or safety. Recent research has shown that LMWCS and GL can reduce the rate of cartilage destruction in equine articular cartilage explants [11]. LMWCS also has been shown to increase synovial HA levels in humans [12]. The combination of GL and LMWCS have shown efficacy in slowing the progression of DJD in herbivore species [13].

Studies in the literature attest to the bioavailability and pharmacokinetic properties of CS and GL in omnivores [14–21]. However, the pharmacokinetic studies of CS and GL in the horse are lacking. Hence, important parameters such as oral bioavailability have not been determined for CS and GL. One of the major challenges associated with assessing the bioavailability and pharmacokinetics of these dietary supplements has been the lack of sensitive and specific analytical methods that can accurately quantify these agents in biological matrices.

The pharmacokinetics of GL are difficult to investigate because it is an endogenous substance which is rapidly utilized by the body for the biosynthesis of other normal constituents and is therefore not recoverable as a parent compound. Previous studies using radiolabelled GL HCl indicate the radioactivity of glucosamine rapidly appeared in most tissues and organs, but with a special tropism for the articular tissues and bone [20]. This wide distribution and subsequent accumulation of GL in various tissues tends to increase its volume of distribution. This in turn yields very low plasma glucosamine levels thus requiring sensitive analytical methods to detect its presence after administration.

Chondroitin sulfate, a much larger molecule than GL, is a GAG made up of glucuronic acid and sulfated *N*-acetyl galactosamine. There are limited studies that report the bioavailability of intact CS. Like GL, it has also been subjected to radiolabelled studies indicating bioavailability and shows tropism for articular cartilage and synovial fluid [18]. Studies using radiolabelled CS suggest that material similar in size to the orally administered CS used in this study appears in plasma within 2 h, along with lower molecular weight material [16,17]. Human studies with unlabelled CS confirm this and appear to show 10%–13% bioavailability [18]. Administration of the same LMWCS used in the present study to humans showed absorption of intact molecules detected with an agarose-gel electrophoretic method [22]. Presently, it is unknown if intact, fragmented or disaccharides are the efficacious component(s) of exogenously administered CS [23]. *In vitro* work where LMWCS was administered intact and not exposed to GAG-degrading enzymes has shown efficacy in stimulating chondrocytes and protecting them from degradation [13].

Conceivably, after oral dosing, some of the CS molecule may be partially digested in the gastrointestinal tract or may be subjected to a large first pass effect, as occurs with other GAGs [24–26].

One of the major challenges associated with assessing the pharmacokinetics and bioavailability of glucosamine and chondroitin sulfate has been the lack of sensitive analytical methods that can quantify these compounds in biological matrices. To overcome this problem, an ultraviolet, high performance liquid chromatography (UV-HPLC) method was developed using pre-column derivatization for glucosamine [14]. This method was found to be specific, accurate, sensitive and the coefficient of variation for the inter-day and intra-day variability was less than 11%. In addition, an assay has been developed and validated to extract and detect disaccharides formed from chondroitin sulfate in the plasma after treatment with chondroitinase ABC. The resultant disaccharides formed after enzymatic treatment are detected using a fluorescent method.

It is proposed that the oral bioavailability and pharmacokinetics of glucosamine and

chondroitin sulfate play an important role in optimizing DJD therapy. Because of the high variability of sources of GL and CS in relation to purity, molecular weight and physiochemical properties [27,28], test materials were chosen that have been shown to be efficacious and also shown to meet the label claim [8,9,13,29–33]. The purpose of this study was to determine the bioavailability and pharmacokinetic properties of GL and two different size LMWCS after single intravenous and oral dosing in horses.

Materials and Methods

Materials and reagents

Glucosamine HCl (FCHG49^{TM}) and LMWCS (TRH122^{TM}) were donated by Nutramax Laboratories® Inc, (Edgewood, MD). The 16.9 kDa material is commercially available (TRH122^{TM}) in Cosequin® Equine Powder. The 8 kDa chondroitin sulfate was an experimental material, not commercially available and donated by Nutramax Laboratories, Inc. D(+) Glucosamine (2-amino-2-deoxy-D-glucose) hydrochloride, chondroitinase ABC, unsaturated chondroitin sulfate disaccharides and dansylhydrazine was purchased from Sigma Chemical Co. (St Louis, MO). Methanol, acetonitrile, phenylisothiocyanate (PITC), sodium phosphate and acetic acid were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). All chemicals and solvents were ACS analytical grade or HPLC grade. Deionized water was prepared by an ultrapure water system Pyrosystem Plus® (Hydro, Research Triangle Park, NC).

Animals

Adult horses were used in the study and were obtained from the Marion duPont Scott Equine Medical Center at Virginia Technical College. Horses were in good health as determined by physical examination and clinical laboratory tests. Horses were kept in a 90 acre pasture or smaller paddocks during the periods before and between dose administration. During the collection period the horses were housed in a stall and fed grass hay *ad libitum* except as described below. The protocol was approved by the Virginia Technical College Animal Care and Use Committee.

Study design

The first study was a randomized four-way crossover design in normal horses ($n = 10$). Horses received each of the following four treatments on separate occasions: (1) i.v. LMWCS (3 g of 8 kDa) (2), p.o. LMWCS (3 g of 8 kDa) (3) i.v. LMWCS (3 g of 16.9 kDa) and (4) p.o. LMWCS (3 g of 16.9 kDa). All four treatments also included the simultaneous administration of 9 g GL HCl. The sampling (20 ml) scheme was 0, 5, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h post-dosing. Plasma was immediately separated by centrifugation and stored at -70°C until analysed.

Because GL could not be quantitated in plasma from the horses in study 1, a subsequent study was conducted. In this study, two horses were included in a two-way crossover study design. Each horse was randomly assigned to receive either i.v. administration of GL HCl (9 g) or p.o administration of GL HCl (125 mg/kg). The sampling (20 ml) scheme after dosing with glucosamine was –10, 5, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8 and 10 h post administration. Plasma was collected in EDTA-tubes and immediately separated by centrifugation and stored at -70°C until analysed. A 7-day washout period separated each treatment. In both studies, food was withheld from the animals for 3 h prior to baseline sampling and 3 hours after dose administration. The i.v. doses were administered in the jugular vein at time zero contralateral to blood sampling, and samples were collected for 24 h. The oral dose was administered via nasogastric tube.

Glucosamine sample analysis

A previously described selective and specific high performance liquid chromatography method was used to quantitate GL HCl in plasma [14]. Horse plasma was used to prepare standard curves in the concentration range 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 1.25–20 µg/ml ($r \geqslant 0.999$). The intra- and inter-day precision was $\leqslant 5.23\%$ and 5.65%, respectively, and the intra- and inter-day accuracy, indicated by relative error (RE), 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) of CS in plasma [34]. All plasma samples were treated with chondroitinase ABC in 1 mM Na_2HPO_4 buffer at pH 7 for 3 h, and subsequently reacted with dansylhydrazine and injected onto the chromatographic system. It should be noted that chondroitinase ABC cleaves CS into the unsaturated disaccharides (ΔDi-OS, ΔDi-4S and ΔDi-6S). The chromatographic conditions consisted of a µ-Bondapack NH_2 column, mobile phase of acetonitrile: 100 mM 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. The calibration curves were found to be linear ($r \geqslant 0.99$) in the range 1.0–20.0 µg/ml. Intra-run precisions were all in the range 90%. The absolute recovery of analytes in horse plasma was $\geqslant 90\%$.

Data analysis

Non-compartmental pharmacokinetic analysis was used to determine the pharmacokinetic parameters of glucosamine as well as total disaccharides formed (ΔDi-OS, ΔDi-4S and ΔDi-6S) after intravenous and oral dosing. 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*), volume of distribution (V_d), bioavailability (F) and apparent bioavailability (F_a). The F_a for the dissacharides of CS was defined as:

$$[AUC_{[\text{total disaccharides}]\text{p.o.}}/AUC_{[\text{total disaccharides}]\text{i.v}}] \times 100.$$

In addition, the bioavailability of glucosamine was normalized to dose. The pharmacokinetic parameters were estimated using the non-linear least squares regression program, Winnonlin™. The pharmacokinetic parameters were compared across treatments using ANOVA with post-hoc analysis (Dunnets). Statistical significance was assessed at a level of $p < 0.05$.

Results and Discussion

Table 1 presents the mean pharmacokinetic parameters observed for glucosamine after oral and intravenous dosing. Figures 1A and 1B display the glucosamine plasma concentration–time profiles after i.v. and oral dosing in each horse, respectively. After intravenous administration (Figure 1A) glucosamine plasma concentrations declined rapidly in a biphasic manner. The biphasic pharmacokinetics of glucosamine is in agreement with reports describing the pharmacokinetics of glucosamine in the dog, rat and man [19–21]. The elimination of glucosamine from the body was fairly rapid with a mean elimination half-life of 0.83 h after intravenous dosing (Table 1). The volume of distribution of glucosamine is exceedingly high (45, 514 L), most likely due to its extensive uptake in tissues. This large V_d observed with glucosamine ($\geqslant 15\%$ of body weight) probably reflects concentration of glucosamine in extravascular tissues. This is in agreement with autoradiographic findings in which glucosamine displayed significant distribution and uptake in liver, kidney, skeletal muscle and articular cartilage in the rat [21].

Table 1 also provides the mean absorption and bioavailability parameters for glucosamine after a 125 mg/kg oral dose. After oral dosing, the mean C_{max} for glucosamine was 10.6 µg/ml and the mean bioavailability was 2.5%. Determination of bioavailability after dosing to horses provides evidence that glucosamine is absorbed orally, albeit low. This is most likely due to extensive first pass metabolism in the gastro-

Table 1. Mean (± SD) Pharmacokinetic parameters for glucosamine HCl (i.*v.* = 9 g, *p.o.* = 125 mg/kg) and total disaccharides after oral and intravenous administration of 8.0 and 16.9 kDa chondroitin sulfate (3 g) to horses. 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^b (l/h)	$V_d{}^b$ (l)	F_a (%)
			Glucosamine				
9 g, i.v.	349	0.083[a]	247	0.83	39,247	45,514	–
	(9.9)	(0.00)	(93.4)	(0.16)	(1,867)	(8533)	
125 mg/kg, p.o.	10.6	2.0	33.2	3.33	–	–	2.5
	(6.9)	(0.7)	(23.8)	(1.52)	–	–	(0.7)
			Total disaccharides				
8.0 kDa, i.v.	83.2*	0.083[a]	36.4*	0.53	90.6	56.5	–
	(0.28)	(0.00)	(0.12)	(0.002)	(0.3)	(0.3)	
8.0 kDa, p.o.	4.53**	2.33	11.82**	2.6	–	–	32.2
	(0.04)	(0.01)	(0.05)	(0.02)			(9.22)
16.9 kDa, i.v.	210*	0.083[a]	253*	1.2	24.5	29.8	–
	(0.42)	(0.00)	(0.85)	(0.01)	(0.2)	(0.3)	
16.9 kDa, p.o.	36.5**	1.32	54.8**	4.8			22
	(0.51)	(0.01)	(0.51)	(0.05)			(22.5)

[a] First sampling time point.
[b] Determined after i.v. dosing, $*p<0.05$, $**p<0.05$.

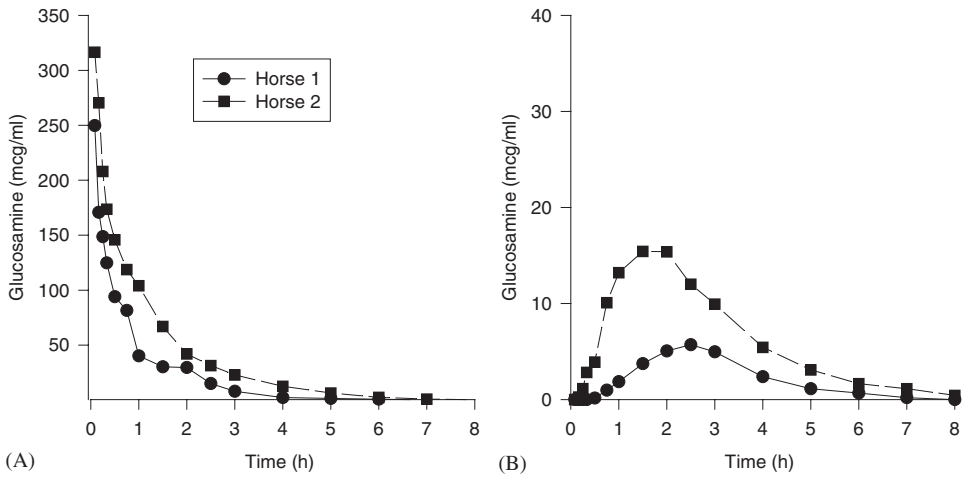

Figure 1. Plasma concentration time profile of glucosamine after (A) intravenous dosing (9 g) and (B) oral dosing (125 mg/kg) to horses

intestinal tract and/or liver prior to systemic availability. This is in contrast to the oral bioavailability of GL in the dog (12%) [15]. The relatively high oral dose administered, approximately a 5–10 fold increase over typical administration levels to the horse, was necessary to achieve levels in the sensitivity range of the assay. The oral dose administered in study 1 (9 g) is a more typical dose. There were no adverse clinical signs associated with the high overdose in the second study.

Figures 2 and 3 display the plasma concentration vs time profiles of individual and total disaccharides after i.v. and oral dosing of 8.0 (Figure 2) and 16.9 (Figure 3) kDa LMWCS to a representative horse. Each of the disaccharides (ΔDi-4S, ΔDi-OS, ΔDi-6S) were found in enzymatically digested plasma after oral and

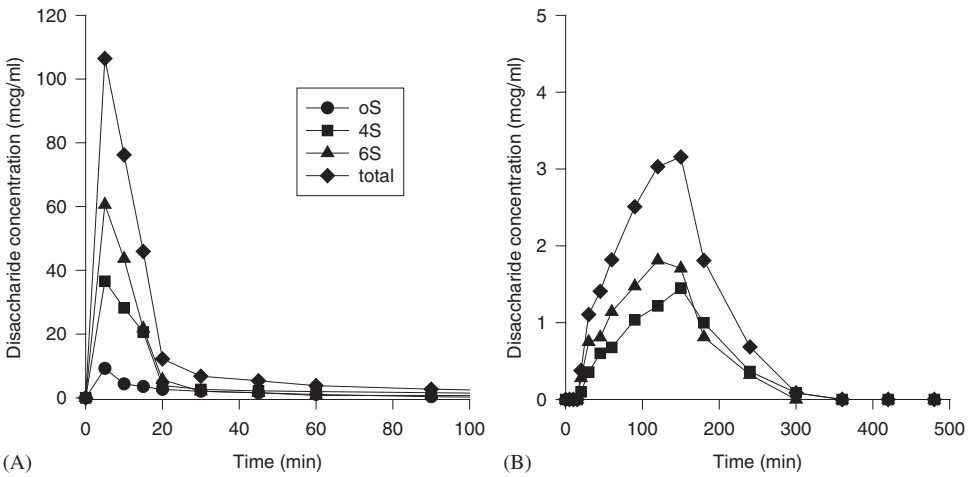

Figure 2. Plasma concentration vs time profiles of total and individual chondroitinase-generated disaccharides of chondroitin sulfate in horse 5 after dosing with 8.0 kDa. (A) ΔDi-OS, ΔDi-4S ΔDi-6S and total disaccharides after intravenous dosing (3 g) of 8.0 kDa chondroitin sulfate and (B) ΔDi-OS, ΔDi-4S ΔDi-6S and total disaccharides after oral dosing (3 g) of 8.0 kDa chondroitin sulfate

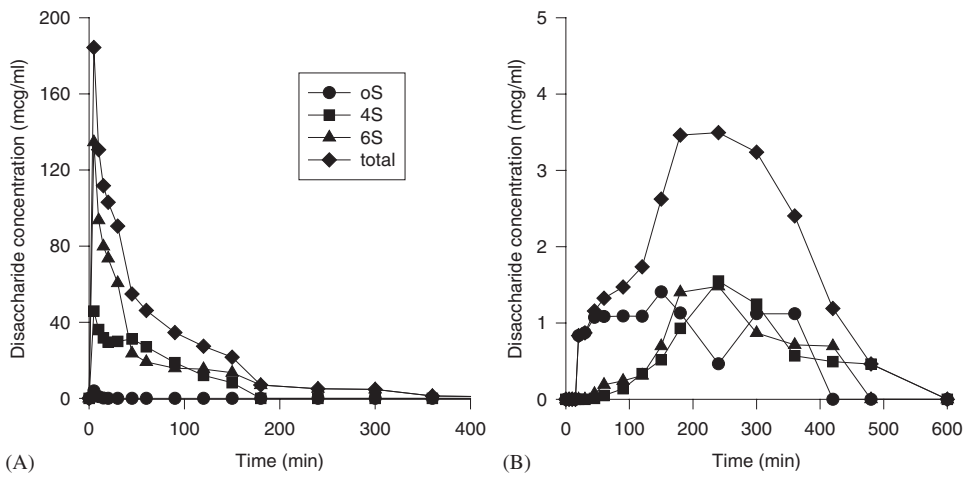

Figure 3. Plasma concentration vs time profiles of total and individual chondroitinase-generated disaccharides of chondroitin sulfate in horse 5 after dosing with 16.9 kDa. (A) ΔDi-OS, ΔDi-4S ΔDi-6S and total disaccharides after intravenous dosing (3 g) of 16.9 kDa chondroitin sulfate and (B) ΔDi-OS, ΔDi-4S ΔDi-6S and total disaccharides after oral dosing (3 g) of 16.9 kDa chondroitin sulfate

intravenous administration of the 8.0 and 16.9 kDa LMWCS. Total chondroitinase-generated disaccharide plasma concentration was highest after the intravenous dosing of the 16.9 kDa LMWCS (Figure 3A). There are no reports on the circulating concentrations of CS or these disaccharides in horses. Each of these disaccharides are found in CS in normal human serum, however, higher proportions of both the ΔDi-4S and ΔDi-OS have been reported with trace levels of ΔDi-6S after enzymatic digestion with chondroitinase [35,36].

Table 1 presents the mean pharmacokinetic parameters observed for the chondroitinase-generated disaccharides of chondroitin sulfate after oral and intravenous dosing with the 8.0 and 16.9 kDa LMWCS. The extent of absorption of LMWCS as indicated by the C_{max} and *AUC* of total disaccharides after dosing with both the 8.0 and 16.9 kDa provides evidence that chondroitin

sulfate of either 8.0 or 16.9 kDa are absorbed orally. As seen in Table 1, mean C_{max} and *AUC* were statistically higher for the 16.9 kDa chondroitin sulfate compared with 8.0 kDa. However, the bioavailability of the higher molecular weight chondroitin sulfate was lower numerically, but not statistically (22%), compared with the 8.0 kDa (32%). These values are consistent with the bioavailability of unlabelled LMWCS in humans [17,25,37] and radiolabelled CS in rats [17], but higher than that found for dogs using the same analytical methodology [34]. In the dog, CS was shown to accumulate with chronic administration [15], possibly explaining why efficacy persists after CS administration ceases [38,39]. It is unknown if this also occurs in the horse. The bioavailability associated with the 8.0 kDa chondroitin sulfate was higher than the 16.9 kDa chondroitin sulfate, which would suggest that the molecular weight influences the oral absorption of chondroitin sulfate. The reasons for this difference may be based on the intrinsic clearance of chondroitin sulfate in the gastrointestinal tract and liver [25,26] and/or differences in the chondroitinase digestibility between the 8.0 and 16.9 kDa materials due to the effect of the depolymerization process necessary to produce the lower molecular weight material for this study. Additional studies are required to assess this disparity in the total CS-derived disaccharides that can be detected after administration of different low molecular weight CS. Nonetheless, this study provides the first determination of the bioavailability of LMWCS in the horse. One factor that may affect the absorption of CS is the chain length of the molecule. Recent in vitro studies using the Caco-2 cell culture system suggested that the molecular weight of CS has a direct influence on its permeability across the gastrointestinal tract, where higher permeability was reported for CS with lower molecular weight [27]. *In vitro* models in herbivores have also shown that high molecular weight CS are not bioavailable [40].

In summary, the purpose of this study was to determine if GL and LMWCS are absorbed after oral administration to horses. Using a validated HPLC method, GL was determined to be absorbed to a limited extent after oral dosing. The absorption of LMWCS was determined by quantifying the disaccharide content using a validated method that combined enzymatic digestion of plasma followed by fluorescence HPLC. LMWCS was absorbed to a higher extent compared with glucosamine and further, its absorption may be influenced by the molecular weight of the polymer.

References

1. Alwan WH, Carter SD, Bennett D, Edwards GB. Glycosaminoglycans in horses with osteoarthritis. *Equine Vet J* 1991; **1**: 44–447.
2. Meschter CL, Gilbert M, Krook L, Maylin G, Corradino R. The effects of phenylbutazone on the intestinal mucosa of the horse: a morphological, ultrastructural and biochemical study. *Equine Vet J* 1990; **22**: 255–263.
3. Meschter CL, Gilbert M, Krook L, Maylin G, Corradino R. The effects of phenylbutazone on the morphology and prostaglandin concentrations of the pyloric mucosa of the equine stomach. *Vet Pathol* 1990; **27**: 244–253.
4. Goodrich LR, Furr MO, Robertson JL, Warnick LD. A toxicity study of eltenac, a nonsteroidal anti-inflammatory drug for horses. *J Vet Pharmacol Ther* 1998; **21**: 24–33.
5. Moskowitz RW, Davis W, Sammarco J. Experimentally induced corticosteroid arthropathy. *Arthritis Rheum* 1996; **13**: S–74.
6. Brand KD. Effects of nonsteroidal anti-inflammatory drugs on chondrocyte metabolism *in vitro* and *in vivo*. *Am J Med* 1987; **83**(suppl 5A): 29–33.
7. Fujii K, Tajiri K, Kajiwara T. Effects of NSAID on collagen and proteoglycan synthesis of cultured chondrocytes. *J Rheumatol* 1989; **16**: 28–31.
8. Hanson RR, Smalley LR, Huff GK. Oral treatment with a glucosamine-chondroitin sulfate compound for degenerative joint disease in horses: 25 cases. *Equine Pract* 1997; **19**: 16–19.
9. Hanson RR, Brawner WR, Blaik MA. Oral treatment with Cosequin in a double-blinded, placebo-controlled, randomized trial as a selective symptom modifying nutraceutical for navicular syndrome. *Vet Ther* 2000; **2**: 148–159.
10. Dorna VI, Guerrero RC. Effects of oral and intramuscular use of chondroitin sulfate in induced equine septic arthritis. *J Equine Vet Sci* 1998; **18**: 548–554.
11. Fenton JI, Chlebek-Brown KA, Peters TL, Caron JP, Orth MW. Glucosamine HCl reduces equine articular cartilage degradation in explant culture. *Osteoarthr Cartil* 2000; **8**: 258–265.
12. Ronca F, Palmieri L, Panicucci P, Ronca G. Anti-inflammatory activity of chondroitin sulfate, *Osteoarth Cartil* 1998; **6**(Suppl. A): 14–21.
13. Lippiello L, Woodward J, Karpman R, Hammad TA. *In vivo* chondroprotection and metabolic synergy of glucosamine and chondroitin sulfate. *Clin Orthoped Rel Res* 2000; **381**: 229–240.
14. Liang Z, Leslie J, Adebowale A, Ashraf M, Eddington ND. Determination of the nutraceutical, glucosamine

hydrochloride, in raw materials, dosage forms and plasma using pre-column derivatization with ultraviolet HPLC. *J Pharmaceut Biomed Anal* 1999; **20**: 807–814.
15. Adebowale A, Du J, Liang Z, Leslie JL, Eddington ND. The bioavailability and pharmacokinetics of glucosamine hydrochloride and low molecular weight chondroitin sulfate after single and multiple doses to beagle dogs. *Biopharm Drug Dispos* 2002; **23**: 217–225.
16. Palmieri L, Conte A, Giovannini L, Lualdi P, Ronca G. Metabolic fate of exogenous chondroitin sulfate in the experimental animal. *Arzneimittelforsch/Drug Res* 1990; **40**: 319–323.
17. Conte A, deBernardi M, Palmieri L, Lualdi P, Mautone G and Ronca G. Metabolic fate of exogenous chondroitin sulfate in man. *Arzneimittelforsch/Drug Res* 1991; **41**: 768–772.
18. Conte A, Volpi N, Palmieri L, Buhousi I, Ronca G. Biochemical and pharmacokinetic aspects of oral treatment with chondroitin sulfate. *Arzneimittelforsch/Drug Res* 1995; **45**: 918–925.
19. Setnikar I, Palumbo C, Zanolo G. Pharmacokinetics of glucosamine in man. *Arzneimittelforsch/Drug Res* 1993; **43**: 1109–1113.
20. Setnikar I, Giacchetti C, Zanolo G. Pharmacokinetics of glucosamine in dog and in man. *Arzneimittelforsch/Drug Res* 1986; **36**: 729–735.
21. Setnikar I, Giachetti C., Zanolo G. Absorption, distribution and excretion of radioactivity after a single intravenous or oral administration of [^{14}C] glucosamine to the rat. *Pharmatherapeutica* 1984; **3**: 8.
22. Volpi N. Disaccharide analysis and molecular mass determination to microgram level of single sulfated glycosaminoglycan species in mixtures following agarose-gel electrophoresis. *Anal Biochem* 1999; **273**: 229–239.
23. DuSouich P, Verges J. Simple approach to predict the maximal effect elicited by a drug when plasma concentrations are not available or are dissociated from the effect, as illustrated with chondroitin sulfate data. *Clin Pharmacol Ther* 2001; **70**: 5–9.
24. Gustafson S, Bjorkman T. Circulating hyaluronan, chondroitin sulphate and dextran sulphate bind to a liver receptor that does not recognize heparin. *Glycoconj J* 1997; **14**: 561–568.
25. Liau YH, Galicki N, Horowitz MI. Degradation of chondroitin 4-sulfate by rat stomach exoglycosidases, sulfohydrolase and hyaluronidase-like enzymes. *Digestion* 1981; **21**: 117–124.
26. Lia YH, Horowitz MI. Desulfation and depolymerization of chondroitin 4-sulfate and its degradation products by rat stomach, liver and small intestine. *Proc Soc Exp Biol Med* 1974; **146**: 1037–1043.
27. Adebowale A, Cox DS, Liang Z, Eddington ND. Analysis of glucosamine and chondroitin sulfate content in marketed products and the Caco-2 permeability of chondroitin sulfate raw materials. *J Amer Nutrac Assoc* 2000; **3**: 37–44.
28. Volpi N. Fast moving and slow moving heparins, dermatan sulfate and chondroitin sulfate: qualitative and quantitative analysis by agarose-gel electrophoresis. *Carbohydr Res* 1993; **247**: 263–278.
29. Das A, Hammad TA. Efficacy of a combination of FCHG49™ glucosamine hydrochloride, TRH122™ low molecular weight sodium chondroitin sulfate and manganese ascorbate in the management of knee osteoarthritis. *Osteoarthri Cartil* 2000; **8**: 343–350.
30. Leffler CT, Philippi AF, Leffler SG, Mosure JC, Kim PD. Glucosamine, chondroitin and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double-blind, placebo-controlled pilot study. *Milit Med* 1999; **164**: 85–91.
31. Johnson KA, Hulse DA, Hart RC, Kochevar D, Chu Q. Effects of an orally administered mixture of chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate on synovial fluid chondroitin sulfate 3B3 and 7D4 epitope in a canine cruciate ligament transection model of osteoarthritis. *Osteoarthr Cartil* 2001; **9**: 14–21.
32. Canapp SO, McLaughlin RM, Hoskinson JJ, Roush JK, Butine MD. Scintigraphic evaluation of dogs with acute synovitis after treatment with glucosamine hydrochloride and chondroitin sulfate. *Am J Vet Res* 1999; **60**: 1552–1557.
33. Beren J, Hill SL, Diener-West M, Rose NR. Effect of preloading oral glucosamine HCl/chondroitin sulfate/manganese ascorbate combination on experimental arthritis in rats. *Exp Biol Med* 2001; **226**: 144–151.
34. Du J, Eddington ND. Determination of the dissacharides of chondroitin sulfate in dog and horse plasma. *Anal Biochem* 2002; **306**: 252–258.
35. Huang Y, Toyoda H, Toida T, Imanari T. Determination of chondroitin sulphates in human whole blood, plasma and blood cells by high performance chromatography. *Biomed Chrom* 1995; **9**: 102–105.
36. Okazaki J, Kamada A, Matsukawa F, Sakaki T, Gonda Y. Disaccharide analysis of chondroitin sulphate in human gingival crevicular fluid using high-performance liquid chromatography. *Arch Oral Biol* 1995; **40**: 777–779.
37. Ronca G, Conte A. Metabolic fate of partially depolymerized shark condroitin sulfate in man. *Int J Clin Pharm Res* 1993; 8(Suppl. 1): 27–34.
38. Mazieres B, Combe B, Phan Van A, Tondut J, Grynfeltt M. Chondroitin sulfate in osteoarthritis of the knee: A prospective, double blind, placebo controlled multicenter clinical study. *J Rheumatol* 2001; **28**: 173–181.
39. Morreale P, Manopulo R, Galati M, Boccanera L, Saponati G, Bocchi L. Comparison of the antiinflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. *J Rheumatol* 1996; **23**:1385-1391.
40. Yamanashi S, Toyoda H, Furuya N, *et al*. Metabolic study on chondroitin sulfates in rabbits. *Yakugaku Zasshi* 1991; **111**: 73–76.

