Synovial Fluid Levels and Serum Pharmacokinetics in a Large Animal Model Following Treatment With Oral Glucosamine at Clinically Relevant Doses

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Objective. To examine the concentration of glucosamine in the synovial fluid and its pharmacokinetics in serum in a large animal model following dosing with glucosamine HCl at clinically relevant levels.

Methods. Eight adult female horses were studied. After an overnight fast, glucosamine HCl (20 mg/kg of body weight) was administered by either nasogastric (NG) intubation or intravenous (IV) injection. Blood samples were collected before dosing and at 5, 15, 30, 60, 120, 180, 240, 360, 480, and 720 minutes after dosing. Synovial fluid samples were collected from the radiocarpal joints 48 hours before dosing and at 1 and 12 hours after dosing. Glucosamine was assayed by fluorophore-assisted carbohydrate electrophoresis.

Results. The maximum concentration of glucosamine in serum reached ~300 μM (~50 μg/ml) following IV dosing and ~6 μM (~1 μg/ml) following NG dosing. Synovial fluid concentrations reached 9–15 μM with IV dosing and 0.3–0.7 μM with NG dosing, and remained elevated (range 0.1–0.7 μM) in most animals even at 12 hours after dosing. Following NG dosing, the median serum maximal concentration of 6.1 μM (range 4.38–7.58) was attained between 30 minutes and 4 hours postdose. The mean apparent volume of distribution was 15.4 liters/kg, the mean bioavailability was 5.9%, and the mean elimination half-life was 2.82 hours.

Conclusion. Clinically relevant dosing of glucosamine HCl in this large monogastric animal model results in serum and synovial fluid concentrations that are at least 500-fold lower than those reported to modify chondrocyte anabolic and catabolic activities in tissue and cell culture experiments. We conclude that the apparent therapeutic benefit of dietary glucosamine on pain and joint space width in humans and animals may be secondary to its effects on nonarticular tissues, such as the intestinal lining, liver, or kidney, since these may be exposed to much high levels of glucosamine following ingestion.

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Glucosamine is now widely taken in the US as a dietary supplement (recommended dosage 20 mg/kg/day) to relieve the discomfort of osteoarthritis (OA)–related joint pain. In Europe, a patented formulation of glucosamine (glucosamine sulfate) is a prescription drug, which is also widely reported to achieve cartilage-protective effects in knee OA (1,2). A quality assessment and meta-analysis of 6 placebo-controlled clinical trials using both glucosamine HCl (2 studies) and glucosamine sulfate (4 studies) concluded that some degree of efficacy appears probable in treating symptoms of knee OA (3). More recently, 2 meta-analyses of a large number of randomized control trials with glucosamine sulfate consistently concluded that the drug is both safe and effective for the treatment of symptomatic OA (4,5). Currently, a 9-center study funded by the National Institutes of Health, the Glucosamine/Chondroitin Ar-

thritis Intervention Trial (GAIT), is under way to examine the possible benefits of dietary glucosamine HCl for knee OA.

Despite this perceived benefit (3,5–11), information on the absorption and serum pharmacokinetics of dietary glucosamine is very limited. Studies in rats, dogs, and humans have been conducted with ^{14}C-glucosamine HCl (mixed with unlabeled glucosamine sulfate), but these did not distinguish between glucosamine and metabolites or degradation products of radiolabeled glucosamine (12–14). Since it was not possible to detect glucosamine by high-performance liquid chromatography in human serum after a single high dose of 7.5 gm of glucosamine sulfate (12), the results suggested poor intestinal absorption or marked first-pass uptake by intestinal or liver cells. Recently, glucosamine pharmacokinetics in serum after oral dosing of rats (15) and horses (16) have been reported. Both of these studies showed that following administration of 125–350 mg/kg (~10 times the recommended clinical dose), the maximum serum glucosamine level was ~10 μg/ml (60 μM).

It has become widely assumed by the general public and the nutriceutical industry that glucosamine achieves its apparent beneficial effects on joint function by acting as a precursor supply for the production of cartilage glycosaminoglycans, such as chondroitin sulfate. However, it is important to note that there have been no published studies of animals or humans in which pre- or postdose concentrations of glucosamine were chemically measured in synovial fluid. This is because there have been no available assay methods, and "native" (undiluted) synovial fluid is very difficult to obtain in humans and small animals.

In the present study, we successfully addressed these problems by using an equine model and a novel assay method for glucosamine, fluorophore-assisted carbohydrate electrophoresis (FACE) (17–19). FACE is currently the most specific, sensitive, and inexpensive method of analyzing monosaccharides in biologic samples, including body fluids. Most importantly, since the reported benefits of dietary glucosamine HCl and glucosamine sulfate are thought to be on the diarthrodial joints, we also measured synovial fluid concentrations of the monosaccharide. In the horse, a sufficient volume of synovial fluid can be sampled without lavage from joints in the standing, awake animal. Fluid collection can be performed without anesthesia, thus avoiding potential drug-induced alterations in circulatory parameters and pharmacokinetics of the compound under study. The successful quantitation of glucosamine in both blood and synovial fluid we report here provides a methodologic framework for additional short-term and long-term pharmacokinetic studies in humans with both glucosamine HCl and patented commercial products such as glucosamine sulfate.

MATERIALS AND METHODS

Materials. Glucosamine HCl and N-acetylglucosamine (GlcNAc) were obtained from Sigma-Aldrich (St. Louis, MO), Dowex-50 H$^+$ resin was from Bio-Rad (Richmond, CA), and FACE reagents were obtained as described elsewhere (17). All other chemicals were of the highest purity available.

Animal studies. Eight adult female horses, with a mean age of 10 years (range 6–15 years) and a mean body weight of 500 kg (range 442–546 kg) were used in a 2-way, intravenous (IV) and nasogastric (NG), application crossover study with a 1-week washout period between IV and NG reverse dosings. All animals were free of clinical evidence of joint disease and were randomly assigned to the experimental groups. Analytical-grade glucosamine HCl (catalog no. G 1514; Sigma-Aldrich) was dissolved at 100 mg/ml in 0.9% sterile saline, pH 6.0, and administered at a dose of 20 mg/kg of body weight by IV injection or NG intubation to animals that had fasted overnight. NG dosing included 500 ml of 0.9% (weight/volume) sterile saline immediately after glucosamine administration. IV injection was via a catheter inserted into the right jugular vein and was followed by a saline flush to assure complete drug administration, and reverse dosing was performed a week later.

For pharmacokinetic evaluations, blood samples were collected into nonheparinized tubes via an IV catheter in the left jugular vein and were obtained immediately before dosing and at 5, 15, 30, 60, 120, 180, 240, 360, 480, and 720 minutes after dosing. Synovial fluid was collected by aseptic arthrocentesis, within 48 hours before dosing (predose) from both radiocarpal joints and at 1 hour postdosing from the left joint and 12 hours postdosing from the right joint. Synovial fluid and blood samples were kept on ice, centrifuged at 5,000g for 20 minutes at 4°C, and the cell-free supernatants were removed and stored at –70°C (for up to 4 weeks) until assayed for glucosamine. Horses were evaluated twice weekly by flexion tests and joint palpation for evidence of heat, pain, or effusion of the radiocarpal joints and lameness.

The experimental protocol was preapproved by the Institutional Animal Care and Use Committee of the University of Montreal. The protocol was designed in accordance with the guidelines of the Canadian Council on Animal Care.

Purification of glucosamine from serum and synovial fluid. Glucosamine levels were determined in quadruplicate portions from samples collected at each time point. Sera (generally, 200 μl) and synovial fluids (generally, 500 μl) were pipetted into 1.7-ml microcentrifuge tubes, placed on ice, and mixed with 3 volumes of ice-cold ethanol, 5 mM sodium acetate, and then maintained at –20°C for 4 hours. Precipitates were pelleted by centrifugation at 15,000g in an Eppendorf microcentrifuge, and the supernatants were recovered and dried by SpeedVac evaporation.

To remove excess glucose and salts from the dried supernatants, they were resuspended in 400 μl of water,

adjusted to pH 4.5–5.5 by addition of 2–5 μl of 5% (volume/volume) acetic acid in water, and mixed with 250 μl of Dowex-50 H^+ resin that was prepacked into a 0.45-μm microfiltration insert (Millipore, Bedford, MA). The mixture was kept at room temperature for 10 minutes, and then the liquid was removed by centrifugation at 10,000g for 1 minute. The resins were washed with a total of 4 ml of deionized water, and resin-bound glucosamine was recovered with 250 μl of 1M HCl.

For acetylation, the acid was removed by SpeedVac evaporation, the residues were dissolved in 50 μl of freshly prepared 200 mM sodium bicarbonate in water, and 30 μl of 1% (v/v) aqueous acetic anhydride was added. Samples were maintained at room temperature for 20 minutes. The mixtures, now containing GlcNAc, were desalted by centrifugation through Dowex-50 H^+ resin (125-μl packed bed-volume) and dried by SpeedVac evaporation. In pilot experiments, ^{3}H-glucosamine (1×10^5 cpm) was included before the first Dowex-50 H^+ step, and the recovery of the radiolabel in ^{3}H-GlcNAc was 65 ± 5% (mean ± SD).

Quantitation of glucosamine as the N-acetyl derivative by FACE. Dried samples containing GlcNAc (see above) were fluorotagged with 2-aminoacridone and sodium cyanoborohydride as described previously (19). The reaction was terminated by addition of 20 μl of 25% (v/v) glycerol, and the samples were mixed and stored at –20°C until electrophoretic separation on 20% acrylamide gels. The gels were prepared as follows. Cassettes were assembled in a plastic pouch using two 10 × 10–cm glass plates separated by 0.8-mm spacers, and then placed into a Joey vertical protein gel caster (Owl Separating Systems, Portsmouth, NH). Each cassette was filled to a height of ~7 cm with degassed separating gel solution (20% acrylamide/N-methylbisacrylamide [38.5:1.5]), 2.5% [v/v] glycerol, 45 mM Tris acetate, pH 7.0, 0.25% [w/v] ammonium persulfate, and 0.5% [v/v] TEMED) and then overlaid with water. After polymerization (5–10 minutes at room temperature), the water was discarded, 2 ml of a degassed stacking gel solution (8% acrylamide/N-methylbisacrylamide [38.5:5], 2.5% [v/v] glycerol, 45 mM Tris acetate, pH 7.0, 0.25% [w/v] ammonium persulfate, and 0.5% [v/v] TEMED) was added, and an 8-well comb was inserted during polymerization. Prepared gels (sealed in the plastic pouch) were stored at 4°C for up to 3 weeks.

For electrophoresis, gels were removed from the pouch, plates were washed with water, and the combs were released from the stacking gel. The sample wells were rinsed with electrophoresis buffer (0.1M Tris base, 0.09M boric acid, 5 mM EDTA, pH 8.3) and cassettes were placed in a Glyco electrophoresis gel tank (Prozyme, San Leandro, CA) that had been filled with precooled electrophoresis buffer. Aliquots (7 μl) of the fluorotagged samples were loaded, and products were separated by electrophoresis at 500V (~40 mA per gel) for 45 minutes at 4°C.

For image analyses, glass plates were removed, and the gel was placed directly onto an ultraviolet light box. Images were captured, recorded, and fluorescent bands were quantitated using the Kodak EDAS Imaging System and corresponding software. The limit of quantitation (LOQ) with this method is ~20 pmoles of glucosamine in 200 μl of biologic fluid, or ~100 nM (19).

Pharmacokinetic analysis. Pharmacokinetic parameters for serum glucosamine were calculated using noncompartmental methods (20). The area under the curve from time 0 to the last measurable concentration (AUC_{0-t}) was calculated using the linear trapezoidal rule. A rate constant of elimination (K_{el}) was calculated using the last measurable concentrations, and the elimination half-life ($T_{1/2}$) was calculated using the formula $0.693/K_{el}$. Sampling was done over a period of at least 5 half-lives. The AUC extrapolated to infinity (AUC_{inf}) was calculated using the formula $AUC_{0-t} + C_{last}/K_{el}$, where C_{last} was the last measurable concentration in serum.

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

RESULTS

Development and validation of the FACE assay for serum glucosamine. The application of the FACE assay to the current project required optimization of the following experimental steps: first, reproducible high-yield purification of glucosamine from biologic fluids (including whole blood, plasma, serum, and synovial fluid) and its subsequent conversion to the N-acetyl derivative; and second, reductive amination of GlcNAc with the fluorophore 2-aminoacridone for FACE analysis.

Validation of these steps (see below) was performed as follows. The percentage recovery of standard glucosamine over a wide range of concentrations (50 pmoles to 9.2 nmoles) through Dowex-50 H^+ purification, acetylation, and desalting was established first (Figure 1). Three sets of 8 samples of GlcNAc (set 1) or glucosamine (sets 2 and 3) were prepared in water. Set 1 samples were dried and directly derivatized with 2-aminoacridone. Set 2 samples were adjusted to 0.3M sodium bicarbonate, acetylated, desalted through Dowex-50 H^+, dried, and derivatized with 2-aminoacridone. Set 3 samples were adjusted to 0.125% (v/v) acetic acid, applied to Dowex-50 H^+, washed with 4 ml of water, and eluted with 250 μl of 1M HCl; the eluent was dried, acetylated, desalted through Dowex-50 H^+, dried again, and derivatized with 2-aminoacridone. All 24 samples were analyzed by FACE, and the images
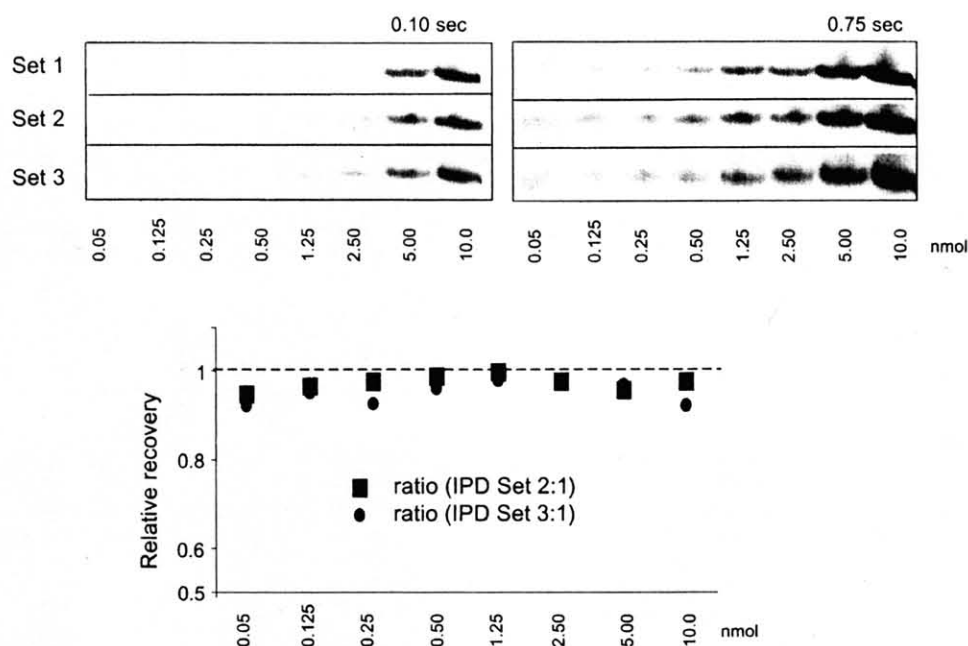
Figure 1. Quantitation of glucosamine as the *N*-acetylated fluorotagged derivative by fluorophore-assisted carbohydrate electrophoresis. A range of concentrations of *N*-acetylglucosamine standard (set 1) and glucosamine HCl standard acetylated and desalted (sets 2 and 3, as described in Materials and Methods) were derivatized with 2-aminoacridone and separated by electrophoresis. **Top,** Typical gel images obtained after 0.1 and 0.75 seconds of exposure. **Bottom,** Relative recovery of the hexosamine at each concentration tested. The range of concentrations calculated from the integrated pixel densities (IPDs) obtained at the different exposure times are shown as a ratio of the IPDs of sets 2 and 3 to the IPDs of set 1. Data for set 1 were defined as 100% recovery (broken line).

were exposed for 0.1, 0.25, and 0.75 seconds (Figure 1 shows 0.1- and 0.75-second exposures).

The integrated pixel density (IPD) data for the 50-pmole to 1.15-nmole range were derived from the 0.75-second exposure, for the 0.25–2.5-nmole range from the 0.25-second exposure (data not shown), and for the 2.5–10-nmole range from the 0.1-second exposure. The ratio of IPD values (where the data for set 1 were defined as 100% recovery) was plotted (Figure 1) and showed that the recovery of glucosamine through the steps of acetylation and desalting was >95% for all samples and the recovery through Dowex-50 H$^+$ binding and acid elution, followed by acetylation and desalting was >90%. These data clearly illustrate that the assay procedure provides both high reproducibility and high recovery of standard glucosamine as its acetylated derivative over the concentration range of interest for analysis of biologic fluids.

Figure 2A shows the procedure for optimizing the experimental steps of the FACE assay in biologic fluids. To examine the validity of this assay method in biologic fluids, 200-μl portions of predose whole blood, plasma, serum, and synovial fluid samples (Figure 2B) were prepared from 2 animals. Samples were supplemented with 0, 0.5, 2.5, or 5.0 nmoles of glucosamine and stored at –70°C for 1 week prior to processing. Analysis of these samples, along with standard GlcNAc that had been derivatized directly, showed that the same reproducible high-yield recovery (>90%) was obtained with each preparation, thereby validating this method for purification and quantitation over a wide concentration range and for a range of biologic fluids.

Quantitation of serum glucosamine following IV dosing. A single IV dose of 20 mg/kg of glucosamine HCl was well tolerated in all animals, and all predose serum concentrations of the monosaccharide were below the LOQ (100 n*M*). Serum concentrations at 5, 15, 30, 60, 120, 180, 240, 360, 480, and 720 minutes following IV
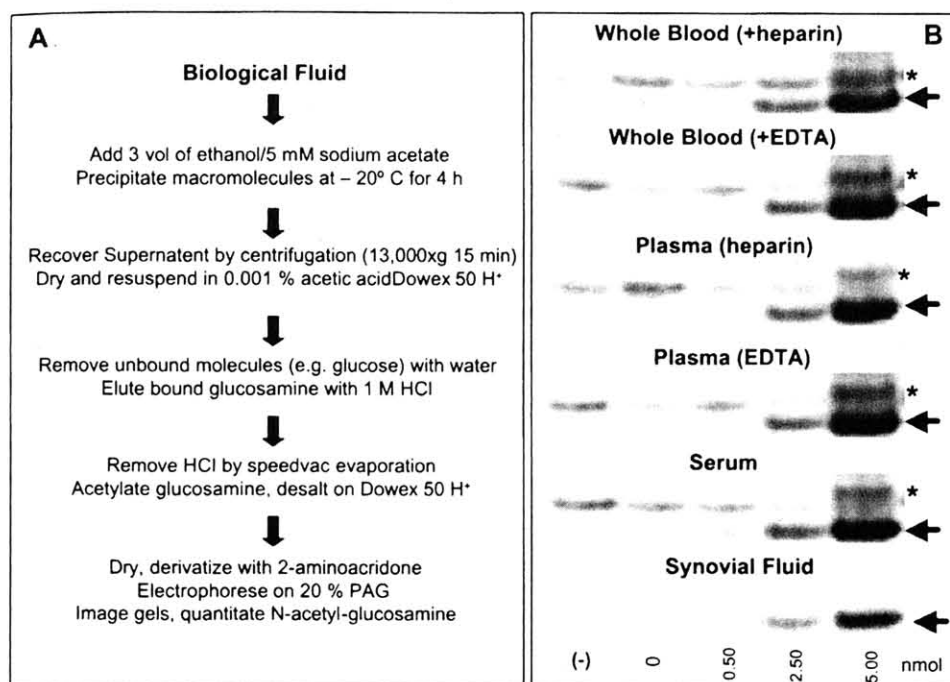
Figure 2. Assay of various concentrations of glucosamine HCl standard added to horse blood, plasma, serum, or synovial fluid. Predose blood (+heparin or +EDTA), plasma (and serum derived from the plasma), and synovial fluid samples were adjusted to the given range of glucosamine HCl concentrations by addition of standard, and then prepared for fluorophore-assisted carbohydrate electrophoresis assay by the procedure outlined in **A** (see Materials and Methods for details). Typical gel images (0.75-second exposure) are shown in **B**. The electrophoretic migration of the *N*-acetylglucosamine is indicated by **arrows**, copurified glucose is indicated with an **asterisk**. Samples in lanes marked – were supplemented with 2.5 nmoles of glucosamine HCl that was not acetylated. PAG = polyacrylamide gels.

administration in all 8 animals are presented on both linear (Figure 3A) and semilogarithmic (Figure 3B) scales. As shown in Figure 3A, the glucosamine concentration declined rapidly over the first 2 hours after IV administration in all animals, but the level remained elevated (at ~50 μM) for the following 1–2 hours and reached baseline levels by 6–12 hours. The serum concentration peaked at 15 minutes for 6 animals and at 5 minutes for 2 animals, and the clearance curves between animals were quite variable. This may be due to the variation in administration times (range 30–60 seconds) of the relatively large injection volumes used (~100 ml), as well as the heterogeneity of the circulation, which may have led to nonuniform mixing of the monosaccharide with the total blood volume.

The data shown in Figure 3 were used to generate a statistical analysis for the pharmacokinetic parameters of IV glucosamine HCl in serum. These values are presented in Table 1. The AUC_{0-t} and AUC_{inf} were

similar, confirming that blood sampling over 12 hours was adequate to characterize the pharmacokinetics for the monosaccharide in this model. The $T_{1/2}$ was highly variable, ranging from 0.78 to 3.96 hours, consistent with major interanimal variation in tissue clearance. The systemic clearance of glucosamine (0.210 liters/hour/kg [3.5 ml/minute/kg]) was markedly lower than the reported hepatic blood flow in horses (~1.2 liters/hour/kg) (21), suggesting a rather low hepatic uptake of glucosamine after IV administration. At the same time, the total volume of distribution (0.347 liters/kg) after IV administration was higher than the reported plasma volume in horses (~0.03 liters/kg) (22), which is consistent with distribution of the monosaccharide into interstitial fluids in the peripheral tissues.

Quantitation of serum glucosamine following NG dosing. Serum concentrations at all time points following NG administration for all 8 animals are presented on
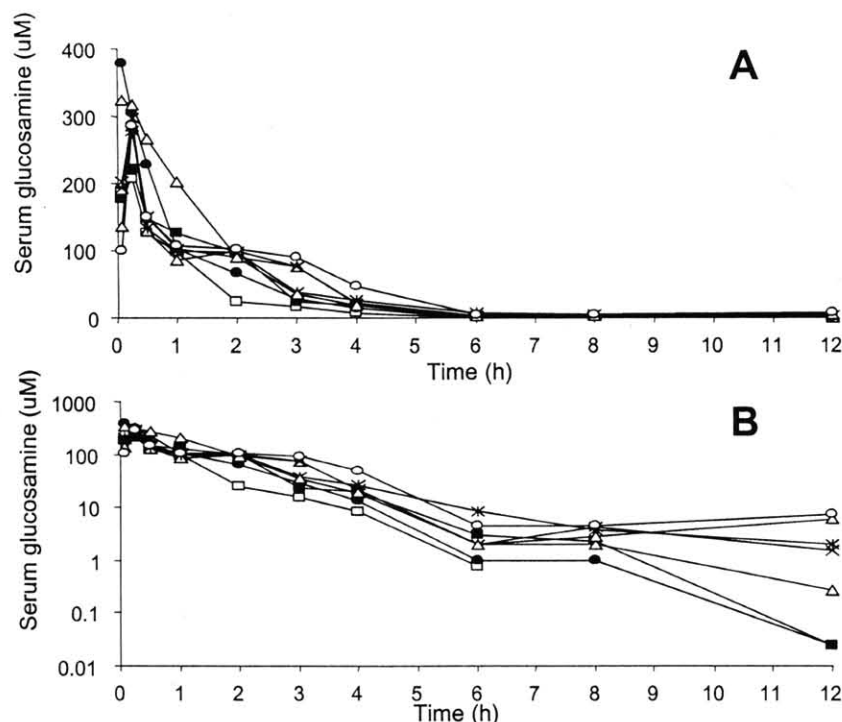
Figure 3. Serum concentrations of glucosamine following intravenous dosing. The concentration of glucosamine in serum was determined by fluorophore-assisted carbohydrate electrophoresis for 8 different horses at various times following a single intravenous dose of glucosamine HCl at 20 mg/kg. Each symbol represents a different animal. For clarity, data are plotted on **A,** linear and **B,** log-linear scales.

both linear (Figure 4A) and semilogarithmic (Figure 4B) scales. Glucosamine was consistently below the LOQ at predose, and not surprisingly, concentrations were markedly (~30-fold) lower than those found after IV dosing with the same amount of glucosamine (Figure 3).

The descriptive statistics for the pharmacokinetic parameters of glucosamine in serum after NG adminis-

Table 1. Pharmacokinetic parameters of glucosamine in the serum of horses (n = 8) following intravenous administration of 20 mg/kg*

	Mean (CV%)	Median (range)
AUC_{0-1}, μmoles/hour/liter	460 (21.2)	484 (269–577)
AUC_{inf}, μmoles/hour/liter	438 (23.3)	453 (269–619)
$T_{1/2}$, hours	2.05 (60.3)	1.82 (0.78–3.96)
CL, liters/hour/kg	0.210 (29.2)	0.190 (0.15–0.344)
MRT, hours	1.80 (38.5)	1.70 (0.98–3.16)
V_d, liters/kg	0.347 (17.6)	0.343 (0.263–0.474)

* CV% = coefficient of variation; AUC_{0-1} = area under the curve from time 0 to the last measurable concentration; AUC_{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.

tration are presented in Table 2. Individual animals exhibited quite variable serum concentration profiles, presumably due to interanimal variation in gastrointestinal function, absorption, and clearance. As a result, the calculated $T_{1/2}$ ranged between 1.45 and 6.17 hours. Following NG administration, the mean bioavailability of glucosamine HCl was 5.9%, a value comparable with the 2.5% and 12% previously determined for horses and dogs, respectively (16,23).

Quantitation of synovial fluid glucosamine following IV and NG dosing. Glucosamine was below the LOQ in all predose synovial fluids, but was detectable after 1 hour with both routes of administration in all animals (except for horse 2 after NG dosing). Concentrations ranged from 9 μM to 15 μM after IV dosing and from 0.3 μM to 0.7 μM after NG dosing (data not shown). These values represent ~4% and ~9%, respectively, of the maximum serum concentrations achieved in the same animals. In contrast to the nearly complete clearance of glucosamine from serum at 6 hours post-dose (Figures 3 and 4), glucosamine was still detectable
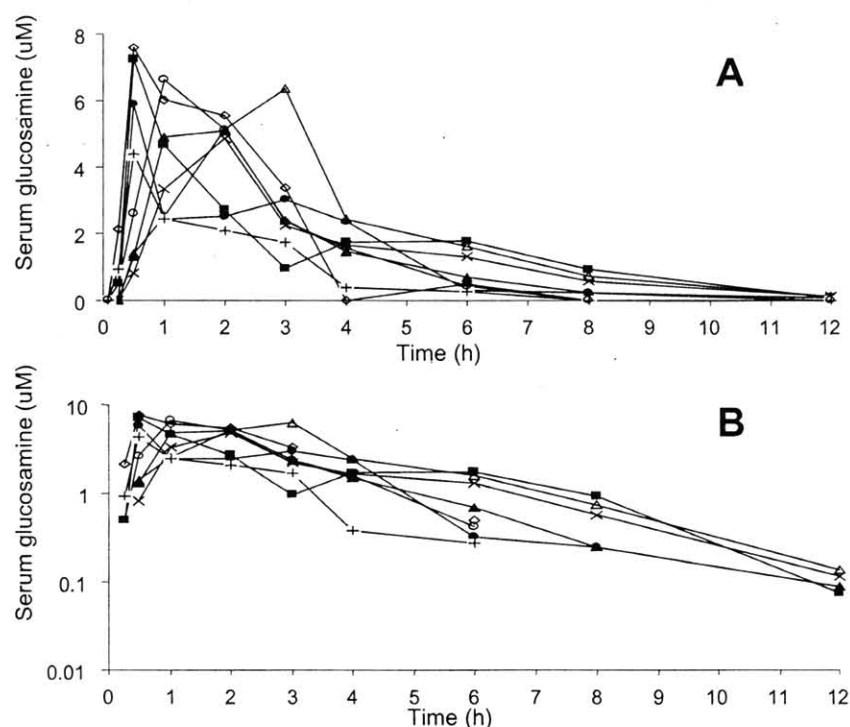
Figure 4. Serum concentrations of glucosamine following nasogastric dosing. The concentration of glucosamine in serum was determined by fluorophore-assisted carbohydrate electrophoresis for 8 different horses at various times following a single nasogastric dose of glucosamine HCl at 20 mg/kg. Each symbol represents a different animal. For clarity, data are plotted on **A**, linear and **B**, log-linear scales.

in synovial fluids even at 12 hours after dosing, at concentrations ranging from 0.1 μM to 0.7 μM (data not shown). Although fluorescence signals obtained with synovial fluids after NG dosing were very low, they were

Table 2. Pharmacokinetic parameters of glucosamine in the serum of horses (n = 8) following nasogastric administration of 20 mg/kg*

	Mean (CV%)	Median (range)
AUC_{0-1}, μmoles/hour/liter	21.4 (23.6)	20.8 (11.8–29.5)
AUC_{inf}, μmoles/hour/liter	23.9 (28.8)	22.9 (12.4–33.3)
C_{max}, μmoles/hour/liter	5.98 (19.3)	6.10 (4.38–7.58)
T_{max}, hours	1.375 (91)	0.75 (0.5–4.00)
$T_{1/2}$, hours	2.82 (57.3)	2.25 (1.45–6.17)
CL/F, liters/hour/kg	4.23 (34.7)	4.06 (2.79–7.48)
V_{area}/F, liters/kg	15.4 (36.1)	14.7 (7.88–24.8)
Bioavailability, %	5.9 (42.8)	4.95 (2.12–9.11)

* CV% = coefficient of variation; AUC_{0-1} = area under the curve from time 0 to the last measurable concentration; AUC_{inf} = area under the curve extrapolated to infinity; C_{max} = maximum serum concentration; T_{max} = time to attainment of maximum serum concentration; $T_{1/2}$ = elimination half-life; CL/F = apparent clearance; V_{area}/F = apparent volume of distribution.

above the LOQ and were, thus, readily quantified by the image analysis software, with IPD values that were at least 3–4 times the background values.

These are the first data to illustrate that dietary glucosamine enters not only the serum, but also the synovial fluid. However, transport of glucosamine from the circulation into the joint cavity appears to be very inefficient, since in all cases, the concentrations in synovial fluid were less than 10% of those in serum obtained at the same time point. However, glucosamine appears to have a longer half-life in synovial fluid than in serum, which suggests a slow or even nonexistent utilization of this monosaccharide by cells in intraarticular joint tissues.

DISCUSSION

The results described here represent the first comprehensive data set on the serum pharmacokinetics of glucosamine and the first analysis of synovial fluid levels of glucosamine following clinically relevant doses

of glucosamine HCl. The clearance data after IV dosing are typical of injected small molecules and showed reasonable reproducibility between and within the 2 experimental groups, thus validating this model and the analytical procedures used for monosaccharide quantitation (Figures 1 and 2). To mimic normal therapeutic usage of dietary glucosamine HCl, the monosaccharide salt was administered through the gastrointestinal system. With this delivery route, a median C_{max} of 6.1 μM (range 4.38–7.58) was reached in the posthepatic circulation, the T_{max} was attained between 30 minutes and 4 hours postdose, and predose levels (below LOQ) were reached between 6 and 12 hours after administration.

The range of serum concentrations measured and the pharmacokinetic profiles derived therefrom are consistent with those of other recently published animal studies in which higher dosages were used. For example, in dogs fed a single dose of glucosamine HCl (~166 mg/kg; 8 times the typical clinical dose) combined with chondroitin sulfate, a mean peak plasma concentration of 7 $\mu g/ml$ (~40 μM) was reached after 1.3 hours (23), which returned to baseline levels after 4 hours. More recently, a study using oral dosing at this high level in horses (16) showed a mean peak plasma concentration of ~10 $\mu g/ml$ (~60 μM) after 2 hours, with a return to baseline values at 6 hours.

The equine model used in our study is very relevant to human physiology since, like humans, the horse is monogastric and equine intestinal absorption of glucose (and glucosamine) occurs through glucose transporters located primarily in the duodenum (24). In addition, we have found that in healthy adult volunteers, ingestion of a single dose of glucosamine HCl at the clinically recommended level of 20 mg/kg generated a C_{max} of ~7 μM in serum, with a postdose T_{max} between 30 minutes and 60 minutes (Plaas AHK, Sandy JD, Thompson V: unpublished observations).

The C_{max} of ~5 μM we found in horses ingesting 20 mg/kg of glucosamine HCl is common for oral dosing of many commonly used drugs in humans, which are taken at a dose of ~2 mg/kg (25–27). This is consistent with the fact that such drugs achieve ~50% bioavailability, whereas glucosamine, as shown by previous studies (16) and the current study, achieves only ~5% bioavailability. Thus, a large proportion of glucosamine taken orally is rapidly eliminated due to poor absorption in the gut, or if absorbed, it is metabolized by cells in the gut lining and liver and/or efficiently cleared by the kidney. Although no substantive data are yet available on the mechanisms of uptake of dietary glucosamine by the intestinal lining, liver, and kidney, it is probably significant that these tissues express a high abundance of the glucose/glucosamine transporter GLUT-2, a "low affinity" transporter of glucose (K_m ~17 mM) but a "high-affinity" transporter of glucosamine (K_m ~0.8 mM) (28).

The finding that synovial fluid levels of glucosamine after both IV and NG dosing are <10% of those in serum collected at the same time point implies that glucosamine does not diffuse readily from the circulation into the joint cavity. While synovial fluid is considered to be an ultrafiltrate of plasma (29), it is possible that the glucosamine is taken up by cells of the fenestrated capillaries or the synovial lining cells. However, the observation that it is still detectable at ~0.5 μM in joint fluid when it is no longer detectable in serum argues for a slow or nonexistent utilization of this sugar by cells in the intraarticular joint tissues. This conclusion is supported by the well-established inefficient utilization of exogenous glucosamine by chondrocytes in cell cultures (30,31) and cartilage explants (32).

While it appears that the synovial fluid concentrations achieved by dietary intake of glucosamine are extremely unlikely to directly affect cartilage metabolism, it has been suggested (33) that glucosamine might be preferentially "targeted" to cartilage and that this could explain its apparent beneficial effects on cartilage health. The studies that have led to this suggestion were conducted with radiolabeled glucosamine, and they did not establish "targeting," but rather, confirmed that chondrocytes (like all other cells in the body) have glucose transporters through which glucosamine can be translocated into cells for utilization in metabolic pathways (34).

In other investigations, a quaternary ammonium conjugate of glucosamine (35) was found to concentrate in cartilage, but its physiologic effects on tissue metabolism were not reported. For in vivo effects of glucosamine on chondrocytes (which are normally exposed to ~5 mM glucose in vivo), the monosaccharide would have to "accumulate" in the cartilage to levels at least 500 times higher than those present in the synovial fluid. Such a process seems very unlikely, even over the extended periods of exposure used in clinical trials (36), since it would require a novel mechanism for partitioning of the glucosamine into tissues such as cartilage. Current Donnan equilibrium theory predicts that synovial fluid cations (such as sodium or glucosamine) will reach tissue concentrations that are typically only ~2.5 times higher than those in the surrounding synovial fluid. In addition, experiments in many independent laboratories using ^3H-glucosamine as a precursor for

glycosaminoglycan biosynthesis in cartilage explants (37–41) have not uncovered any high-affinity binding partners for glucosamine in cartilage, but suggest that glucosamine behaves much like other freely diffusible nutrients. When taken together, these considerations strengthen the argument that glucosamine-mediated effects on joint health are probably indirect and due to glucosamine affecting cells in tissues other than those in the diarthrodial joint.

It is conceivable that the documented beneficial effects of oral glucosamine HCl and glucosamine sulfate on joint health may originate from altered metabolic activities in tissues, where its extracellular concentrations postingestion can be expected to reach levels that are high enough to modify cell behavior. Based on the glucosamine HCl pharmacokinetic data reported here and by other investigators (33), such tissues could include the intestine, liver, and potentially, the kidney because of urinary concentration.

In this regard, many glucosamine-mediated effects on cells (32,42–44) are related to its known stimulation (as glucosamine-6-phosphate [GlcN6P]) of the intracellular hexosamine biosynthetic pathway (HBP) (45–47). The end product of this pathway, UDP-GlcNAc, is the active donor for intracellular protein glycosylation, including O-linked GlcNAc modification of serine and threonine residues (48). This type of protein glycosylation is somewhat analogous to kinase-mediated phosphorylation, in that its targets are generally involved in signal transduction pathways, such as transcription factors, nuclear pore proteins, and cytoskeletal components (49,50). Indeed, it should be noted that glucosamine per se is not a normal dietary component, nor is it generated as such in vivo or in vitro by metabolic pathways inside cells. However, GlcN6P, the precursor for the HBP, is normally synthesized from precursor glucose (via fructose-6-phosphate and glutamine) by the enzyme glutamine:fructose-6-phosphate amidotransferase (51). Following oral dosing, concentrations of glucosamine might exceed 1 mM in the intestine and liver and therefore be taken up into the cells by glucose transporters (28,52), where it is efficiently converted to GlcN6P (53), and thereby stimulate the HBP and its associated signal transduction events. In this regard, it has been shown that diabetic hyperglycemia, with attendant increases in HBP activity in kidney and liver cells, causes marked increases in the circulating levels of systemic mediators such as angiotensin (54,55).

It is therefore entirely possible that the apparent beneficial effect of oral glucosamine on joint health may result from changes in levels of as-yet-unidentified mediators of systemic disease. Indeed, such a mechanism is consistent with a recent report that oral glucosamine HCl blocks the formation of fibrotic capsular contractures following breast surgery (56). In addition, we have found that long-term (8-week) dosing of rabbits with clinical levels of glucosamine HCl can block anterior cruciate ligament transection–induced accumulation of biglycan and hyaluronan in the synovial membranes of operated knee joints (Plaas AHK, Laverty S: unpublished observations).

There is an ongoing debate about the relative efficacy of glucosamine HCl and the proprietary glucosamine sulfate in human OA (4). If glucosamine sulfate (i.e., glucosamine as the sulfate salt; see US patent no. 5,847,107 and attachments) and glucosamine HCl have different effects, this must presumably be explained by the different salt composition of the 2 formulations, since we have shown by FACE analysis (Laverty S, et al: unpublished observations) that both contain only bona fide glucosamine. The hydrochloride salt would not be expected to markedly influence the uptake or metabolism of glucosamine, since the stomach typically contains ~20 ml of 50 mM HCl, which would not be markedly altered by the HCl ingested with a single dose of glucosamine HCl. However, the glucosamine sulfate preparation contains sulfate, Na^+, K^+, Ca^{2+}, and Mg^{2+}, and these might conceivably influence the GLUT-2–mediated uptake and utilization of glucosamine by intestinal lining cells. So far, there is no published evidence that the sulfate anion or metal cations can directly influence GLUT-2. In addition, since the serum levels of sulfate (~0.8 mM) are largely controlled by the intestinal sodium/sulfate cotransporter (57), more research on the effect of sulfate on the metabolism of intestinal lining cells may provide new insight into optimizing the treatment of joint diseases with dietary glucosamine (58).

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