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Synovial and plasma glucosamine concentrations in osteoarthritic patients following oral crystalline glucosamine sulphate at therapeutic dose[1]

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Summary

Objective: We investigated the synovial and plasma glucosamine concentrations in osteoarthritic patients following oral administration of crystalline glucosamine sulphate at the therapeutic dose of 1500 mg once-a-day for 14 days.

Design: Twelve osteoarthritic patients (six males and six females) received 14 consecutive once-daily oral administrations of crystalline glucosamine sulphate soluble powder (1500 mg), in an open fashion. Plasma and synovial fluid were collected simultaneously from the same patient, at baseline and, at steady state (3 h after the last dose). Glucosamine was determined in plasma and synovial fluid by liquid chromatography–tandem mass spectrometry.

Results: Median endogenous glucosamine concentrations in plasma and synovial fluid were 52.0 ng/ml (0.29 μM) and 36.5 ng/ml (0.21 μM), respectively ($P = 0.001$), and varied substantially among patients (41–121 ng/ml and <10–67 ng/ml, respectively). Three hours after the last dose, glucosamine concentrations resulted increased from baseline in all patients with median increases of 20.5 and 21.5 folds in plasma and synovial fluid, respectively, the difference being not statistically significant ($P = 0.11$). In plasma, the median post-treatment value was 1282 ng/ml (7.17 μM) and ranged from 600 to 4061 ng/ml (3.35–22.7 μM). The median post-treatment synovial glucosamine concentration was 777 ng/ml (4.34 μM), i.e., significantly lower than in plasma ($P = 0.001$), and ranged from 577 to 3248 ng/ml (3.22–18.1 μM). Plasma and synovial glucosamine concentrations were highly correlated and were in the 10 μM range.

Conclusions: Glucosamine is bioavailable both systemically and at the site of action (the joint) after oral administration of crystalline glucosamine sulphate in ostaeoarthritis patients. Steady state glucosamine concentrations in plasma and synovial fluid were correlated and in line with those effective in selected *in vitro* studies.

Key words: Glucosamine sulphate, Synovial fluid, Bioavailability, Mechanism of action, Human.

Introduction

Several clinical studies have indicated that crystalline glucosamine sulphate is effective in controlling osteoarthritis (OA) symptoms and disease progression[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 suggested that the drug also has a structure-modifying effect, as assessed by measurement of joint space narrowing using validated techniques on standardised plain radiographs[4,5–7]. Another recently completed trial (the GUIDE study[8]), confirmed the symptomatic results described above and indicated that, at the dose of 1500 mg once-a-day, crystalline glucosamine sulphate provided a symptomatic effect that was significantly superior to that observed after the administration of placebo[8]. In the same study and depending on the selected outcome measures, the effect observed after the administration of the currently preferred symptomatic medication in OA (acetaminophen) was not always different from that observed after the administration of placebo[8]. The formulation used in the above pivotal studies[4,5,8] is the original crystalline glucosamine sulphate 1500 mg once-a-day soluble powder preparation which is a prescription drug in most European and extra-European countries and differs from glucosamine formulations available in the USA and other countries. In fact, the US Dietary Supplements Health and Education Act of 1994[9] favoured the appearance of several poorly characterised dietary supplements containing either inadequate active ingredient quantity[10], or other glucosamine salts (e.g., hydrochloride), derivatives (e.g., *N*-acetyl-glucosamine), or dosage forms and regimens. This might also provide an explanation for the finding that when other salts, formulations and/or daily regimens have been used in clinical trials, the results have not been favourable[11–15]. In particular, the recently completed National Institutes of

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Health (NIH)-sponsored Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) trial in knee OA, indicated that the symptomatic effect of glucosamine hydrochloride at the dose of 500 mg t.i.d. did not differ significantly from placebo[15]. This confirmed the skepticism not only on the several confounders and problematic study design for some trials, but also on the possible suboptimal exposure of the patients to the active drug that might also come from the adopted dose and dosing interval[16].

The selection of the optimal dose and dosing schedule for glucosamine is made difficult by the fact that the mechanism of action by which the drug exerts its clinical effects has not been fully elucidated. A major problem in this respect has been the limited knowledge of glucosamine pharmacokinetics after systemic and especially oral administration of glucosamine sulphate. We recently investigated the oral bioavailability and dose proportionality of glucosamine in healthy volunteers after administration of crystalline glucosamine sulphate at the doses of 750, 1500, and 3000 mg once daily[17]. The study demonstrated that after repeated oral administrations of crystalline glucosamine sulphate the active drug glucosamine is available to the systemic circulation. Glucosamine pharmacokinetics resulted linear in the dose interval 750–1500 mg and the steady state peak plasma concentrations at the 1500 mg dose were in the 10 μM range[17]. Possible limitations of that investigation were the lack of information regarding the distribution of the drug at the site of action (i.e., the joint) and the fact that the study was performed in healthy volunteers and not in patients. Even though other studies investigated the serum and synovial levels of glucosamine in horses after oral administration of glucosamine hydrochloride at clinically relevant doses[18], whereas other studies investigated the serum concentrations of glucosamine in OA patients receiving a single 1500 mg dose of glucosamine as sulphate[19] or hydrochloride[20], it is currently unknown if glucosamine reaches the joint and the possible biological target within the joint, after oral administration of crystalline glucosamine sulphate in OA patients.

The bioanalytical method for the determination of glucosamine in human plasma[21] used in our previous investigation in healthy volunteers[17], has been recently validated for the determination of the drug in human synovial fluid. Therefore, the availability of these bioanalytical methods for the determination of glucosamine in both matrices made possible the present investigation that was aimed at assessing if glucosamine reaches the systemic circulation and the joint after oral administration of crystalline glucosamine sulphate at the therapeutic dose of 1500 mg once-a-day for 14 days in knee OA patients. The present paper reports the results obtained and thus addresses most of the unresolved issues related to the absorption and distribution of glucosamine in OA patients in a study designed to mimic the conditions found during the therapeutic use of this drug.

Methods

PATIENTS

Twelve Caucasian patients (six males and six females) fulfilling the American College of Rheumatology criteria for knee OA were recruited from the Istituti Ortopedici Rizzoli of Bologna, Italy (Table I). A complete medical history was obtained from each patient to exclude endocrine, metabolic, liver, kidney, and cardiac diseases and acute illness such as infection and respiratory disorders. Thus, except for OA, the patients were considered otherwise healthy at screening based on physical examination including concomitant drug use.

Table I
Subject demographic characteristics

Randomisation n°	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Females				
2	57	155	54	22.5
3	64	168	67	23.8
7	47	163	75	28.2
8	49	160	95	37.1
10	49	163	64	24.1
12	81	150	60	26.7
Median	53	161.5	65.5	25.4
Range	47–81	150–168	54–95	22.5–37.1
Males				
1	48	180	85	26.2
4	61	172	76	26.0
5	65	178	105	33.1
6	56	174	92	30.4
9	25	178	78	24.7
11	61	168	91	32.2
Median	58.5	176	88	28.3
Range	25–65	168–180	76–105	22.5–33.1
Overall				
Median	56.5	168	77	26.5
Range	25–81	150–180	54–105	22.5–37.1

The patients 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 Istituti Ortopedici Rizzoli 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).

The study design was open-label, to investigate the plasma and synovial concentrations of glucosamine before and after repeated oral administration of the original crystalline glucosamine sulphate formulation at the once-a-day dose of 1500 mg for fourteen consecutive days. Previous studies have shown that the pharmacokinetics of glucosamine were at steady state after 3 consecutive days of administration of this formulation and dose regimen[17]. However, to avoid unnecessary discomfort to the patients during the second synovial fluid collection, this was separated from the first collection by a 14-day interval during which treatment with glucosamine sulphate was continued. This provided the additional benefit of a study design that more closely mimics the conditions found during the long-term therapeutic use of crystalline glucosamine sulphate in OA patients.

Crystalline glucosamine sulphate (Dona, Viartril-S, Xicil or other trademarks by the Rottapharm Group, Monza, Italy) is a defined, pure and stable substance in which glucosamine, sulphate, 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 sulphate. The standard formulation used in the present study consists of an oral

soluble powder presented as a sachet containing 1500 mg of the active ingredient to be taken once daily. As per current guidelines[22] the powder was dissolved each day in 240 ml of water. During the entire study period, the food and fluid intake of the patients was maintained as per instructions given at enrolment, to ensure adherence to the inclusion/exclusion criteria. Apart from these restrictions, the lifestyle of the patients remained unchanged in order to mimic as much as possible the conditions encountered in clinical practice. Blood was collected from the antecubital vein into heparinised tubes, whereas synovial fluid was collected by aseptic arthrocentesis from the knee joint. For ethical reasons, the investigators were instructed to collect a minimum amount of synovial fluid from the joint to allow the bioanalytical determinations (approximately 1 ml) regardless of the total volume of fluid available. Blood and synovial fluid were always collected from each patient at the same time during the same visit. Each patient underwent two blood and synovial fluid collections that were carried out during the two scheduled visits. The first collection of blood and synovial fluid was conducted at enrolment (time 0), i.e., before the start of the treatment period with the study drug. Glucosamine concentrations determined in plasma and synovial fluid collected before the start of treatment allowed to assess the endogenous glucosamine concentrations in these biological fluids. After the first visit the patients started the treatment period with the study drug that lasted for 14 consecutive days during which they received oral crystalline glucosamine sulphate at the standard prescription dose of 1500 mg once-a-day. On the last day of treatment (day 14) the patients returned at the study centre for the second scheduled visit to undergo the second and last blood and synovial fluid collection that was carried out 3 h after the last drug intake. The sampling time of 3 h after the last dose was chosen based on the previous healthy volunteer study that had shown that the steady state peak plasma concentrations ($C_{ss,max}$) occurred on average, 3 h after drug administration[17]. Aliquots of plasma (obtained by blood centrifugation at 2000 $\times$ g at 4°C) and synovial fluid 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 and synovial fluid concentrations were determined using identical liquid chromatography methods with mass spectrometry detection (LC−MS/MS). Details of the method in plasma have been reported elsewhere[21]. Briefly, plasma and synovial fluid samples (0.49 ml) were added with 0.01 ml of a solution of the internal standard (^{13}C-glucosamine) at a final concentration of 250 ng/ml and subjected to a protein precipitation step by the addition of 0.25 ml of a 200 mg/ml solution of trichloro acetic acid. The samples were then stirred on a vortex and centrifuged at 2000 $\times$ g for 20 min. The supernatants were transferred into injection vials and a 3 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 high performance liquid chromatography (HPLC) system from Alliance Waters (model 2695). This system was equipped with a Shodex Asahipak NH_2P-50G 2D column (150 mm $\times$ 2.0 mm Internal Diameter; particle size: 5 μm) fitted with a Shodex Asahipak NH_2P-50G 2D (30 mm $\times$ 2.0 mm Internal Diameter; particle size: 5 μm) guard column. The HPLC system was connected to a mass spectrometer from Micromass (model Quattro-LC) 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 and synovial fluid spiked with glucosamine at concentrations ranging from 10 ng/ml (the limit of quantitation [LOQ] of the methods), corresponding to a 0.06 μM concentration (based on a glucosamine Molecular Weight of 179.17 g/mol as a free base) up to 1000 ng/ml. Recovery was calculated using six replicate analyses at three concentrations (10, 100, and 800 ng/ml) and ranged from 96.1% to 107.6% in plasma and from 93.5% to 99.3% in synovial fluid. The assay precision relative standard deviation% (RSD%) calculated as mean experimental concentration/standard deviation $\times$ 100 and accuracy (BIAS%) calculated as (mean experimental concentration − theoretical concentration)/theoretical concentration $\times$ 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 4.1% to 13.8% in plasma and from 4.6% to 9.7% in synovial fluid. Its accuracy ranged from −3.0% to 7.0% in plasma and from −1.3% to 7.3% in synovial fluid. At the LOQ (10 ng/ml), the within assay precision averaged 13.8% in plasma and 9.7% in synovial fluid, whereas the within assay accuracy averaged 4.0% in plasma and 7.3% in synovial fluid.

The methods were validated according to current bioavailability guidelines[22] including bench top, long-term and repeated freeze/thawing cycles stabilities, as well as 1:10 v/v dilution with blank human plasma and synovial fluid to be able to analyse plasma and synovial samples containing glucosamine at concentrations as high as 9000 ng/ml.

STATISTICAL ANALYSIS

Because of the small number of patients, the main descriptive statistics used for demographic data, as well as for baseline and post-treatment glucosamine concentrations, were the median values and ranges. This also allowed to appropriately account for concentrations below the LOQ at baseline. The correlation between glucosamine concentrations in plasma and synovial fluid was investigated by the Pearson's test or, in case of values below the LOQ at baseline, by the Spearman's rank test and by linear regression analysis. The Wilcoxon matched-pairs test was used to compare the absolute plasma and synovial fluid concentrations at baseline and after treatment, as well as the median folds increase in concentrations between plasma and synovial fluid after treatment. $P < 0.05$ was taken as statistically significant. All inferential statistical analyses were performed for the overall group of patients and not by gender, since the power of such analysis would be too low, given the small number of subjects in each gender group. However, gender data are presented descriptively.

Results

The subject demographic characteristics are reported in Table I, where they are grouped by gender. These characteristics span over a broad range as in the general patient population.

The actual collection times post-dosing did not differ by more than 10% from the nominal collection time (3 h),

therefore it was concluded that the post-dosing collection procedure was conducted according to the study protocol (data not shown).

Endogenous glucosamine was detected in plasma collected from all 12 patients, whereas in synovial fluid endogenous glucosamine was detected in 10 subjects as one female and one male had endogenous levels below the LOQ of the method (Table II). Baseline plasma and synovial levels were characterised by a high degree of inter-subject variability as sometimes observed for endogenous substances[23]. There was a good correlation between the endogenous glucosamine concentrations in plasma and synovial fluid (Spearman's $\rho = 0.78$, $P < 0.01$). On the other hand, endogenous synovial fluid concentrations were lower ($P = 0.001$) than those in plasma (median value 45.5% lower; Table II). In both plasma and synovial fluid the endogenous concentrations of glucosamine appeared to be similar in males and females, with only a numerical trend for higher values in males (Table II).

After 14 consecutive days of treatment with glucosamine sulphate at the dose of 1500 mg/day, the drug was absorbed and was well detectable in plasma and synovial fluid collected from all the enrolled patients. Figures 1 and 2 show the individual increases of glucosamine concentrations after the 14-day treatment period compared to pre-treatment (baseline) values in plasma and synovial fluid, respectively. In both compartments, glucosamine concentrations resulted increased from baseline in all 12 enrolled patients. As summarised in Table III, the relative increases from baseline were similar in the two compartments, with only a marginally higher median increase in the synovial fluid compared with plasma: 21.5 folds vs. 20.5 folds ($P = 0.11$), suggesting that the drug has a similar distribution between the two compartments. Nevertheless, the same Table III shows that, similarly to endogenous levels at baseline, absolute post-treatment glucosamine concentrations were higher in plasma than in the synovial fluid ($P = 0.001$), with a median synovial/plasma concentration ratio of 76.5% (i.e., the median concentration in synovial fluid was only 23.5% lower than that in plasma). Figure 3 reports that there was a very high degree of correlation between post-treatment concentrations in the two compartments (Pearsons $r = 0.96$, $P < 0.0001$).

Post-treatment glucosamine concentrations were similar between males and females in plasma and synovial fluid, with a numerical trend for slightly higher concentrations in females (Table III), i.e., differently than the situation at baseline (Table II). In particular, women tended to experience higher increases from baseline concentrations in the synovial fluid compared to men.

There were no safety issues during or after treatment in the present study.

Discussion

To our knowledge, this is the first study describing the plasma and synovial fluid bioavailability of glucosamine in OA patients receiving the medication by the oral route. This study was conducted after repeated oral doses of the prescription crystalline glucosamine sulphate formulation shown to be effective in OA pivotal clinical trials[4,5,8] and thus was designed to mimic the conditions encountered during the clinical use of the drug. The study confirmed previous results that have indicated that glucosamine is available to the systemic circulation after oral administration[17] and adds new data demonstrating that the drug is also available at the site of action, i.e., the joint.

In addition, the study confirmed that endogenous glucosamine already found in plasma collected from healthy volunteers[17], is also present in plasma and in synovial fluid collected from OA patients. Previous studies failed to detect

Table II
Baseline glucosamine levels in plasma and synovial fluid before the start of the treatment period

Randomisation n°	Plasma		Synovial fluid		Synovial/plasma concentration × 100
	ng/ml	μM	ng/ml	μM	
Females					
2	50	0.28	<10	<0.06	>5
3	45	0.25	20	0.11	44
7	47	0.26	33	0.20	70
8	41	0.23	22	0.18	54
10	121	0.68	67	0.37	55
12	86	0.47	38	0.21	44
Median	48.5	0.27	27.5	0.19	49
Range	41–121	0.23–0.68	<10–67	<0.06–0.37	>5–70
Males					
1	47	0.26	<10	<0.06	>4.7
4	112	0.63	53	0.30	47
5	99	0.55	52	0.29	53
6	47	0.26	35	0.20	74
9	56	0.31	44	0.25	79
11	54	0.30	57	0.32	106
Median	55	0.31	48	0.27	64
Range	47–112	0.26–0.63	<10–57	<0.06–0.32	>4.7–106
Overall					
Median	52	0.29	36.5	0.21	54.5
Range	41–121	0.23–0.68	<10–67	<0.06–0.37	>4.7–106

In the overall population the difference between plasma and synovial fluid concentrations is statistically significant ($P = 0.001$).

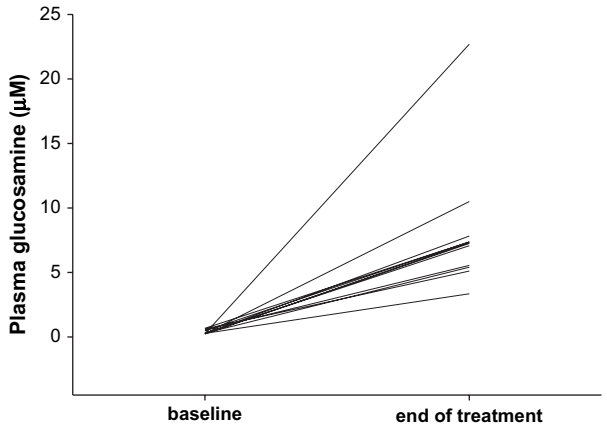

Fig. 1. Individual glucosamine concentration in plasma samples collected at baseline and at the end of treatment.

endogenous glucosamine in human and animal plasma or serum[18,19,20,24–29] and in horse synovial fluid[18]. This might be due to the LOQ of the bioanalytical methods employed in our studies (10 ng/ml in plasma and synovial fluid) that is much lower than those of these other investigations. Another reason for the discrepancy in the results obtained in different studies might be due to the fact that our study determined glucosamine in plasma, whereas some of the previous investigations in horses[18] and OA patients[19] determined glucosamine in serum. Unpublished results obtained in our laboratories, have indicated that glucosamine is unstable in blood left at room temperature to allow clotting.

The endogenous concentrations in both plasma and synovial fluid varied considerably between patients (high inter-subject variability). This finding has been reported previously in plasma of healthy volunteers[17] and thus indicates that OA is unlikely to be the cause for the observed variability. On the other hand, the presence of endogenous glucosamine in synovial fluid might be of pathophysiological relevance for different disease characteristics, given the role of glucosamine in the biology of the joint[30] and its therapeutic effects[1–8]. Further studies are warranted to investigate the possible prognostic and predictive value of endogenous glucosamine in plasma and synovial fluid in OA.

Despite a good correlation between the two compartments, endogenous glucosamine is present in synovial fluid

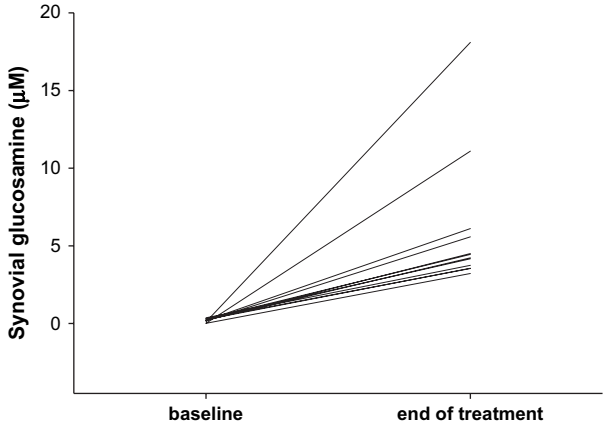

Fig. 2. Individual glucosamine concentration in synovial fluid samples collected at baseline and at the end of treatment.

of OA patients at concentrations that resulted around 50% lower than those determined in plasma and were even not detectable in two subjects. Conversely, the correlation was stronger between the levels of exogenous glucosamine determined at steady state, after repeated doses of crystalline glucosamine sulphate, in plasma and synovial fluid, and they were only approximately 25% lower in the latter, although the difference was still statistically significant. The reason why glucosamine concentrations tend to be lower in the synovial fluid compared with the plasma is unknown and this might be of pathophysiological relevance for the endogenous levels (see above) where the differences are higher. It is worth mentioning that contrarily to plasma, the total synovial fluid volume is not constant in OA patients and may vary significantly depending on the disease characteristics and severity. In the present investigation the total synovial fluid volume was not determined as it was not foreseen in the study protocol for ethical reasons. It is therefore conceivable that inter-subject variation in the synovial fluid volume might have influenced the glucosamine concentrations determined, perhaps resulting in lower (and variable) estimates of glucosamine concentrations. As a support of this hypothesis is the finding that the overall increases from baseline levels after treatment with crystalline glucosamine sulphate were similar in plasma and synovial fluid (20.5 and 21.5 median folds increases, respectively, Table III) and did not differ significantly. This indicates that the increase from baseline values may represent a more robust estimate of the bioavailability of the drug in this compartment as this parameter takes also into account the variations in the synovial volumes. Further studies are needed to assess the total amount of glucosamine in synovial fluid in addition to the glucosamine concentrations in this compartment by collection of all the available fluid from the joint by articular lavage. This study design has also been suggested for studies aimed at assessing various OA biomarkers in this compartment as described in the currently ongoing NIH OA Initiative[31]. In addition, it is worth considering that while the previous healthy volunteer study[17] indicated that peak plasma concentrations were actually achieved 3 h after dosing with 1500 mg crystalline glucosamine sulphate, i.e., the sampling time in the present investigation, it is not known whether also in the synovial fluid the peak concentrations are reached 3 h after dosing. If equilibrium between plasma and synovial fluid occurs at later times, the concentrations reported here in synovial fluid are likely to have been underestimated.

The finding that the increases in the concentrations of glucosamine from baseline in plasma and in synovial fluid were similar after repeated administration of crystalline glucosamine sulphate is clinically relevant, as it indicates that for glucosamine, monitoring of the plasma drug concentration is a valid surrogate to study the fate of the drug at the site of action. Therefore, future studies of the possible correlation between the plasma (and therefore synovial) glucosamine concentration increases and the therapeutic effects, can be designed and may assess if the exposure to glucosamine represents a predictive factor of response. The results observed in the present study, if confirmed in larger trials, provide also the rationale for the conduction of such investigations since they indicated that the concentrations of endogenous as well as exogenous glucosamine are characterised by a high degree of inter-subject variability, this being in line with the response rate of OA patients that also appears to vary[3].

Previous studies in horses using glucosamine hydrochloride have found that synovial fluid glucosamine

Table III
Glucosamine levels in plasma and synovial fluid at the end of the treatment period, three hours after the last drug administration. Folds increase from baseline values are also shown between parentheses

Randomisation n°	Plasma		Synovial fluid		Synovial/plasma concentration × 100
	ng/ml	μM	ng/ml	μM	
Females					
2	600 (12)	3.35	577 (>57.7)	3.22	96
3	4061 (90)	22.7	3248 (162)	18.1	80
7	1401 (30)	7.83	1093 (33)	6.11	78
8	1309 (32)	7.31	999 (45)	5.58	76
10	1319 (11)	7.37	672 (10)	3.75	51
12	1025 (12)	5.73	758 (20)	4.23	74
Median	1314 (21)	7.34	878.5 (39)	4.91	77
Range	600–4061 (11–90)	3.35–22.7	577–3248 (10–162)	3.22–18.1	51–96
Males					
1	1896 (40)	10.5	1978 (>197.8)	11.1	104
4	914 (8)	5.11	633 (12)	3.54	69
5	993 (10)	5.55	796 (15)	4.45	80
6	1297 (28)	7.25	808 (23)	4.51	62
9	1267 (23)	7.08	638 (15)	3.56	50
11	968 (18)	5.41	746 (13)	4.17	77
Median	1130 (20.5)	6.32	771 (15)	4.31	73
Range	914–1896 (8–40)	5.11–10.5	633–1978 (12–>197.8)	3.54–11.1	50–104
Overall					
Median	1282 (20.5)	7.17	777 (21.5)	4.34	76.5
Range	600–4061 (8–90)	3.35–22.7	577–3248 (10–>197.8)	3.22–18.1	50–104

In the overall population, the difference between plasma and synovial fluid concentrations is statistically significant ($P = 0.001$). Conversely, the fold increase in plasma and synovial fluid concentrations did not differ significantly ($P = 011$).

concentrations were 90% lower than those observed in serum and negligible in absolute values[18]. In our present study with glucosamine sulphate the median drug concentration in synovial fluid were only about 25% lower than those in plasma and not negligible in absolute values, as they were between 4 and 5 μM, or even higher if the mean values are considered. Therefore, the glucosamine plasma and synovial fluid concentrations were important and in the same order of magnitude (i.e., in the 10 μM range). Besides the use of a different glucosamine salt, this apparent discrepancy could be due to a specie-specific difference and, as stated above, to the different bioanalytical method and biological matrix used[17,18].

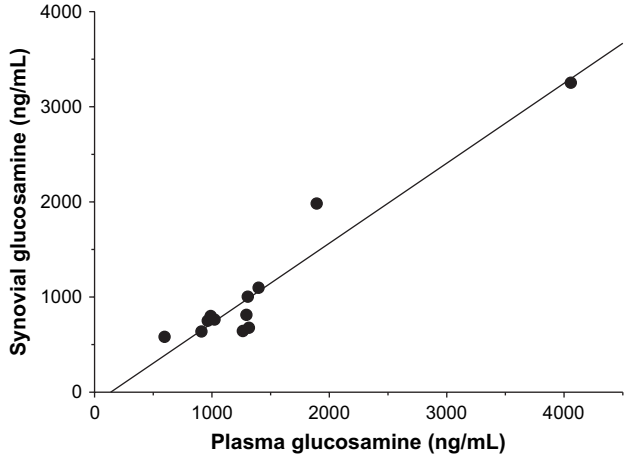

Fig. 3. Correlation between plasma and synovial fluid glucosamine concentrations at the end of treatment. The correlation was linear and described by the equation $y = 0.84x - 115.6$ ($r = 0.96$).

Previous pharmacological studies have shown that glucosamine is preferentially incorporated by chondrocytes into the components of the glycosaminoglycan chains in the intact cartilage[32], stimulates the synthesis of physiological proteoglycans[33–35] and decreases the activity of catabolic enzymes, including matrix metalloproteinases (MMPs)[33,35,36]. Selected *in vitro* models[33] showed that glucosamine was metabolically effective at concentrations hundred-folds lower than the average concentrations determined in plasma and synovial fluid in the present study while, on the contrary, other studies suggested that glucosamine was not able to stimulate glycosaminoglycan synthesis at concentrations below 1 mM[37]. On the other hand, it is unlikely that the clinical effects of glucosamine sulphate, with particular regard to the symptom-modifying effects achieved over relatively short treatment courses[3,8], but also to the putative disease-modifying activity[4,5], are linked to a mere stimulation of glycosaminoglycan synthesis. In fact, this outdated hypothesis has been recently replaced by the theory that the compound inhibits interleukin-1 (IL-1)-induced gene expression, possibly via the suppression of the cytokine intracellular signalling pathway and nuclear factor-kappaB activation[38,39] thus reversing the pro-inflammatory and joint degenerating effects of IL-1[36,40].While these initial evidences were obtained at high *in vitro* glucosamine concentrations, more recent studies have shown these effects at concentrations only slightly above those described in the present study[41], or exactly in the same 10 μM range[42]. Actually, crystalline glucosamine sulphate inhibited IL-1-stimulated gene expression of cyclooxygenase 2 (Cox-2), inducible nitric oxide synthase (iNOS), tumour necrosis factor-α, IL-6, IL-1, MMP-3 and

aggrecanase 2, with glucosamine IC_{50} ranging between 3 and 14 μM[42], close or even slightly lower than those found in both plasma and synovial fluid in the present study.

Thus, the present investigation provides compelling evidence that after oral administration of crystalline glucosamine sulphate, the active drug glucosamine reaches pharmacologically relevant concentrations in the systemic circulation and at the site of action.

The present study has been conducted using the once-a-day soluble powder formulation of crystalline glucosamine sulphate used in pivotal clinical trials[4,5,8] 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]. In fact, these uncontrolled formulations often have a much lower glucosamine content than reported in their label claims and are thus commonly underdosed[10]. In addition, there is currently no clinical justification to use different glucosamine compounds or even other glucosamine salts, e.g., hydrochloride, as pivotal trials failed to show the same benefit[3,11,15]. In addition, a single-dose pharmacokinetic study with the glucosamine hydrochloride solid preparation, at the same 1500 mg unit dose, used in the GAIT, found glucosamine peak concentrations in plasma that were only 3 μM[20], i.e., almost three-fold lower than the steady state concentrations reached with 1500 mg of crystalline glucosamine sulphate and they might be even lower in the synovial fluid and, especially, when the 1500 mg daily dose is fractioned as 500 mg three times a day as done in GAIT and in other studies. These lower concentrations found with glucosamine hydrochloride are less effective *in vitro* on the putative mechanism of action of glucosamine described above[42] and may explain the unsatisfactory clinical trial results of glucosamine hydrochloride[11,15] compared with the prescription glucosamine sulphate[43]. There are several reasons why glucosamine hydrochloride formulations might have a lower bioavailability than the standard prescription glucosamine sulphate formulation. First of all, the latter is formulated as a soluble powder for oral administration, thus providing an extemporary liquid formulation that has by definition, optimal and higher bioavailability compared with a solid formulation. In addition, the different nature of the formulation involves the use of different excipients that may also affect bioavailability, similarly to the presence and stoichiometric ratio of different ions in the different glucosamine salts and stabilisation processes[17].

Unfortunately, there are no pharmacokinetics and bioavailability studies of other glucosamine sulphate preparations and it is therefore impossible to comment on their clinical value relative to the prescription formulation.

Finally, the sulphate supplementation provided by glucosamine sulphate may significantly contribute to its effects[44,45] and it would be interesting to asses if the sulphate concentrations in synovial fluid increase after the administration of glucosamine sulphate. It is intriguing that the only clinically relevant results in GAIT were seen in the subgroup of more severe patients when glucosamine hydrochloride was combined with chondroitin sulphate, providing further support to the hypothesis that increasing the sulphate concentrations may have therapeutic effects[45].

Another hypothesis generated by the present study is that of the higher plasma and synovial fluid glucosamine concentrations in females compared to males after treatment with glucosamine sulphate. The difference could not be analysed statistically (due to the small sample size), and might be only apparent as it was not presents at baseline (Table II). This issue should be assessed in larger, suitably designed trials taking into consideration that in the present study, this hypothetical difference does not appear to be due to gender related differences in body mass index (BMI) that, as shown in Table I, was similar in males and females (27.9 and 27.1, respectively).

A possible limitation of the present study is that the bioanalytical determinations although relevant from a pharmacological point of view, did not allow the determination of glucosamine concentrations in the cartilage tissue. It has been demonstrated that 2 h after administration of radiolabelled glucosamine to rats and dogs, the radioactivity accumulated in the knee cartilage of the treated animals with values being 13-folds higher than those determined in plasma[46]. If this is the case also for humans, the glucosamine concentrations achieved in the cartilage of OA patients after oral administration of crystalline glucosamine sulphate might be much higher than those determined in plasma and synovial fluid in the present investigation.

A number of other potential limitations should be acknowledged. First of all, the study is of limited size, due to the difficulties and ethical restrictions in providing not only blood, but also synovial fluid samples on repeated occasions. Larger patient cohorts might be recruited in future studies to further assess the clinical effects of glucosamine sulphate, as long as the collection of plasma and synovial fluid does not affect the efficacy and safety evaluation, which may be problematic. Larger patient samples might also offer the opportunity to test possible differences for gender, age groups and concomitant diseases, including gastrointestinal function disorders. Finally, a direct comparison of the bioavailability of glucosamine sulphate and glucosamine hydrochloride might also be useful.

In conclusion, we have described the plasma and synovial bioavailability of glucosamine after oral administration of crystalline glucosamine sulphate in man. Our findings indicate that the drug is available systemically and at the site of action (the joint) at concentrations that are in line with those found to be effective in *in vitro* models on the putative mechanism of action of the drug, thus supporting the favourable clinical results in OA. Future studies should asses the total amount of glucosamine in the joint and the existence of a possible correlation to treatment response. Additional studies should compare the bioavailability of glucosamine at the site of action after the administration of the prescription glucosamine sulphate formulation used here with that of other glucosamine salts, derivatives, formulations, or dose regimens. This might provide some clues to explain the observed discrepancies in therapeutic effects.

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