Sulfate Could Mediate the Therapeutic Effect of Glucosamine Sulfate

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Glucosamine sulfate is a controversial osteoarthritis remedy that is presumed to stimulate articular cartilage glycosaminoglycan synthesis by increasing glucosamine concentrations in the joint space. However, this is not plausible because even large oral doses of the product have no effect on serum glucosamine concentrations. We propose instead that sulfate could mediate the clinical benefit attributed to this treatment. Sulfate is required for glycosaminoglycan synthesis, and unlike glucosamine, its serum level can be modified by dietary and other factors. In this study, we tested whether oral glucosamine sulfate increases serum sulfate concentrations and whether the sulfate concentration in the synovial fluid reflects that in the serum. The serum sulfate concentration of 7 normal subjects was 331 ± 21 μmol/L before ingestion of 1.0 g glucosamine sulfate and 375 ± 17 μmol/L 3 hours after ($P < .05$). Serum sulfate concentrations decreased from 325 ± 19 to 290 ± 19 μmol/L when the same dose of glucosamine sulfate was ingested with 1.0 g of the analgesic drug acetaminophen, which is largely metabolized by sulfation ($P < .05$). Unlike glucosamine sulfate, oral sodium sulfate did not significantly increase the serum sulfate concentration. Synovial fluid and serum sulfate concentrations were closely similar when measured in 15 patients undergoing diagnostic needle aspiration of a knee effusion ($r = .99$, slope = .97, $P < .0001$). These results do not prove that glucosamine sulfate improves osteoarthritis, but considered with other data, they do provide a plausible biochemical mechanism for its reported beneficial effects. This hypothesis is clinically relevant because it predicts that nonsulfate salts of glucosamine will be ineffective and that renal function, diet, and concurrent acetaminophen therapy could confound clinical trials of this therapy.

GLUCOSAMINE SULFATE is a nutritional supplement widely used to treat the symptoms of osteoarthritis. Notwithstanding the skepticism of many experts, critical reviews of prospective clinical trials[1,2] and a recent meta-analysis[3] report evidence that glucosamine sulfate has mild to large beneficial effects in osteoarthritis. However, the clinical trial data are not entirely consistent, and defects in the individual trials have been pointed out.[1-3] Large, definitive trials are advocated.[4-6]

The mechanism of action of glucosamine sulfate (and that of a related product, chondroitin sulfate) is not understood, but antiarthritic effects have been reported in cell culture and whole-animal testing systems.[7-9] These are presumed to result from the provision of glucosamine as a substrate for articular cartilage glycosaminoglycan (GAG) synthesis.[3,4,10] However, this mode of action is virtually impossible in vivo because even large oral doses of glucosamine do not affect serum glucosamine concentrations.[10,11] If oral glucosamine does not increase serum glucosamine concentrations, it cannot increase glucosamine availability within the joint space, where GAG synthesis takes place.

In light of the clinical evidence that glucosamine sulfate may benefit patients with osteoarthritis, it is worthwhile to consider alternative explanations for its effectiveness. We wondered whether sulfate, rather than glucosamine, is the active component. Sulfate is required for GAG synthesis, and van der Kraan et al[12,13] have shown that sulfate depletion inhibits GAG synthesis in rat articular cartilage. Indeed, GAG synthesis in human articular cartilage is particularly sensitive to sulfate deficiency.[14]

In this study, we examined 2 simple underpinnings of the hypothesis that sulfate mediates the biologic effects of glucosamine sulfate therapy. First, we determined whether oral glucosamine sulfate increases the serum sulfate concentration. Second, we determined whether there is agreement between the concentration of sulfate in serum and that in the synovial fluid, which is the compartment from which the substrates for GAG synthesis are drawn. We also tested whether use of the analgesic drug acetaminophen, which is largely metabolized by sulfation, affects the serum sulfate response to glucosamine sulfate administration.

SUBJECTS AND METHODS

Seven healthy adults (4 women and 3 men, age 34 ± 4 years, weight 65 ± 4 kg, body mass index 22 ± 1 kg/m^2), with normal biochemistries and using no medications, had venous blood drawn to measure serum sulfate before and 3 hours after they ingested 1 g of glucosamine sulfate (1.65 mmol sulfate; SISU Enterprises Co Inc, Burnaby, BC, Canada) and, at least 2 days later, before and 3 hours after they ingested 1 g of glucosamine sulfate together with 1 g (6.62 mmol) of acetaminophen. Six subjects (3 women and 3 men, age 31 ± 5 years; weight 70 ± 4 kg; body mass index 24 ± 1 kg/m^2) had serum sulfate measured before and 3 hours after they ingested 1 g of anhydrous sodium sulfate (3.5 mmol sulfate). These studies were carried out in the postabsorptive state. Fifteen nonfasting outpatients (9 women and 6 men, age 65 ± 4 years, weight 75 ± 4 kg, body mass index 27 ± 1.4 kg/m^2) with osteoarthritis who were about to undergo diagnostic needle aspiration of a knee effusion gave permission to measure the sulfate concentration in their synovial fluid and in a sample of venous blood drawn within 1 hour of obtaining the synovial fluid. All volunteers gave written consent for the study, which was approved by the Research Ethics Committee of the Sir Mortimer B. Davis Jewish General Hospital.

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Clotted blood was centrifuged at 1,400 × *g* for 10 minutes, and the serum was transferred into screw-cap vials and stored at −30°C. A sample of the synovial fluid was transferred immediately into an SST Gel and Clot Activator Vacutainer blood collection tube (Becton-Dickinson, Franklin Lakes, NJ), then centrifuged at 1,400 × *g* for 20 minutes at 4°C. The supernatant was transferred into screw-cap vials and stored at −30 °C. Serum and synovial fluid inorganic sulfate were detected and measured by ion-exchange high-performance chromatography with conductivity detection (IEC-CD) using a Dionex 2110i chromatography system (Dionex, Sunnyvale, CA), as previously described.[15] The serum was diluted 10-fold and the synovial fluid diluted 20-fold with type 1 or ultrapure water (purified with Milli-RO Plus and Milli-Q UF Plus systems, resistivity > 18 Mohm/cm; Millipore, Bedford, MA), then passed through an Amicon filter (10,000 molecular weight cut-off; Beverley, MA) by centrifugation (1,400 × *g*), and the filtrate was injected onto the chromatographic column. Serum and synovial fluid urea and creatinine were measured with a Hitachi 917 automated analyzer (Laval, QC, Canada).

The amount of sulfate in the glucosamine sulfate product we used and its ionic form were measured by dissolving 10 mg of the product in 200 mL of deionized water. The solution was sonicated for 10 minutes, filtered (0.45-μm Millipore filter), and analyzed as described above. The amount of sulfate measured precisely matched the label claim, showing that glucosamine sulfate is an ionic salt. To check for the presence of organic sulfate esters, we measured total sulfate in the product by mixing 0.5 mL of the solution described above with 1 mL of 0.04 mol/L HCl in a sealed container, which was incubated in a boiling water bath for 30 minutes. This treatment hydrolyzes organic sulfate molecules to release inorganic sulfate.[16] The sulfate content did not increase, demonstrating the absence of organic sulfate in the product.

Two-way repeated-measures analysis of variance (ANOVA) or the paired *t* test were used to determine the significance of differences in the serum sulfate response to glucosamine sulfate, glucosamine sulfate plus acetaminophen, or sodium sulfate (SigmaStat version 1.0; Jandel Corporation, San Rafael, CA). When ANOVA results showed significance, Newman-Keuls test was used post hoc to determine the source of difference. The paired *t* test was used to compare serum and synovial fluid sulfate concentrations. All results are presented as means ± SEM.

RESULTS

The serum sulfate concentration of normal subjects was comparable to concentrations previously reported by others (290 to 350 μmol/L).[17-20] As shown in Table 1, serum sulfate was significantly increased 3 hours after ingestion of 1.0 g of glucosamine sulfate. The effect was reversed when glucosamine sulfate and 1.0 g acetaminophen were ingested at the same time. In a separate study, the serum sulfate concentration was 345 ± 15 μmol/L before and 369 ± 22 μmol/L 3 hours after ingestion of 1.0 g (3.5 mmol) of anhydrous sodium sulfate ($P = .15$).

Table 1. Serum Sulfate Concentrations Before and 3 Hours After Oral Ingestion of 1 g Glucosamine Sulfate or Glucosamine Sulfate Plus 1 g Acetaminophen

Treatment	Baseline (μmol/L)	3 Hours (μmol/L)	% Change	*P*
Glucosamine sulfate	331 ± 21	375 ± 17	+14.6 ± 4.4	<.05
Glucosamine sulfate and acetaminophen	325 ± 19	290 ± 19*	−10.3 ± 3.9	<.05

NOTE. Data are presented as means ± SEM.
*Significantly different from glucosamine sulfate alone ($P < .05$)

Table 2. Sulfate, Urea, and Creatinine Concentrations in Serum and Synovial Fluid

	Serum	Synovial Fluid
Sulfate (μmol/L)	404 ± 35	402 ± 34
Urea (mmol/L)	6.6 ± 0.5	6.5 ± 0.4
Creatinine (μmol/L)	77 ± 5	63 ± 5*

NOTE. Data are presented as mean ± SEM.
*Significantly different from serum ($P < .002$).

All synovial fluid samples were noninflammatory, with no calcium pyrophosphate or sodium urate cyrstals present. Serum and synovial fluid sulfate and urea concentrations were closely similar, whereas serum creatinine was higher than synovial fluid creatinine concentration (Table 2). Serum and synovial fluid sulfate and urea concentrations were virtually identical among individuals (Fig 1). Serum urea was highly predictive of serum and synovial fluid sulfate concentrations (Fig 2). Serum creatinine concentration also predicted the synovial fluid sulfate concentration ($r = .85$, $P < .0001$).

DISCUSSION

We have shown that oral glucosamine sulfate increases serum inorganic sulfate concentration in normal persons and that this effect is reversed by coingestion of acetaminophen, a widely used analgesic drug that is largely metabolized by sulfation.[21] For reasons that are not clear, sodium sulfate that provided more than twice the sulfate dose as glucosamine sulfate failed to increase the serum sulfate concentration. This interesting observation needs further confirmation but suggests that intestinal absorption of sulfate may be improved when it is ingested as the glucosamine salt. In large doses, sodium sulfate is an osmotic laxative, although small amounts of it are well absorbed from the gastrointestinal tracts of humans[22] and rats.[23] Specific information about the relative bioavailabilities of the different sulfate salts is lacking.

It is not known whether coingestion of food with glucosamine sulfate would further boost the serum sulfate concentration. Serum sulfate concentrations increase after consumption of a mixed diet[19] or a protein load[24] as a consequence of sulfur amino acid catabolism.

Serum and synovial fluid sulfate concentrations were found to be virtually identical. This is important because the synovial fluid is the presumed source of the sulfate used for articular cartilage GAG synthesis. It would have been desirable but was not possible to measure synovial fluid sulfate concentrations at a timed interval after consumption of glucosamine sulfate. In light of the essentially perfect agreement between sulfate concentrations measured in matched serum and synovial fluid samples and sulfate's rapid distribution in the extracellular fluid space,[25] we consider it highly likely that changes in serum sulfate concentration are matched by similar changes in synovial fluid sulfate concentration. Synovial fluid sulfate concentrations were recently reported to be slightly higher than serum sulfate concentrations measured in a separate reference population.[20] The

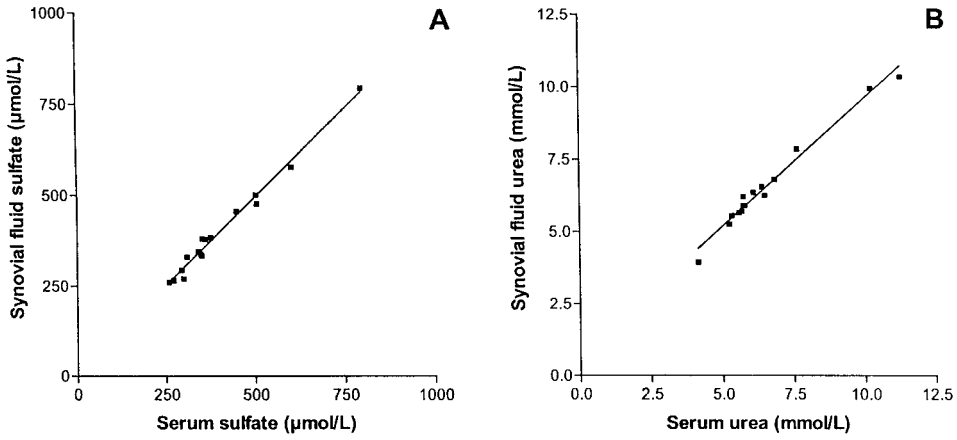

Fig 1. (A) Serum versus synovial fluid sulfate concentrations ($r = .99$, $P < .0001$, slope = 0.97). (B) Serum versus synovial fluid urea concentrations ($r = .99$, $P < .0001$, slope = 0.88).

present data should be considered more reliable because they are from matched serum and synovial fluid samples. The close relationship we observed between serum urea or creatinine and serum sulfate is not surprising because urinary sulfate clearance diminishes as renal function decreases.[21]

The present results are not proposed as providing strong evidence that sulfate mediates the therapeutic effects of glucosamine sulfate, nor do they bear directly on the question of whether glucosamine has therapeutic effects at all. However, they do indicate that sulfate is a far more plausible candidate than glucosamine for any such beneficial effects. In the only studies of glucosamine kinetics available, radioactively labeled glucosamine tracer was absorbed from the gastrointestinal tract, but an oral *substrate* dose of 6,000 mg failed to increase the plasma glucosamine concentration into the detectable range of the assay used, 45 µmol/L,[10] perhaps because of first-pass hepatic metabolism.[11] This should allay any concern that standard oral doses of glucosamine (which are less than one tenth of this) could incur insulin resistance,[4] but it also makes it impossible for oral glucosamine to change the glucosamine concentration in the synovial fluid, as it must to stimulate GAG synthesis in articular cartilage.

Unlike glucosamine, which is readily synthesized from molecules ubiquitous in the normal intracellular environment, extracellular (and hence, intra-articular) sulfate concentrations can vary considerably.[19,24] Sulfate depletion inhibits GAG synthesis in rat articular cartilage,[12,13] and human articular cartilage GAG synthesis is particularly sensitive to sulfate deficiency.[14] The extracellular sulfate pool of humans is amongst the smallest of all the species,[26] and it is readily depleted by consumption of a low-protein diet[27] or by drugs that, like acetaminophen, are metabolized by sulfation.[18,20,26] The basic studies often cited as providing evidence that glucosamine stimulates GAG biosynthesis could represent responses to sulfate rather than to glucosamine.[7-9]

These considerations underscore the importance of basing the evaluation of any new therapy on a biologically plausible rationale. Glucosamine is currently available in pharmacies and health food stores as the sulfate, hydrochloride, *N*-acetyl, or chlorohydrate salt.[28] Earlier clinical trials reporting a beneficial effect of glucosamine in osteoarthritis used glucosamine sulfate, but this is no longer the case. One recent carefully designed randomized, prospective clinical trial used glucosamine chloride.[29] The form of glucosamine used in another trial was not specified.[30] Results of both trials were essentially negative.

An important implication of the hypothesis that sulfate mediates the reported beneficial effects of glucosamine sulfate is that concurrent use of the analgesic drug acetamino-

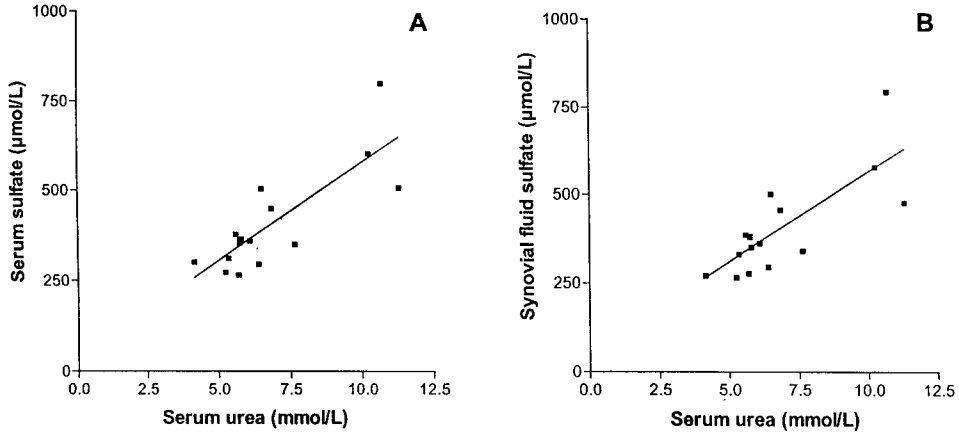

Fig 2. (A) Serum urea versus serum sulfate ($r = .82$, $P = .0001$). (B) Serum urea versus synovial fluid sulfate ($r = .78$, $P = .0003$).

phen could confound clinical trial results. As the present study reconfirms, acetaminophen is known to reduce serum sulfate concentrations.[18,20,26] Van de Kraan et al[12] and de Vries et al[31] have carefully detailed the potential adverse effects of acetaminophen and other sulfate-lowering drugs on cartilage metabolism in arthritis. Conversely, sulfate administration can increase the metabolic clearance of acetaminophen in previously sulfate-depleted persons, potentially reducing its analgesic potency.[21] These mutually neutralizing effects of glucosamine sulfate and acetaminophen do not appear to have been considered in the design of any clinical trials involving glucosamine sulfate, despite the high risk of a false-negative result.

In conclusion, we have shown that oral glucosamine sulfate increases serum sulfate concentrations in humans. This effect is reversed by concurrent ingestion of acetaminophen, an analgesic medication commonly used to treat the symptoms of arthritis. Sulfate concentrations in synovial fluid closely match those in serum. We have outlined the reasons sulfate is more likely than glucosamine to mediate the therapeutic effects of glucosamine sulfate therapy in osteoarthritis. This hypothesis should considered in evaluating the results of clinical trials of glucosamine therapy that use nonsulfate forms of glucosamine, allow study participants to use acetaminophen, or include patients with impaired renal function.

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