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Comparison of pharmacokinetics of glucosamine and synovial fluid levels following administration of glucosamine sulphate or glucosamine hydrochloride[1]

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Summary

Objective: To compare the pharmacokinetics of glucosamine and the synovial fluid levels attained following treatment with glucosamine sulphate or glucosamine hydrochloride in a large animal model at clinically relevant doses.

Methods: Eight adult female horses were used. Crystalline glucosamine sulphate (Dona®) or glucosamine hydrochloride was administered at a dose of 20 mg/kg by either intravenous (IV) injection or nasogastric (NG) intubation. Plasma samples were collected before dosing and at 5, 15, 30, 60, 120, 360, 480 and 720 min after dosing. Synovial fluid samples were collected from the radiocarpal joints within 48 h before dosing and at 1, 6 and 12 h post-dosing. Glucosamine was assayed by Liquid Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS).

Results: Plasma concentrations reached ~50 μg/mL after IV injection and ~1 μg/mL after NG administration of both types of glucosamine. The median oral bioavailability was 9.4% for glucosamine sulphate and 6.1% for glucosamine hydrochloride. Synovial fluid concentrations were significantly higher at 1 and 6 h following oral treatment with glucosamine sulphate compared to glucosamine hydrochloride. Twelve hours following oral administration, glucosamine levels in the plasma and the synovial fluid were still significantly higher than baseline for the glucosamine sulphate preparation, but not for the hydrochloride preparation.

Conclusion: Following oral administration of a clinically recommended dose of glucosamine sulphate (Dona®), significantly higher synovial fluid concentrations of glucosamine are attained, when compared to an equivalent dose of glucosamine hydrochloride. Whether this difference is translated into a therapeutic effect on the joint tissues remains to be elucidated.

Key words: Glucosamine, Cartilage, Osteoarthritis, Neutraceutical, Horse.

Introduction

An ideal therapeutic agent for osteoarthritis (OA) would be both disease and symptom-modifying and have minimum side effects. Several investigations suggest that glucosamine therapy meets this ideal[1–3], however, to the contrary, a number of studies have not been able to detect any beneficial effects[4–6]. Consequently, glucosamine therapy for OA remains a contentious issue. Results of two prospective 3 year, randomised, controlled clinical trials in humans demonstrated that oral glucosamine sulphate slowed the radiographic progression of OA, supporting the concept that it may be a disease-modifying OA drug[2,3]. Recently, the oral administration of glucosamine hydrochloride was shown to have a site-specific, but partial disease–modifying effect in an experimental animal model of OA[7]. However, it was emphasised in this study that the effects were modest, as ulcerations of cartilage still occurred in some animals receiving glucosamine[7].

Despite multiple double-blind controlled clinical trials on the use of glucosamine in OA, the controversy on its efficacy related to symptomatic improvement continues[8,9]. The recently published Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) indicated that the symptomatic effect of glucosamine hydrochloride at the dose of 500 mg three times daily did not differ significantly from placebo[10]. However, in a subgroup of patients with moderate-to-severe knee pain, the rate of response to a combination of 500 mg glucosamine hydrochloride and 400 mg chondroitin sulphate three times daily was significantly higher than the

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response to placebo[10]. In contrast, the Glucosamine Unum in Die Efficacy (GUIDE) trial reported a significant improvement in the Lequesne algofunctional index when glucosamine sulphate, at the dose of 1500 mg once-a-day, was compared to placebo[11]. The discrepancies in outcome of these two studies fuel the debate about the efficacy of glucosamine therapy in OA.

In Europe, a patented formulation of glucosamine sulphate is a prescription drug, whereas in North America glucosamine is considered to be a dietary supplement. A crystalline glucosamine sulphate formulation (Dona®, Viartril-S®, Xicil® or other trademark) is patented by the Rottapharm Group (Monza, Italy) with glucosamine, sulphate, chloride and sodium ions present in stoichiometric ratios of 2:1:2:2. Over-the-counter glucosamine formulations, on the other hand, may contain glucosamine sulphate, glucosamine hydrochloride or *N*-acetylglucosamine and poor product quality has been reported for a variety of these formulations with only a small percentage of the products actually containing the amount of product listed on the label[12,13].

Information on the absorption, serum pharmacokinetics and synovial fluid concentrations of glucosamine has been limited until recently. In a large animal model Laverty *et al.* demonstrated that glucosamine enters the synovial fluid following administration of recommended oral doses of glucosamine hydrochloride[14]. The concentrations obtained in the synovial fluid were less than 10% of those in serum, at the same time points[14], suggesting that the majority of the absorbed glucosamine is excreted or metabolised by other tissues and therefore not available for transportation from the circulation into the synovial fluid. Recently, synovial fluid glucosamine concentrations have been measured in human OA patients, following repeated oral crystalline glucosamine sulphate administration at therapeutic doses and the median synovial fluid glucosamine concentrations were only 23.5% lower than those in plasma[15].

In a Cochrane review it was speculated that the conflicting trial results might be attributed to the use of different glucosamine formulations, and it was concluded that the most favourable trial results were associated with the prescription glucosamine sulphate preparation[16]. Whether there is a difference in bioavailability and efficacy between glucosamine sulphate and glucosamine hydrochloride is presently unknown. It has been proposed that eventual differences in absorption could be explained by a different salt composition of the two formulations[14].

The objectives of the present study were twofold: to compare the pharmacokinetics of glucosamine following treatment with glucosamine sulphate or glucosamine hydrochloride at clinically recommended doses and to compare the synovial fluid levels attained when each formulation was administered.

Methods

MATERIALS

Glucosamine sulphate (Dona®) was purchased from Rotta Pharmaceuticals Inc. (Wall, NJ, USA). Glucosamine hydrochloride (catalogue no. G1514, purity $\geq$ 99%) and the internal standard (theophylline) were purchased from Sigma–Aldrich (St-Louis, MO, USA). Other chemicals, including acetonitrile and formic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA).

ANIMAL STUDIES

Eight adult female horses, with a mean $\pm$ SD body weight of 459 $\pm$ 42 kg and a mean $\pm$ SD estimated age of 14.4 $\pm$ 2.1 years were used in this study. These animals were free of clinical evidence of joint disease (absence of synovial effusion in target joint and no lameness attributable to this joint). The study was executed over a 4-week period. On week one, following a 12 h fast, all horses were administered glucosamine sulphate by nasogastric (NG) intubation which was followed 1 week later by intravenous (IV) administration of the same compound. On the third and fourth week the same protocol of administration was followed for glucosamine hydrochloride.

Crystalline glucosamine sulphate, a branded dietary supplement (Dona®) or analytical-grade glucosamine hydrochloride was dissolved at 100 mg/mL in 0.9% sterile saline and adjusted to a pH of 6.0. The final solution was filtered through a 0.2 μm filter and administered at a dose of 20 mg/kg body weight by NG intubation or IV injection. Each horse received 20 mg glucosamine sulphate or glucosamine hydrochloride per kg bodyweight. NG dosing included 500 ml of 0.9% sterile saline immediately after compound administration. IV injection was via a catheter inserted into the right jugular vein and was followed by a 20 mL saline flush to assure complete drug administration.

Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes via an IV catheter in the contralateral jugular vein and were obtained within 48 h before dosing and at 5, 15, 30, 60, 120, 360, 480 and 720 min post-dosing. Synovial fluid was collected in EDTA tubes by aseptic arthrocentesis, within 48 h pre-dose from both radiocarpal joints and at 1, 6 and 12 h from only one radiocarpal joint. After NG administration, synovial fluid was obtained at 1 and 12 h post-dosing from the left and at 6 h post-dosing from the right radiocarpal joint. The alternate sequence was used after IV administration. Synovial fluid and blood samples were centrifuged at 1000 g for 20 min within 30 min of collection and the cell-free supernatants were removed and stored at −80°C until assayed for glucosamine.

Horses were evaluated daily for signs of discomfort or joint problems, for 2 days following the interventions and then every other day.

The experimental protocol was approved by the Institutional Animal Care and Use Committee.

QUANTIFICATION OF GLUCOSAMINE FROM PLASMA AND SYNOVIAL FLUID

The concentrations of glucosamine in the plasma and synovial fluid samples were assessed using Liquid Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS). Details of this method in equine plasma have been reported elsewhere[17]. The reproducibility of the method was evaluated by analysing a minimum of six replicates of plasma and synovial fluid at the nominal glucosamine concentration of 20, 40 and 60 ng/mL with a calibration curve made in saline solution (0.9% (w/v) NaCl in water) (Fig. 1). In order to calculate the accuracy, the endogenous level needed to be subtracted from the observed concentration of fortified equine plasma and synovial fluid (%NOM = ([measured concentration] − [endogenous concentration])/[fortified concentration]100). The precision (%CV) and accuracy (%NOM) observed ranged from 5.3 to 11.3% and from 87.8 to 107.2% in equine plasma and from 6.2 to 11.2% and from 85.5 to 113.1% in synovial fluid. The interbatch precision observed ranged from 5.2 to 8.2%, whereas the accuracy ranged from 90.8 to 112.9%, respectively. Precision and accuracy in samples prepared in saline solution were also calculated and were well within ±15% acceptance criteria. The limit of quantification (LOQ) was set at 10 ng/mL and, according to the bioanalytical validation guideline published by the FDA in May 2001[18], acceptable precision and accuracy results were achieved.

PHARMACOKINETICS

Pharmacokinetic parameters of glucosamine in equine plasma were calculated using non-compartmental methods[19]. The area under the curve from time 0−12 h (AUC_{0-12h}) was calculated using a linear trapezoidal rule. A terminal rate constant of elimination (k_{el}) was calculated using a minimum of three measurable plasma concentrations and the terminal elimination half-life ($T_{1/2}$) was calculated ($T_{1/2} = (\ln 2)/k_{el}$). The area under the curve extrapolated to infinity (AUC_{0-inf}) was calculated using $AUC_{0-12h} + C_{last}/k_{el}$, where C_{last} was the last measurable plasma concentration.

After IV administration, the systemic clearance (CL) was calculated by dividing the actual dose administrated by the AUC_{0-inf}. The mean residence time (MRT) was obtained by dividing the area under the first moment−time curve ($AUMC_{0-inf}$) by the AUC_{0-inf}. The total volume of distribution (V_d) was calculated using CL × MRT. After NG administration, the apparent clearance (CL/*F*) was calculated by dividing the dose by the AUC_{0-inf}. The maximal serum concentration (C_{max}) and the time to attain it (T_{max}) were also determined. The bioavailability (*F*) of glucosamine sulphate and glucosamine hydrochloride was calculated using the formula ($AUC_{0-inf\ NG}/AUC_{0-inf\ IV}$) × ($Dose_{IV}/Dose_{NG}$).

STATISTICS

For each pharmacokinetic parameter, the median value in each treatment group was compared using Wilcoxon Signed Rank Tests. Endogenous

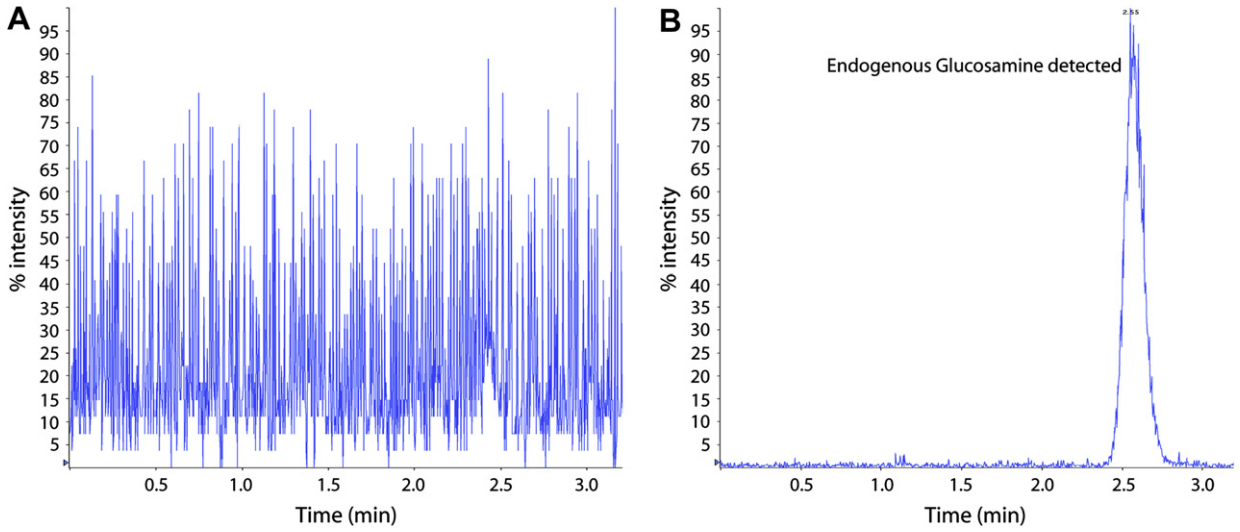

Fig. 1. Quantification of glucosamine by Liquid Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS). (A) Chromatogram of a blank sample. (B) Chromatogram of an extracted horse plasma sample with endogenous glucosamine.

levels of glucosamine were examined using a repeated-measures linear model with compartment (plasma or synovial fluid) and type of glucosamine (sulphate and hydrochloride) as repeated factors. A repeated-measures linear model, with time (1, 6 and 12 h) and type of glucosamine as repeated factors, was used to investigate changes in synovial fluid glucosamine concentrations following either IV or NG dosing. We used *a priori* contrasts to examine differences between treatments at each time period. The actual dose administered was normalised according to product potency (free glucosamine) for statistical comparisons of the AUC of both formulations.

Results

All procedures were well tolerated by the animals and no adverse reactions were observed following the administration of both glucosamine formulations. One horse alone, with a nervous temperament, was sedated prior to the arthrocentesis procedures.

QUANTIFICATION OF PLASMA GLUCOSAMINE FOLLOWING IV DOSING

The individual data of each animal were used to generate the pharmacokinetic parameters of IV glucosamine sulphate and hydrochloride in plasma (Table I). Mean ± SD plasma glucosamine concentrations at selected time points following IV administration of glucosamine sulphate and glucosamine hydrochloride are presented in Fig. 2. For both types of glucosamine, the pharmacokinetic profile was characterised by a rapid decline over the first 2 h after IV administration, followed by a more progressive elimination in the following hours to reach a mean ± SD plasma concentration of 433.3 ± 245.1 ng/mL for glucosamine sulphate and 305.0 ± 136.4 ng/mL for glucosamine hydrochloride at 12 h post-treatment.

There was no significant difference between the median AUC_{0-12h} or the median AUC_{0-inf} of both IV treatments. The $T_{1/2}$ for both treatments was variable between animals, ranging from 1.7 h to 2.9 h for glucosamine sulphate and from 1.7 h to 2.6 h for glucosamine hydrochloride, but the variability was consistent with inter-animal variation in tissue clearance. The median $T_{1/2}$ was not significantly different between treatments, nor was the median CL, the median MRT or the median V_d.

QUANTIFICATION OF PLASMA GLUCOSAMINE FOLLOWING NG DOSING

Mean ± SD plasma glucosamine concentrations at all time points following NG administration of glucosamine

Table I
Mean (±SD) and median (range) pharmacokinetic parameters of glucosamine in equine plasma (n = 8) following IV administration of 20 mg/kg glucosamine sulphate or glucosamine hydrochloride

	Glucosamine sulphate		Glucosamine hydrochloride	
	Mean (±SD)	Median (range)	Mean (±SD)	Median (range)
AUC_{0-12h}, mg/h/L	56.08 (±14.01)	53.71 (41.11–77.10)	51.16 (±12.66)	53.62 (34.45–66.99)
AUC_{0-inf}, mg/h/L	57.64 (±14.42)	55.47 (41.47–77.67)	52.28 (±12.79)	55.41 (35.05–67.55)
$T_{1/2}$, h	2.30 (±0.40)	2.40 (1.70–2.90)	2.16 (±0.35)	2.10 (1.68–2.60)
CL, L/h/kg	0.29 (±0.07)	0.29 (0.20–0.38)	0.34 (±0.09)	0.30 (0.25–0.47)
MRT, h	2.14 (±0.53)	2.10 (1.48–3.10)	1.85 (±0.34)	1.70 (1.43–2.37)
V_d, L/kg	0.61 (±0.17)	0.60 (0.30–0.81)	0.62 (±0.20)	0.66 (0.35–0.86)

AUC_{0-12h} = area under the curve from time 0 to 12 h; AUC_{0-inf} = area under the curve extrapolated to infinity; $T_{1/2}$ = elimination half-life; CL = systemic clearance; MRT = mean residence time; V_d = total volume of distribution.

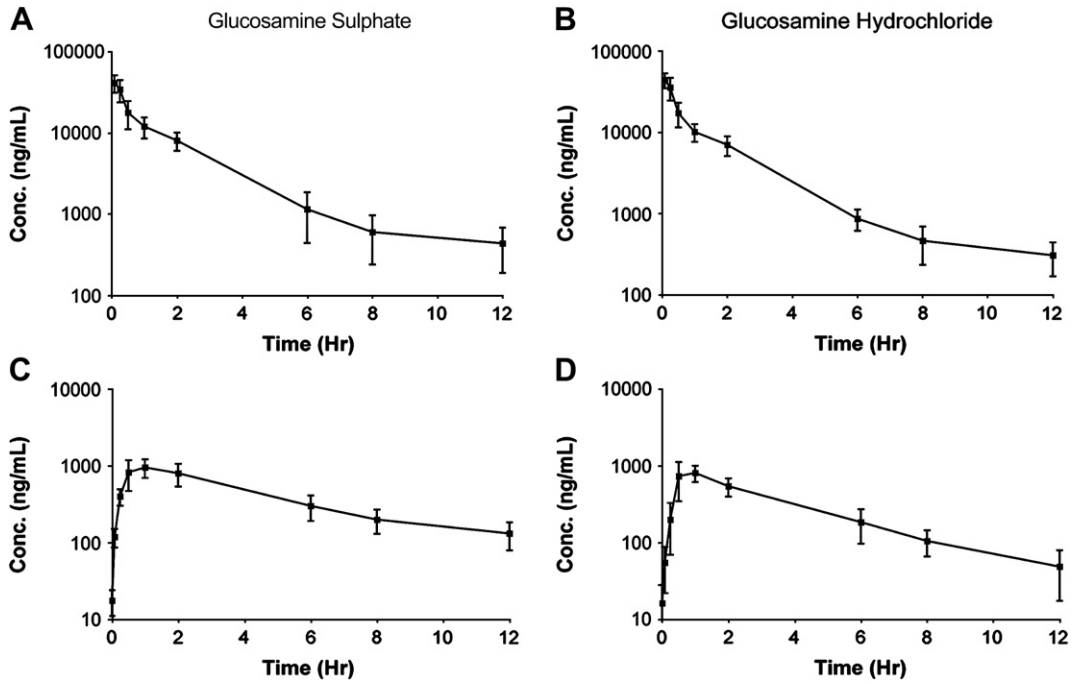

Fig. 2. Mean (±SD) plasma concentrations of glucosamine in horses ($n = 8$) following IV administration of 20 mg/kg glucosamine sulphate (A) or glucosamine hydrochloride (B); following NG administration of 20 mg/kg glucosamine sulphate (C) or glucosamine hydrochloride (D).

hydrochloride and glucosamine sulphate are shown in Fig. 2. At pre-dose, glucosamine plasma concentrations ranged from <10 ng/mL (below LOQ) to 39.1 ng/mL. The difference in endogenous plasma concentrations at baseline between treatment groups was not statistically different.

Following NG administration both glucosamine formulations were readily absorbed, reaching maximum plasma concentrations 30 min to 2 h post-treatment. The mean ± SD maximal plasma concentration was 1077.8 ± 171.0 ng/mL for glucosamine sulphate compared to 935.7 ± 267.8 ng/mL for glucosamine hydrochloride. The difference in median maximal plasma concentrations between the two treatments was not statistically significant. The peak plasma concentrations were followed by a progressive decline to mean ± SD plasma concentrations of 131.0 ± 52.1 ng/mL for glucosamine sulphate and 48.4 ± 31.0 ng/mL for glucosamine hydrochloride at 12 h post-dosing. At this time point, the plasma glucosamine levels were not significantly different from baseline levels in the glucosamine hydrochloride group, in contrast with the plasma concentrations 12 h post-treatment in the glucosamine sulphate treatment group ($P = 0.03$).

The descriptive statistics for the pharmacokinetic parameters of both types of glucosamine in plasma after NG dosing are presented in Table II. The median elimination half-life of glucosamine sulphate and glucosamine hydrochloride after oral treatment was 4.0 h (range: 2.8–4.7) and 3.0 h (range: 1.8–4.1), respectively ($P = 0.055$). The median AUC_{0-12h} and the median AUC_{0-inf} were higher ($P = 0.02$ and $P = 0.008$) following NG administration of glucosamine sulphate when compared to glucosamine hydrochloride. The median CL/F for glucosamine, on the other hand, was lower after oral glucosamine sulphate treatment compared to glucosamine hydrochloride treatment ($P = 0.008$). When the bioavailabilities of both formulations were compared, the median bioavailability was marginally,

Table II
Mean (±SD) and median (range) pharmacokinetic parameters of glucosamine in equine plasma (n = 8) following NG administration of 20 mg/kg glucosamine sulphate or glucosamine hydrochloride

	Glucosamine sulphate		Glucosamine hydrochloride	
	Mean (±SD)	Median (range)	Mean (±SD)	Median (range)
AUC_{0-12h}, mg/h/L	4.86 (±1.42)	4.58* (3.29–7.76)	3.23 (±0.79)	3.36* (1.86–4.27)
AUC_{0-inf}, mg/h/L	5.61 (±1.69)	5.08* (3.91–9.01)	3.47 (±0.86)	3.60* (2.01–4.59)
C_{max}, μg/mL	1.08 (±0.17)	1.13 (0.83–1.30)	0.94 (±0.27)	0.90 (0.58–1.30)
T_{max}, h	0.86 (±0.52)	0.80 (0.50–2.00)	0.81 (±0.26)	1.00 (0.50–1.00)
$T_{1/2}$, h	3.90 (±0.70)	4.00 (2.80–4.60)	2.88 (±0.80)	3.00 (1.80–4.10)
CL/F, L/h/kg	2.99 (±0.77)	3.09* (1.74–4.01)	5.10 (±1.54)	4.62* (3.61–8.27)
Bioavailability, %	10.80 (±5.60)	9.40 (5.20–21.70)	6.90 (±2.10)	6.10 (3.90–10.30)

AUC_{0-12h} = area under the curve from time 0 to 12 h; AUC_{0-inf} = area under the curve extrapolated to infinity; C_{max} = maximum plasma concentration; T_{max} = time to attainment of maximum plasma concentration; $T_{1/2}$ = elimination half-life; CL/F = apparent clearance.
*$P \leq 0.05$.

but not significantly higher for the glucosamine sulphate formulation compared to the hydrocholoride salt ($P = 0.08$). The median oral bioavailability of glucosamine sulphate was 9.4 and 6.1% for glucosamine hydrochloride.

QUANTIFICATION OF SYNOVIAL FLUID GLUCOSAMINE FOLLOWING IV AND NG DOSING

Endogenous glucosamine concentrations in synovial fluid prior to NG glucosamine administration ranged from <10 ng/mL (below LOQ) to 24.6 ng/mL. These values were not significantly different from endogenous plasma concentrations and the measured levels of endogenous glucosamine were not significantly different between treatment groups.

Following NG administration mean ± SD maximal synovial fluid glucosamine concentration was 153.6 ± 32.9 ng/mL with glucosamine sulphate administration and 92.7 ± 34.9 ng/mL following glucosamine hydrochloride administration (Table III). These values represent 14.3 and 9.9% of the mean maximal plasma concentrations achieved in the same groups, respectively. Twelve hours post-dosing mean ± SD synovial fluid concentrations were lowered to 36.4 ± 25.0 and 12.3 ± 6.2 ng/mL, respectively. The difference in synovial fluid concentrations attained following NG administration of glucosamine sulphate and glucosamine hydrochloride was statistically significant at 1 ($P = 0.0004$) and 6 h ($P = 0.02$), but not at 12 h ($P = 0.07$) post-treatment. However, 12 h post-treatment, the glucosamine levels in the synovial fluid were still significantly higher than baseline levels following oral administration of glucosamine sulphate ($P = 0.03$). In contrast, no significant difference in glucosamine levels between baseline and 12 h post-treatment occurred in the glucosamine hydrochloride group.

Following IV administration mean ± SD maximal synovial fluid concentrations were 1687.4 ± 809.2 ng/mL for glucosamine sulphate and 1487.7 ± 830.8 ng/mL for glucosamine hydrochloride, respectively, 4.02 and 3.38% of the mean maximal plasma concentrations. The difference in synovial fluid concentrations attained between treatment groups was not statistically significant following IV treatment.

Discussion

The present study demonstrates that significantly higher synovial fluid levels of glucosamine are attained following the administration of the crystalline glucosamine sulphate formulation (Dona®) when compared to glucosamine hydrochloride at the same therapeutic dose. Interestingly, the differences were observed following NG but not IV administration, indicating that absorption or metabolic factors may account for this difference. This could be explained by a higher absolute oral bioavailability of glucosamine with the glucosamine sulphate formulation compared to the hydrochloride salt. However, neither the difference in bioavailability nor the difference in mean maximal plasma concentrations observed reached statistical significance.

In the present study the median oral bioavailability of glucosamine following administration of glucosamine sulphate and glucosamine hydrochloride was 9.4 and 6.1%, respectively. Previous animal studies reported glucosamine bioavailability following glucosamine hydrochloride administration of 19% in rats[20], 12% in dogs[21] and 2–5% in horses[14,22]. These differences could be due to inter-species effects but also due to differences in study design and the use of different formulations as well as analytical methods in the various investigations. The absolute oral bioavailability of glucosamine following glucosamine sulphate administration in man has, in the past, been estimated to be 44%, following radiolabelled glucosamine administration[23]. This method does not allow differentiation between the unchanged drug and its metabolites, and consequently systemic availability may be overestimated[20,21].

It is possible that glucosamine could exert its beneficial effects on either the synovial membrane or other peri-articular structures. Glucosamine hydrochloride at a clinically relevant dose has been shown to partially inhibit the high turnover of the subchondral bone in OA[24] providing support for peri-articular effects. The plasma levels, rather than synovial levels, could therefore be more relevant to the debate on its action. In this study oral glucosamine administration resulted in maximal plasma concentrations that were higher following NG treatment with glucosamine sulphate (mean $C_{max} = 1077.8$ ng/ml or 6.2 μM) compared to glucosamine hydrochloride (mean $C_{max} = 935.7$ ng/ml or 5.2 μM), but, again, the difference was not statistically significant. Therefore the maximal plasma concentrations after oral administration of both glucosamine formulations cannot account for the more favourable clinical trial results observed with the prescription glucosamine sulphate formulation.

The AUC_{0-12h} and AUC_{0-inf} were significantly higher and the clearance of glucosamine from the plasma was significantly lower following NG administration of glucosamine sulphate compared to oral treatment with glucosamine hydrochloride, but there was no significant difference after IV administration. These data suggest that differences observed are likely due to the different composition of both formulations. The hydrochloride salt was administered as

Table III
Mean (±SD) and median (range) glucosamine levels (ng/mL) in equine synovial fluid (n = 8) following IV or NG administration of 20 mg/kg glucosamine sulphate or glucosamine hydrochloride

Time (h)	Glucosamine sulphate		Glucosamine hydrochloride	
	Mean (±SD)	Median (range)	Mean (±SD)	Median (range)
IV				
1	1687.4 (±809.2)	1493.9 (431.0–3169.0)	1487.7 (±830.8)	1565.4 (299.5–2539.5)
6	517.9 (±266.8)	469.3 (126.3–1023.4)	505.2 (±239.4)	521.7 (173.0–801.6)
12	500.7 (±296.6)	409.2 (208.8–1153.0)	480.2 (±251.8)	398.5 (235.5–890.0)
NG				
1	153.6 (±32.9)	158.3* (104.9–199.5)	92.7 (±34.9)	88.9* (50.0–156.7)
6	52.3 (±17.3)	57.3* (21.4–69.9)	19.9 (±10.3)	14.4* (10.4–35.8)
12	36.4 (±25.0)	24.2 (16.1–84.1)	12.3 (±6.2)	10.5 (blq–20.7)

Blq = below LOQ (<10 ng/mL).
*$P \leq 0.05$.

a pure substance and would not be expected to markedly influence the uptake or metabolism of glucosamine[14]. Glucosamine sulphate, on the other hand, was a commercially available crystalline formulation (Dona®) that contained other substances such as Na^+, Cl^-, aspartame, citric acid, sorbitol, polyethylene glycol 400 (PEG 400) and phenylalanine. Most of these ingredients are used to improve the palatability of the compounds. However, PEG 400 could potentially alter glucosamine solubility and release, and consequently influence its absorption, distribution and clearance[19]. Therefore, the beneficial effects attributed to glucosamine sulphate could in some way be related to its formulation and to the length of exposure to overall higher concentrations of glucosamine, that are available for penetration into the joints resulting in higher intra-synovial concentrations.

It is interesting to note that the mean maximal steady state concentration ($C_{ss,max}$) reported in humans following an equivalent, but repeated, dose of glucosamine sulphate was 1601.9 ng/mL (8.9 μM)[25] suggesting a higher bioavailability of glucosamine sulphate in humans. In the latter study plasma concentrations of glucosamine were still above baseline levels 48 h post-dosing and pharmacokinetics were at steady state after 3 consecutive days of once-a-day oral treatment with crystalline glucosamine sulphate[25]. The data in the study herein were generated following a single dose administration of glucosamine products. Based on the glucosamine half-life and elimination time of our study a ''steady state'' situation is not feasible without a dramatic increase in dosage or administration times. The same conclusion was made by Biggee *et al.* in an investigation in humans[26], and also in an investigation in dogs where no significant differences were present between single and multiple dose pharmacokinetics following oral administration of glucosamine hydrochloride[21].

Following NG administration mean ± SD maximal synovial fluid concentration was 153.6 ± 32.9 ng/mL (0.86 μM) for glucosamine sulphate and 92.7 ± 34.9 ng/mL (0.52 μM) for glucosamine hydrochloride and the difference in synovial fluid concentrations attained was statistically significant for up to 6 h post-treatment. However, the attained synovial fluid concentrations with both types of glucosamine were only ~10% of those obtained in plasma following NG dosing. These values confirm the data in our previous study on glucosamine hydrochloride in the same large animal model[14] but using different analytical methods fluorophore-assisted carbohydrate electrophoresis (FACE) and contrast with findings in a human study of glucosamine sulphate where synovial fluid concentrations were only approximately 25% lower than plasma concentrations[15]. In the latter study 1500 mg of crystalline glucosamine sulphate was administered orally, once daily, for 14 consecutive days and plasma and synovial fluid concentrations were considered to be at steady state. In the present study, on the other hand, the attained synovial fluid levels are the result of a single dose administration of glucosamine and clearance data indicate that a steady state would be impossible to achieve with the same formulations and dose regimens. Another possible explanation for the discrepancy between the plasma/synovial fluid concentration ratio between equine and human studies is a species difference. In the present study we used an equine model that has proven its suitability for pharmacokinetic studies of this type[14]. This model is particularly useful because of the ability to harvest large quantities of synovial fluid without lavage in normal animals. Persiani *et al.*, on the other hand, studied glucosamine sulphate pharmacokinetics in human OA patients[15]. While glucosamine is considered to be an ultrafiltrate of plasma[27], results from the latter study suggest that glucosamine does not diffuse readily from the circulation into the joint cavity. A species-specific difference in diffusion would be difficult to explain. The joints of the horses in the present study were clinically normal. In OA joints the synovial lining demonstrates non-specific changes of chronic, mild inflammation[28]. Previous experimental animal studies have demonstrated that the permeability of different substances through the synovial membrane changes in response to inflammation[29,30]. The observed difference in intra-articular glucosamine diffusion might therefore be explained as the result of inflammation of the synovium in OA joints. However, in the above mentioned study, no information was supplied on the inflammation status of the joints[15]. To the contrary, one could also argue that moderate joint distension in response to inflammation could have a dilutional effect on the measured glucosamine levels[31]. Further studies are warranted to investigate the effect of joint inflammation on the concentrations of glucosamine attained in the synovial fluid.

Most of glucosamine's beneficial effects were demonstrated in the past by *in vitro* studies with concentrations far exceeding those that can be obtained in plasma or synovial fluid after oral administration of recommended doses. Recent investigations demonstrated that glucosamine may also have beneficial effects on articular cartilage explants at biologically relevant doses[32,33]. However, the glucosamine concentrations employed in the latter experiments approximate the plasma concentrations that can be achieved after NG dosing of glucosamine, but are higher than the concentrations we achieved in the synovial fluid. Although the treatment of horses with oral crystalline glucosamine sulphate resulted in significantly higher synovial fluid concentrations of glucosamine compared to glucosamine hydrochloride, it remains questionable if the difference in attained synovial fluid concentrations, based on current *in vitro* studies, explains the favourable results attained with glucosamine sulphate administration in some clinical trials in humans. The levels attained were very low and below those observed to have *in vitro* effects on cartilage.

It could be argued that a limitation of the study design was that an effect of time on glucosamine pharmacokinetics could not be measured. However, no differences were detected between baseline glucosamine levels measured at the outset of the different arms of the study in the plasma or synovial fluid indicating that the design was robust for the questions addressed. An additional limitation is that multiple arthrocenteses at weekly intervals could alter the levels of glucosamine measured in synovial fluid by inducing inflammation. In a previous study in horses from our laboratory[34] the effect of repeated synoviocentesis on synovial inflammation was assessed and no differences in inflammation parameters were detected 1 week after synoviocentesis. Another equine study indicated that the timing and duration of the arthrocentesis effects on biomarkers of joint inflammation varied per biomarker and the author recommended waiting 1 week before repeating arthrocenteses when studying markers of joint disease[35]. Combined, these studies suggest that repeated arthrocentesis at 1-week intervals in this animal model does not alter inflammation parameters and consequently should not alter kinetics (entry and clearance) of the glucosamine in the joint.

In conclusion, following oral administration of crystalline glucosamine sulphate (Dona®) significantly higher synovial fluid concentrations of glucosamine are attained when compared to an equivalent dose of glucosamine hydrochloride. Whether this difference in synovial levels of glucosamine attained is translated into a therapeutic effect on the joint tissues remains to be elucidated.

Conflict of interest

None of the authors has received any financial contribution from commercial sources for this work, nor do we have other financial interests that would create a potential conflict of interest with regards to the work.

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References

1. Bruyere O, Pavelka K, Rovati LC, Gatterova J, Giacovelli G, Olejarova M, *et al*. Total joint replacement after glucosamine sulphate treatment in knee osteoarthritis: results of a mean 8-year observation of patients from two previous 3-year, randomised, placebo-controlled trials. Osteoarthritis Cartilage 2008;16:254–60.
2. Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacovelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. Arch Intern Med 2002;162(18):2113–23.
3. Reginster JY, Deroisy R, Rovati LC, Lee RL, Lejeune E, Bruyere O, *et al*. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. Lancet 2001; 357(9252):251–6.
4. Hughes R, Carr A. A randomized, double-blind, placebo-controlled trial of glucosamine sulphate as an analgesic in osteoarthritis of the knee. Rheumatology (Oxford) 2002;41(3):279–84.
5. Cibere J, Kopec JA, Thorne A, Singer J, Canvin J, Robinson DB, *et al*. Randomized, double-blind, placebo-controlled glucosamine discontinuation trial in knee osteoarthritis. Arthritis Rheum 2004; 51(5):738–45.
6. Cibere J, Thorne A, Kopec JA, Singer J, Canvin J, Robinson DB, *et al*. Glucosamine sulfate and cartilage type II collagen degradation in patients with knee osteoarthritis: randomized discontinuation trial results employing biomarkers. J Rheumatol 2005;32(5):896–902.
7. Tiraloche G, Girard C, Chouinard L, Sampalis J, Moquin L, Ionescu M, *et al*. Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. Arthritis Rheum 2005;52(4):1118–28.
8. Reginster JY, Bruyere O, Neuprez A. Current role of glucosamine in the treatment of osteoarthritis. Rheumatology (Oxford) 2007;46(5):731–5.
9. Altman RD, Abramson S, Bruyere O, Clegg D, Herrero-Beaumont G, Maheu E, *et al*. Commentary: osteoarthritis of the knee and glucosamine. Osteoarthritis Cartilage 2006;14(10):963–6.
10. 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(8):795–808.
11. Herrero-Beaumont G, Ivorra JA, Del Carmen Trabado M, Blanco FJ, Benito P, Martin-Mola E, *et al*. Glucosamine sulfate in the treatment of knee osteoarthritis symptoms: a randomized, double-blind, placebo-controlled study using acetaminophen as a side comparator. Arthritis Rheum 2007;56(2):555–67.
12. Russell AS, Aghazadeh-Habashi A, Jamali F. Active ingredient consistency of commercially available glucosamine sulfate products. J Rheumatol 2002;29(11):2407–9.
13. Oke S, Aghazadeh-Habashi A, Weese JS, Jamali F. Evaluation of glucosamine levels in commercial equine oral supplements for joints. Equine Vet J 2006;38(1):93–5.
14. 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(1):181–91.
15. 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(7):764–72.
16. Towheed TE, Maxwell L, Anastassiades TP, Shea B, Houpt J, Robinson V, *et al*. Glucosamine therapy for treating osteoarthritis. Cochrane Database Syst Rev 2005;2:CD002946.
17. Beaudry F, Vachon P. Determination of glucosamine in horse plasma by liquid chromatography tandem mass spectrometry. Biomed Chromatogr 2008;22(1):1–4.
18. Bioanalytical method validation. In: Guidance for Industry. Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), http://www.fda.gov/cder/guidance/4252fnl.pdf; May 2001.
19. Rowland M, Towser TN. Clinical Pharmacokinetics: Concepts and Applications. Philadelphia: Lippincott Williams and Wilkins; 1995.
20. Aghazadeh-Habashi A, Sattari S, Pasutto F, Jamali F. Single dose pharmacokinetics and bioavailability of glucosamine in the rat. J Pharm Pharm Sci 2002;5(2):181–4.
21. 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(6):217–25.
22. Du J, White N, Eddington ND. The bioavailability and pharmacokinetics of glucosamine hydrochloride and chondroitin sulfate after oral and intravenous single dose administration in the horse. Biopharm Drug Dispos 2004;25(3):109–16.
23. Setnikar I, Palumbo R, Canali S, Zanolo G. Pharmacokinetics of glucosamine in man. Arzneimittelforschung 1993;43(10):1109–13.
24. Wang SX, Laverty S, Dumitriu M, Plaas A, Grynpas MD. The effects of glucosamine hydrochloride on subchondral bone changes in an animal model of osteoarthritis. Arthritis Rheum 2007;56(5):1537–48.
25. Persiani S, Roda E, Rovati LC, Locatelli M, Giacovelli G, Roda A. Glucosamine oral bioavailability and plasma pharmacokinetics after increasing doses of crystalline glucosamine sulfate in man. Osteoarthritis Cartilage 2005;13(12):1041–9.
26. Biggee BA, Blinn CM, McAlindon TE, Nuite M, Silbert JE. Low levels of human serum glucosamine after ingestion of glucosamine sulphate relative to capability for peripheral effectiveness. Ann Rheum Dis 2006;65(2):222–6.
27. Levick JR. Microvascular architecture and exchange in synovial joints. Microcirculation 1995;2(3):217–33.
28. Moskowitz RW. Osteoarthritis: Diagnosis And Medical/Surgical Management. 4th edn. Philadelphia: Wolters Kluwer Health/Lippincott Williams and Wilkins; 2007.
29. Errecalde JO, Carmely D, Marino EL, Mestorino N. Pharmacokinetics of amoxycillin in normal horses and horses with experimental arthritis. J Vet Pharmacol Ther 2001;24(1):1–6.
30. Firth EC, Klein WR, Nouws JF, Wensing T. Effect of induced synovial inflammation on pharmacokinetics and synovial concentration of sodium ampicillin and kanamycin sulfate after systemic administration in ponies. J Vet Pharmacol Ther 1988;11(1):56–62.
31. Kraus VB, Huebner JL, Fink C, King JB, Brown S, Vail TP, *et al*. Urea as a passive transport marker for arthritis biomarker studies. Arthritis Rheum 2002;46(2):420–7.
32. Neil KM, Orth MW, Coussens PM, Chan PS, Caron JP. Effects of glucosamine and chondroitin sulfate on mediators of osteoarthritis in cultured equine chondrocytes stimulated by use of recombinant equine interleukin-1beta. Am J Vet Res 2005;66(11):1861–9.
33. Chan PS, Caron JP, Rosa GJ, Orth MW. Glucosamine and chondroitin sulfate regulate gene expression and synthesis of nitric oxide and prostaglandin E(2) in articular cartilage explants. Osteoarthritis Cartilage 2005;13(5):387–94.
34. Celeste C, Ionescu M, Robin Poole A, Laverty S. Repeated intraarticular injections of triamcinolone acetonide alter cartilage matrix metabolism measured by biomarkers in synovial fluid. J Orthop Res 2005;23(3):602–10.
35. van den Boom R, van de Lest CH, Bull S, Brama RA, van Weeren PR, Barneveld A. Influence of repeated arthrocentesis and exercise on synovial fluid concentrations of nitric oxide, prostaglandin E2 and glycosaminoglycans in healthy equine joints. Equine Vet J 2005; 37(3):250–6.