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Glucosamine oral bioavailability and plasma pharmacokinetics after increasing doses of crystalline glucosamine sulfate in man[1]

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Summary

Objective: Pharmacokinetic data on glucosamine are scant, limiting the understanding of glucosamine sulfate mechanism of action in support of its treatment effects in osteoarthritis. This study investigated the oral pharmacokinetics and dose-proportionality of glucosamine after administration of the patented crystalline glucosamine sulfate in man.

Methods: Twelve healthy volunteers received three consecutive once-daily oral administrations of glucosamine sulfate soluble powder at the doses of 750, 1500, and 3000 mg, in an open, randomised, cross-over fashion. Glucosamine was determined in plasma collected up to 48 h after the last dose by a validated Liquid Chromatography method with Mass Spectrometry detection. Pharmacokinetic parameters were calculated at steady state.

Results: Endogenous plasma levels of glucosamine were detected (10.4–204 ng/ml, with low intra-subject variability). Glucosamine was rapidly absorbed after oral administration and its pharmacokinetics were linear in the dose range 750–1500 mg, but not at 3000 mg, where the plasma concentration–time profiles were less than expected based on dose-proportionality. Plasma levels increased over 30-folds from baseline and peaked at about 10 μM with the standard 1500 mg once-daily dosage. Glucosamine distributed to extravascular compartments and its plasma concentrations were still above baseline up to the last collection time. Glucosamine elimination half-life was only tentatively estimated to average 15 h.

Conclusions: Glucosamine is bioavailable after oral administration of crystalline glucosamine sulfate, persists in circulation, and its pharmacokinetics support once-daily dosage. Steady state peak concentrations at the therapeutic dose of 1500 mg were in line with those found to be effective in selected *in vitro* mechanistic studies. This is the only glucosamine formulation for which pharmacokinetic, efficacy and safety data are now available.

Key words: Glucosamine sulfate, Pharmacokinetics, Bioavailability, Mechanism of action, Human.

Introduction

Glucosamine sulfate was found to be effective in controlling osteoarthritis (OA) symptoms in several clinical studies[1–3]. In particular, two randomised, placebo-controlled, double-blind trials of 3-year duration in knee OA patients, showed that this symptom-modifying effect is sustained over long-term treatment courses[4,5]. Moreover, both studies indicated that the drug also has a structure-modifying effect, as assessed by measurement of joint space narrowing on standardised plain radiographs[4,5] by a valid technique[6] and consistently within studies and patient populations[7].

However, the mechanism of action by which glucosamine sulfate exerts these clinical effects has not been fully elucidated. A major problem in this respect has been the limited knowledge about glucosamine pharmacokinetics after systemic and especially oral administration of glucosamine sulfate, due to the lack of suitable bioanalytical methods able to determine with sufficient sensitivity and specificity the compound concentrations in biological fluids[8]. Outcries from the medical community have recently focussed the attention on this limitation[9]. In fact, it is currently unclear how much of the unchanged compound reaches the systemic circulation and thus the possible biological target within the joint, after exogenous administration. The determination of the amounts of drug reaching the systemic circulation is further complicated by the fact that glucosamine is a normal constituent of the extracellular matrix of mammalian articular cartilage and synovial fluid[10], and therefore endogenous concentrations of glucosamine may be present in blood as a result of this and other connective tissue turnover. Another currently unaddressed issue regards the persistence of the drug in the circulation after dosing, to ensure adequate exposure during treatment and optimal administration intervals. Finally, the dose-proportionality of glucosamine pharmacokinetics is also

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unknown, which limits considerations about possible use of oral doses other than the standard 1500 mg/day glucosamine sulfate dosage used in most clinical studies.

Besides its relevance for the understanding of glucosamine biological mechanisms, the lack of sensitive and specific bioanalytical methods made impossible so far to test the bioequivalence of different glucosamine formulations, thus creating a major public health concern. In fact, the patented crystalline glucosamine sulfate 1500 mg once-a-day soluble powder preparation used here and in the most relevant clinical trials[4,5] is a prescription drug in most European and extra-European countries, whereas the Dietary Supplement Health and Education Act of 1994[11] favoured the appearance on the dietary supplement market in the USA and other countries of several undocumented glucosamine salts (e.g., hydrochloride), derivatives (e.g., *N*-acetyl-glucosamine), or dosage forms and regimens. Besides the questionable active ingredient content of these uncontrolled commercially available supplements[12], when other salts, formulations and/or daily regimens have been used in clinical trials, the results have not been favourable[13–16], casting doubts not only on the several confounders and problematic study design for some of these trials, but also on possible suboptimal bioavailability of the preparations used[17].

Our group has been involved in early efforts to elucidate the pharmacokinetics and metabolism of oral glucosamine sulfate in rats, dogs and humans, using ^{14}C-labelled glucosamine[8]. Although these studies provided compelling information about the absorption, distribution and elimination of radioactivity, they were not able to differentiate the unchanged drug from its metabolites and/or degradation products. When tentative specific methods for the determination of glucosamine in human plasma were developed, they were not sensitive enough to monitor the plasma concentration of the unchanged compound after oral administration of therapeutic doses[8,18].

Other authors have recently investigated the pharmacokinetics of some form of oral glucosamine in rats[19], dogs[20], and horses[21]. Due to the high limit of quantitation (LOQ) of the assays employed, they had to use doses much higher than those currently used in humans, whose relevance to the treatment of OA is unknown. More recently, the synovial fluid and serum concentrations of glucosamine have been determined in adult female horses following treatment with oral and intravenous glucosamine at clinically relevant doses (20 mg/kg), with reasonable assay sensitivity[22].

With the availability of hyphenated bioanalytical methods such as Liquid Chromatography with Mass Spectrometry detection (LC–MS/MS), it is now possible to accurately and specifically determine a large variety of drugs in biological fluids after administration of therapeutic doses. We recently developed and validated a sensitive LC–MS/MS method for the determination of unchanged glucosamine in human plasma. Due to the low LOQ obtained, the method is able to determine possible endogenous plasma concentrations of glucosamine. In the present study, therefore, glucosamine plasma levels were determined before and after administration of increasing oral doses of crystalline glucosamine sulfate, with the aim to investigate glucosamine endogenous concentrations and whether unchanged glucosamine reaches the systemic circulation after oral administration, in amounts that are pharmacologically relevant and dose-proportional. The study design was selected to mimic the conditions found during the therapeutic use of glucosamine in OA patients. In addition, the study allowed to assess glucosamine pharmacokinetics, including drug persistence in the circulation and the inference about its distribution into extravascular compartments.

Method

SUBJECTS

Twelve healthy Caucasian volunteers (six males and six females) were enrolled in the present study. A complete medical history was obtained from each volunteer. They were considered healthy at screening based on physical examination including vital signs recording and routine laboratory tests on blood and urine.

Subjects were non- or mild smokers $\leq$10 cigarettes/day and drank $\leq$5 cups of xanthine-containing beverages (coffee/tea) per day. They understood and signed the Informed Consent Form.

STUDY DESIGN

The study protocol and related material were approved by the Local Ethics Committee of the S. Orsola-Malpighi Hospital in Bologna, Italy. The study was carried out in accordance with the current revision of the Declaration of Helsinki concerning medical research in humans, and with current Good Clinical and Laboratory Practice Guidelines (USA and EU).

This was a randomised, open, cross-over design study, to investigate the dose-proportionality of glucosamine pharmacokinetics after repeated oral administration of the patented crystalline glucosamine sulfate formulation at the once-a-day doses of 750, 1500, and 3000 mg for 3 consecutive days, i.e., the time needed to reach steady state conditions based on pilot experiments. The possible presence of endogenous glucosamine in plasma makes mandatory a multiple dose, steady state pharmacokinetic study to allow for homeostatic adjustments. This makes possible to measure the net increase in plasma concentrations afforded by exogenous administration[23,24]. Crystalline glucosamine sulfate (Dona, Viartril-S, Xicil or other trademarks by the Rottapharm Group, Monza, Italy and Rotta Pharmaceuticals Inc. Wall, NJ) is a defined, pure and stable substance in which glucosamine, sulfate, chloride and sodium ions are present in stoichiometric ratios of 2:1:2:2. Doses are defined in relation to the net content in glucosamine sulfate. The subjects received each of the three selected dose levels in three different study periods whose order was randomised according to a Williams variance-balanced design for three formulations[25]. Drug treatment consisted of three consecutive once-daily administrations of crystalline glucosamine sulfate formulated as an oral soluble powder presented as a sachet, under fasting condition and dissolved in 240 ml of water. The three study periods were separated by a washout of at least 3 days after the last blood collection (48 h post-dosing) on each dosing period. Thus, the minimum washout period observed in the present study between two different dose levels was 5 days. Fasting was maintained from the evening before and up to 4 h after drug intake, when a light meal was served consisting of 40 g of boiled rice with 10 g of butter and 10 g of parmesan cheese, 150 g of chicken, 50 g of bread, 1 stowed apple (150 g), 200 ml of mineral water. The meal provided a total of 584 calories (26.4% proteins, 19.4% lipids, and 54.2% glucides). After this light meal and up to 48 h after drug intake, the food and fluid intake of the volunteers was monitored to ensure adherence to the

inclusion/exclusion criteria. During each study period, blood was collected from the antecubital vein by indwelling catheters into heparinised tubes before (0 h) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h after the third and last dose. Blood was also collected before the first and second drug administrations. Aliquots of plasma (obtained by blood centrifugation at 2000 × *g* at 4°C) were stored at −20°C pending analysis.

Safety and tolerability were monitored throughout the study by adverse events recording and by repeating all the screening procedures at a follow-up visit carried out within 7 days after the study end.

BIOANALYSIS

Glucosamine plasma concentrations were determined by LC−MS/MS. Briefly, plasma samples (1 ml) were added with the internal standard (^{13}C-glucosamine) at a final concentration of 250 ng/ml and subjected to a liquid/liquid extraction by the addition of 1 ml of acetonitrile. The samples were then stirred on a vortex and centrifuged at 2000 × *g* for 20 min. The supernatants were transferred into injection vials and a 20 μL sampling volume was injected into the LC−MS/MS instrumentation. Separation was achieved using a mixture of acetonitrile and water as the mobile phase in a gradient mode on an Alliance system 2690 model (Waters). This system was equipped with a Shodex Asahipak NH_2P-50G 2D column (150 mm × 2.0 mm internal diameter (ID); particle size: 5 μm) fitted with a Shodex Asahipak NH_2P-50G 2D (30 mm × 2.0 mm ID; particle size: 5 μm) guard column. The high performance liquid chromatography system was connected to an API 2000 (Applied Biosystems) MS/MS instrument operating in the positive ion mode. Quantitative determination of glucosamine was performed in the Multiple Reaction Monitoring mode to follow the transitions 180 → 72 for glucosamine and 181 → 73 for ^{13}C-glucosamine. Calibration curves were generated using calibration samples obtained from glucosamine free plasma spiked with glucosamine at concentrations ranging from 6.25 ng/ml (the LOQ of the method, corresponding to 0.03 μM based on a glucosamine molecular weight of 179.17 as a free base) upward. Recovery was calculated using six replicate analyses at three concentrations within the calibration curve and averaged 92.4%, 101.1% and 98.4%, respectively. The overall recovery was, therefore, 97.3%. The assay precision (RSD%) calculated as mean experimental concentration/standard deviation × 100 and accuracy (BIAS%) calculated as (mean experimental concentration − theoretical concentration)/theoretical concentration × 100 were assessed both intra- and inter-day using three concentration levels within the calibration range analysed in six replicates. The assay precision ranged from 1.5% to 13.9%. Its accuracy ranged from −0.7% to 18.4%. At the LOQ the precision ranged from 4.7% to 13.9% and the accuracy from 13.2% to 18.4%.

The method was validated according to current guidelines including bench top, long-term and repeated freeze/thawing cycles stabilities, as well as 1:10 v/v dilution with blank human plasma.

PHARMACOKINETIC ANALYSIS

Glucosamine plasma concentrations vs time profiles were analysed using the Kinetica 2000 software version 4.2 (Innaphase, Philadelphia, USA) by standard non-compartmental methods. Possible concentrations of endogenous glucosamine determined at baseline were subtracted from the concentrations determined in all subsequent samples collected from the same individual in the same treatment period. Subtraction of endogenous levels of a substance that is also administered exogenously is requested by current guidelines[23,24]. Together with a multiple dose study design to allow for homeostatic adjustment, this ensure the estimation of the net increase in the circulating levels of glucosamine afforded by the oral administration of crystalline glucosamine sulfate.

Maximum plasma concentrations at steady state ($C_{ss,max}$) and the corresponding times (T_{max}) were taken directly from the raw data. At steady state, the area under the plasma concentration−time curve within a dosing interval (AUC_{ss}) and up to the last time at which the glucosamine concentration was measurable (AUC_t) were calculated using the mixed log-linear trapezoidal rule. Glucosamine plasma concentrations determined before the second and third drug administrations and 24 h after the third and last drug administration (day 4), denoted as $C_{min,day\,2}$, $C_{min,day\,3}$ and $C_{min,day\,4}$, respectively, were taken from the raw data in order to asses the time to steady state. Glucosamine elimination half-life was only tentatively estimated based on the time to reach steady state conditions[26].

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) after log-transformation was used to assess any gender or period effect on glucosamine baseline levels, i.e., comparing basal concentrations between males and females and among the three different treatment periods. In addition, the possible carryover effect was also included as a factor in the General Linear Model (GLM) ANOVA for the Williams variance-balanced design conducted on the pharmacokinetic parameters compared across doses (see below). Investigation of dose-proportionality of glucosamine pharmacokinetics was one of the objectives of the present study, i.e., to assess that the increase in plasma levels is directly related and proportional to the increase in the drug dose. In case of dose-proportional, and thus linear pharmacokinetics, a doubling of the dose produces a doubling of the plasma concentrations. To test the dose-proportionality of glucosamine pharmacokinetics, the relevant pharmacokinetic parameters $C_{ss,max}$, AUC_{ss}, and AUC_t, determined at the three dose levels were normalised to the dose of 1 mg, and subjected to the above mentioned GLM-ANOVA with Tukey HSD test for multiple comparisons after log-transformation with dose as factor. Presence of any gender differences in these parameters was also assessed by ANOVA after log-transformation. T_{max} were compared by the non-parametric Kruskall−Wallis test without log-tranformation or dose adjustment. To assess the time to steady state the $C_{min,day\,2}$, $C_{min,day\,3}$ and $C_{min,day\,4}$ values were pair-wise compared by paired *t* test. $P < 0.05$ was taken as statistically significant in all tests.

Results

The subject demographic characteristics are reported in Table I, grouped by gender. All subjects were young healthy volunteers, with normal body mass index (BMI).

Endogenous glucosamine was detected in the plasma of all subjects (Table II). Baseline plasma levels ranged between 10.4 and 204.0 ng/ml (corresponding to 0.06 and 1.1 μM, respectively), i.e., with a high degree of

Table I
Subject demographic characteristics

Randomisation no. (with treatment order)	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Women				
1 (750, 3000, 1500 mg)	27	160	52	20.3
2 (1500, 750, 3000 mg)	26	160	50	19.5
4 (750, 1500, 3000 mg)	23	162	52	19.8
6 (1500, 3000, 750 mg)	23	153	49	20.9
9 (3000, 1500, 750 mg)	23	150	50	22.2
12 (750, 3000, 1500 mg)	31	165	69	25.3
Mean ± SD	25.5 ± 3.2	158 ± 5.7	53.7 ± 7.6	21.3 ± 2.2
Range	23–31	150–165	49–69	19.5–25.3
Men				
3 (3000, 1500, 750 mg)	31	172	80	27.0
5 (3000, 750, 1500 mg)	38	163	63	23.7
7 (3000, 750, 1500 mg)	24	173	65	21.7
8 (1500, 3000, 750 mg)	26	185	75	21.9
10 (750, 1500, 3000 mg)	40	168	70	24.8
11 (1500, 750, 3000 mg)	30	174	65	21.5
Mean ± SD	31.5 ± 6.4	172.5 ± 7.3	69.7 ± 6.7	23.4 ± 2.2
Range	24–40	163–185	63–80	21.5–27.0
Overall				
Mean ± SD	28.5 ± 5.7	165 ± 9.7	61.7 ± 11	22.4 ± 2.3
Range	23–40	150–185	49–80	19.5–27.0

inter-subject variability, especially within women although it appeared that there were no significant gender differences. However, analysis of baseline levels before the three treatment periods revealed a low degree of intra-subject variability since the concentrations before each treatment period were very similar for each subject after the appropriate washout. Indeed, there were no significant differences between periods (Table II). Even though the endogenous glucosamine concentrations were much lower compared with those determined after administration of glucosamine sulfate at all doses and they were similar for each subject at the start of the three treatment periods, the baseline subtraction was applied as described since it represents a conservative approach.

Table II
Baseline glucosamine levels in plasma (ng/ml), before each treatment period

Randomisation no.	Period I	Period II	Period III
Women			
1	45.3	51.2	89.8
2	75.3	39.9	46.0
4	26.2	23.6	10.4
6	54.8	64.7	204.0
9	107.2	41.0	95.6
12	56.2	53.0	44.3
Mean ± SD	60.8 ± 27.8	45.6 ± 14.1	81.7 ± 67.8
Range	26.2–107.2	23.6–64.7	10.4–204.0
Men			
3	31.9	39.7	33.2
5	41.9	47.0	39.6
7	47.9	50.2	61.0
8	51.2	79.5	25.6
10	63.6	51.3	42.0
11	61.4	54.2	70.2
Mean ± SD	49.7 ± 11.9	53.7 ± 13.6	45.3 ± 17.0
Range	31.9–63.6	39.7–79.5	25.6–70.2
Overall			
Mean ± SD	55.2 ± 21.2	49.6 ± 13.8	63.5 ± 50.8
Range	26.2–107.2	23.6–79.5	10.4–204.0

Through concentrations, i.e., those obtained 24 h after the first ($C_{min,\ day\ 2}$) and second ($C_{min,\ day\ 3}$) dose, increased over baseline as described in Table III. Within each dose level, the $C_{min,\ day\ 3}$ was not significantly different from the concentration detected 24 h after the third and last administration ($C_{min,\ day\ 4}$), indicating that glucosamine pharmacokinetics were at steady state with the third glucosamine sulfate oral intake.

Figure 1 shows the baseline-subtracted average plasma concentration vs time profiles with each of the three once-daily doses of glucosamine sulfate at steady state. It appears that glucosamine is rapidly absorbed and available to the systemic circulation after oral administration. Steady state mean peak concentrations were achieved 3–4 h after administration of each dose (median T_{max}, Table IV) and they were in the 10 μM range with the standard therapeutic once-daily dosage of 1500 mg glucosamine sulfate; the median $C_{ss,\ max}$ at this dose was actually 9.92 μM (1777.6 ng/ml). Thereafter, the plasma concentrations of glucosamine slowly decreased and were still measurable and consistently above baseline levels in the last samples collected 48 h after dosing in all subjects at all doses. This

Table III
Mean (±SD) plasma concentrations of glucosamine at baseline, before the second and third drug administration ($C_{min,\ day\ 2}$ and $C_{min,\ day\ 3}$) and 24 h after the third and last administration ($C_{min,\ day\ 4}$)

	Administered dose		
	750 mg	1500 mg	3000 mg
Baseline (ng/ml)	61.8 ± 48.6	52.7 ± 17.7	53.8 ± 24.6
$C_{min,\ day\ 2}$ (ng/ml)	166.9 ± 66.2*	185.8 ± 41.4*	182.2 ± 72.7*
$C_{min,\ day\ 3}$ (ng/ml)	210.1 ± 86.3	245.7 ± 58.9	267.4 ± 70.9
$C_{min,\ day\ 4}$ (ng/ml)	225.6 ± 85.0	277.3 ± 67.5	308.7 ± 97.9

*$P < 0.05$ vs $C_{min,\ day\ 3}$ and $P < 0.01$ vs $C_{min,\ day\ 4}$. There were no significant differences between $C_{min,\ day\ 3}$ and $C_{min,\ day\ 4}$.

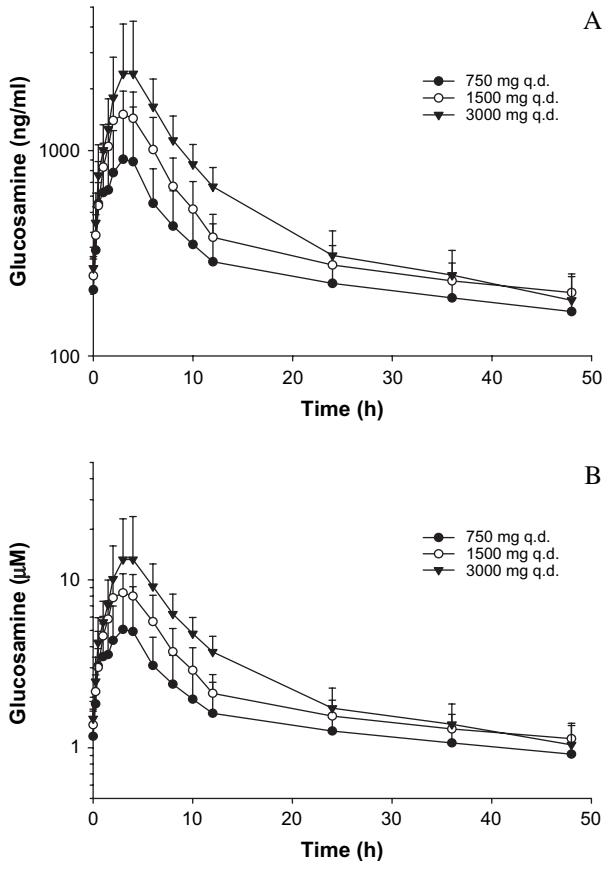

Fig. 1. Mean glucosamine plasma concentration vs time profiles at steady state after repeated once-daily doses of glucosamine sulfate 750, 1500 and 3000 mg ($n = 12$ for each dose level). Concentrations are baseline-subtracted and expressed as ng/ml (panel A) or μM (panel B). Bars represent standard deviations.

precluded an unequivocal estimation of the terminal phase of the plasma concentration vs time profile from which the elimination half-life is calculated. For this reason, this parameter could be only tentatively estimated based on the time to reach the steady state and therefore resulted to be on average 15 h.

Further visual inspection of the plasma concentration vs time profiles of glucosamine at all doses and in all the subjects, showed that, after a rapid rise during the absorption phase, the plasma concentrations decreased in a multi-exponential fashion (i.e., the plasma concentrations decreased rapidly soon after T_{max} and then decreased at a slower pace from 10 h post-dosing onward). This indicates a significant distribution of the drug into extravascular compartments.

Analysis of the pharmacokinetic parameters reported in Table IV shows that although mean peak concentrations at steady state ($C_{ss,\ max}$) apparently increase with increasing doses, they are linear only within the 750–1500 mg dose range, while they deviate from linearity at 3000 mg, whose dose-normalised $C_{ss,\ max}$ value was different from that observed after the lowest dose ($P < 0.05$). This deviation from linearity with the highest dose was even more evident for the extent of bioavailability, represented by the AUC_{ss} and AUC_t values. In fact, the dose-normalised values for the 3000 mg dose were both significantly lower than the corresponding values calculated at the dose of 750 mg ($P < 0.01$). In addition, the AUC_t also differed significantly from the corresponding value calculated at the dose of 1500 mg ($P < 0.05$). This indicates that the increase in the plasma concentrations of glucosamine observed at the dose of 3000 mg was less than proportional based on the dose increase. The evaluation of any possible carryover effect was included as a factor in the statistical test utilised for the comparison of the pharmacokinetic parameters across doses (GLM-ANOVA). The test did not detect any carryover effect. These results and the lack of any significant difference between the baseline glucosamine plasma concentrations in the three study periods, indicated that the washout period observed between each period (at least 5 days) was sufficient to exclude any carryover effect.

Individual baseline-subtracted plasma concentration vs time profiles (Fig. 2) clearly suggest lower concentrations with higher variability after the 750 mg dose compared with the standard 1500 mg dose, that provided sustained concentrations in the 10 μM range in most of the subjects. Further doubling the dose to 3000 mg did not provide further apparent advantage, perhaps with the exception of more consistent concentrations in the 10 μM range in all subjects. Glucosamine average plasma concentrations 24 h after administration were still over 5-fold higher than mean baseline concentrations with all doses.

There were no statistically significant gender differences in pharmacokinetics parameters at any dose level (data not shown). There were no safety issues during or after treatment with any of the tested doses.

Discussion

To our knowledge, this is the first study describing the bioavailability and pharmacokinetics of glucosamine in humans. This study was performed after repeated oral doses of the patented crystalline glucosamine sulfate

Table IV
Glucosamine pharmacokinetic parameters at steady state for all tested doses of glucosamine sulfate

	Administered dose		
	750 mg	1500 mg	3000 mg
$C_{ss,max}$ (ng/ml)	1069.2 ± 675.3	1601.9 ± 424.9	2503.1 ± 1835.3†
T_{max}* (h)	3 (0.5–6)	3 (1.5–4)	4 (3–4)
AUC_{ss} (ng·h/ml)	9697.0 ± 4215	14,564 ± 4138	22,095 ± 6984‡
AUC_t (ng·h/ml)	14,323 ± 5582	20,216 ± 5021	27,991 ± 8035‡§

Data are mean ± SD, except *median and range.
†$P < 0.05$ vs 750 mg when dose-normalised.
‡$P < 0.01$ vs 750 mg when dose-normalised.
§$P < 0.05$ vs 1500 mg when dose-normalised.

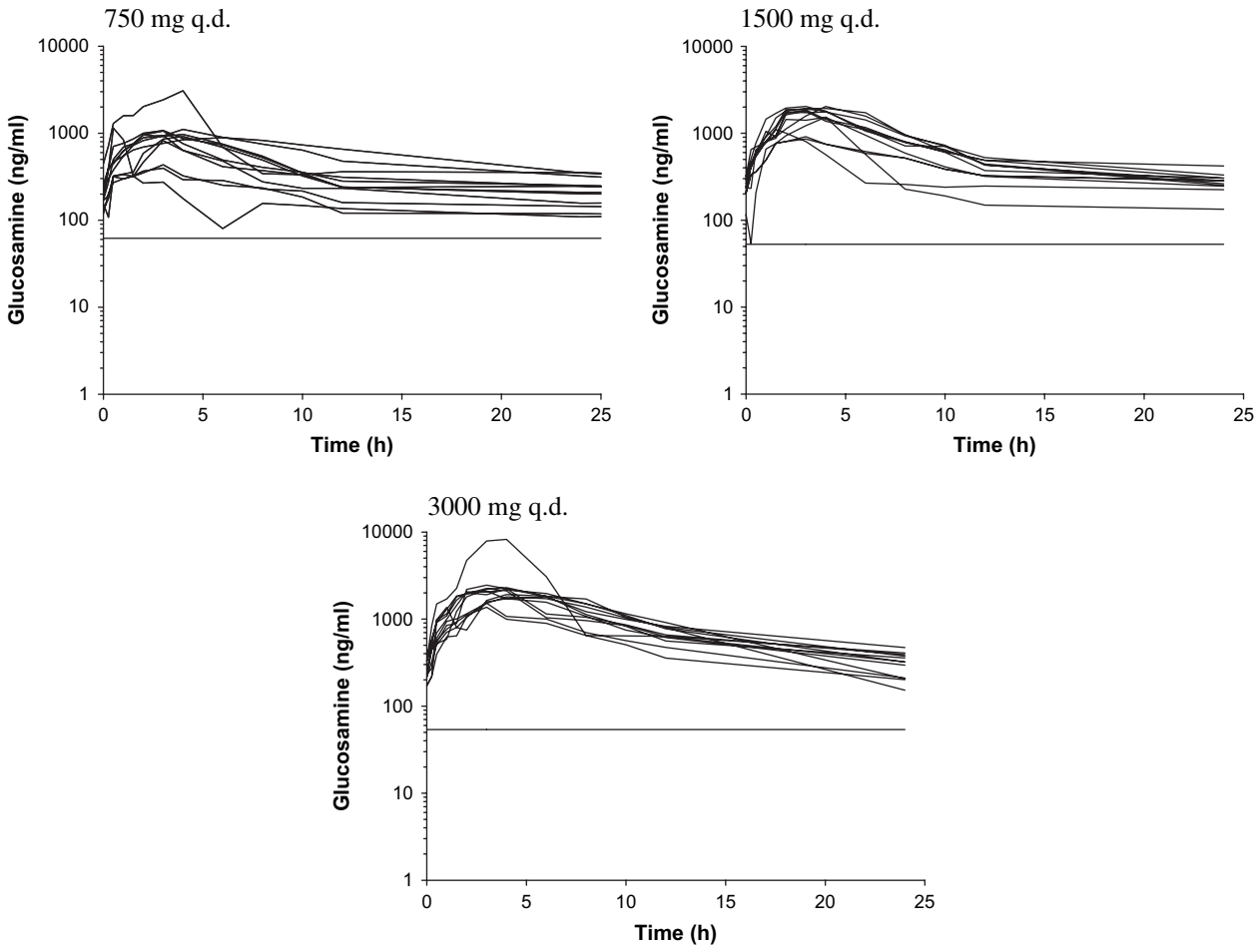

Fig. 2. Individual glucosamine plasma concentration vs time profiles at steady state within a dosing interval after repeated once-daily doses of glucosamine sulfate 750, 1500 and 3000 mg ($n = 12$ for each dose level). Concentrations are baseline-subtracted and expressed in ng/ml. Horizontal lines indicate mean endogenous plasma concentrations at baseline.

formulation shown to be effective in OA pivotal clinical trials[4,5] and indicated that glucosamine is available to the systemic circulation.

In addition, the low LOQ of the developed LC−MS/MS method allowed to detect and quantify the endogenous glucosamine levels in plasma. Previous animal studies failed to detect circulating endogenous glucosamine: this might be due to the poor sensitivity of the bioanalytical methods[19–21] and/or to species differences[22]. Endogenous glucosamine circulated in human plasma at concentrations ranging from as low as 10 to as high as 200 ng/ml. The endogenous concentrations varied considerably between individuals (high inter-subject variability), especially between women, but they were rather consistent within individuals (low intra-subject variability, although with a slight trend for some degree of variability again within women). This might be of pathophysiological relevance for future studies in diseased subjects. Given the role of glucosamine in the biology of the joint[10] and its therapeutic effects[1–5], further studies are warranted to investigate possible correlations between endogenous levels and articular disorders, with particular regard to OA, and possible intrinsic sources of variability (e.g., disease severity, or treatment response).

Baseline endogenous glucosamine levels were subtracted in this study to allow measurement of the net increase after exogenous administration[23,24]. Even though the endogenous concentrations were low compared with those achieved after exogenous oral administration, the correlations between glucosamine concentrations and therapeutic effects in patients should be made on total plasma concentrations.

A possible limitation of our study is that it did not provide indications of glucosamine absolute oral bioavailability as the intravenous route of administration could not be investigated. The absolute oral bioavailability of glucosamine has been estimated between 3% and 6% in horses[21,22], 12% in dogs[20] and 21% in rats[19] using glucosamine hydrochloride. The data in the present study suggest that glucosamine absolute bioavailability in man is probably higher since the C_{max} are higher and concentrations much more sustained compared to those found in horses after the administration of comparable doses[22].

In the present study, glucosamine was rapidly absorbed and bioavailable at steady state. Glucosamine plasma concentrations increased on average over 30-folds from baseline after repeated oral administrations of the standard 1500 mg once-a-day dose of glucosamine sulfate and reached a maximum in the 10 μM range.

Glucosamine is preferentially incorporated by chondrocytes into the components of the glycosaminoglycan chains in the intact cartilage[27], stimulates the synthesis of

physiological proteoglycans[28–30] and decreases the activity of catabolic enzymes, including matrix metalloproteases (MMP)[29–31]. As common in mechanistic studies, most of the *in vitro* experiments used glucosamine concentrations higher than those found in human plasma after therapeutic doses in the present study, also because the latter were previously unknown. However, selected *in vitro* models showed that glucosamine was metabolically effective at concentrations hundred-folds lower than the 10 μM average plasma peak found here[30]. On the other hand, it was recently suggested that glucosamine was not able to stimulate glycosaminoglycan synthesis at concentrations below 1 mM[32]. Whatever the concentration needed, it is unlikely that the possible metabolic effects of glucosamine and especially the mere incorporation in glycosaminoglycans are able to fully explain the pattern of effects shown in clinical trials. In particular, even though a metabolic hypothesis might be attractive to support the putative joint structure-modifying effects observed after long-term treatments[4,5], it could not readily explain the short-term[1,33,34] and long-term[2–5] symptom-modifying effects, which have been indicated as being of major clinical relevance[35,36].

A unifying hypothesis has been recently proposed and related to glucosamine-induced reversal of the pro-inflammatory and joint-degenerating effects of interleukin-1 (IL-1)[31,37,38], via an inhibitory effect on the IL-1 intracellular signalling cascade and specifically by the reduction in the activation of the transcription factor nuclear factor-kappa B (NF-KB)[39,40]. Effects connected with NF-kB decreased activation such as chondrocyte-specific scavenging effects[39], are evident already at concentrations of 10 μM[41]. Moreover, IL-1-induced prostaglandin E_2 release from cartilage explants and cytokine-induced gene expression of MMP-13 and aggrecanase 1 and 2 have been recently shown to be inhibited already at 5 μg/ml glucosamine[42,43], i.e., a concentration only slightly above the range described in the present study.

An alternative hypothesis has been recently put forward by Laverty and coworkers[22], who suggested looking at altered metabolic activities in tissues where extracellular glucosamine concentrations should be higher, including the intestine, liver and kidney, and that might modulate the compound anti-arthritic effects.

The pharmacokinetic profile of glucosamine suggests a distribution between the vascular and extravascular compartments, as shown by the multi-exponential decay of plasma concentration–time profiles. Previous studies[8,44,45] indeed showed that orally administered radiolabelled glucosamine distributes to several organs and selectively to the joint and the articular cartilage. It is therefore conceivable that glucosamine concentrations at the articular site of action may be higher than those found in plasma. While recently published results obtained in horses have indicated that the synovial concentrations of glucosamine were, on average, 10% of those observed in serum[22], preliminary data we are obtaining in knee OA patients suggest that plasma and synovial fluid levels are equal, i.e., in a 1:1 relationship (Persiani *et al.*, unpublished observations) and deserve further investigation.

In addition, it should be taken into account that glucosamine sulfate is continuously administered to patients for months or years and extrapolation of clinical effects solely based on concentrations may be misleading, as it does not consider the duration of exposure, i.e., the product of concentration and time.

The elimination half-life of glucosamine could not be calculated in the present study due to the sustained plasma concentrations observed. However, the pharmacokinetics of glucosamine were at steady state after three consecutive daily oral administrations. Since after a constant dose and dosing interval, steady state conditions are reached after five elimination half-lives[26], the latter was tentatively estimated to be approximately 15 h for glucosamine in this study. These findings support the once-daily oral dosages of glucosamine sulfate used here and in pivotal efficacy and safety trials[4,5].

The finding that the plasma concentrations of glucosamine persisted above baseline for 48 h after dosing was taken into consideration when assessing if the washout period between treatments was sufficient. Indeed, it appears that the levels had returned to baseline virtually in all subjects at the start of each treatment period, occurring at least 5 days post-dosing. This was also confirmed by the absence of any statistically significant difference between the baseline glucosamine concentrations in each of the three study periods and by the lack of any carryover effect during the comparison of the pharmacokinetic parameters across doses.

Glucosamine pharmacokinetics appeared to be linear, i.e., dose-proportional, up to the glucosamine sulfate standard dose of 1500 mg once-a-day. Half the recommended dose (750 mg) provided approximately half plasma levels and may have proportionally lower pharmacological and clinical effects, further challenged by a much greater variability than with the standard dose. Higher doses, e.g., the 3000 mg tested here, deviated from linearity for both the rate and the extent of bioavailability, in that this dose increase failed to produce proportionally higher increases in maximum concentrations and area under the concentration vs time curve. This may be due to a saturation of the absorption process, as can be also evinced from the higher T_{max} values observed at this dose. This implies that doses higher than 1500 mg glucosamine sulfate, by producing proportionally lower plasma concentration–time profiles may not be clinically worth.

The present study has been conducted using the once-a-day soluble powder formulation of crystalline glucosamine sulfate used in pivotal clinical trials[4,5], which is a prescription drug in most European countries. Transfer of the efficacy and safety data obtained with this substance and formulation to common dietary supplements, has already been discouraged[4,5,35]. In fact, these uncontrolled formulations often have a much lower glucosamine content than reported in their label claims and are thus commonly underdosed[12]. In addition, there is currently no clinical justification to use different glucosamine compounds or even glucosamine salts, e.g., hydrochloride, as pivotal trials failed to show the same benefit[3,13]. While some authors suggested that sulfate levels increased after administration of glucosamine sulfate and may significantly contribute to its effects[46,47], pharmacokinetic considerations should also be taken into account. In fact, the bioavailability of different salts of the same compound may vary significantly, especially when they are included in solid oral formulations whose comparative dissolution profile and interaction with excipients are unknown[48]. The question of the unit dose is also relevant. In fact, we have clearly shown that unit doses lower than 1500 mg give proportionally lower plasma levels: therefore, appropriate pharmacokinetic and pharmacological studies should be performed before fractioning the 1500 mg once-a-day standard dose[4,5] into smaller and more frequent doses is justified. Such fractioning is done in some currently ongoing trials[49], after recent studies failed to show the clinical relevance of this approach[13–16]. In addition,

glucosamine sulfate is not stable as such and should be adequately stabilised, as achieved by a patented process[50] for the formulation used here and in the majority of trials[3–5]. Doubts in this respect may explain the negative findings obtained in short-term clinical studies of previously untested glucosamine sulfate formulations[14–16], besides their many confounders in terms of sample size and characteristics, disease severity, use of concomitant medications and other problematic study design features[3,17].

In conclusion, we have described the pharmacokinetic profile of glucosamine after administration of crystalline glucosamine sulfate in man. Our findings indicate that the drug is orally bioavailable at concentrations that are in line with those found to be effective in selected *in vitro* models that may explain the favourable clinical results in OA. Future studies should compare the bioavailability of this patented formulation with that of other glucosamine salts, derivatives, formulations, or dose regimens.

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