Glucosamine sulfate for knee osteoarthritis: science and evidence-based use

The pharmacological treatment of osteoarthritis is traditionally accomplished with nonspecific symptomatic agents that are generally effective only for acute symptom relief. Compounds are currently being searched for that might exert specific effects on osteoarthritis pathogenesis and thus induce at least a similar short-term symptomatic effect, but also control the progression of the disease in the long-term. Glucosamine sulfate is, so far, the only drug that has demonstrated a combined symptom-modifying and potential structure-modifying effect in knee osteoarthritis when used as the prescription crystalline glucosamine sulfate formulation at the dose of 1500 mg once daily. These effects may be explained by reversal of the proinflammatory and joint-degenerating effects of IL-1 through the inhibition of the cytokine intracellular signaling pathway. However, efficacy data obtained with this prescription glucosamine sulfate formulation may not be applicable to all glucosamine products available as generics or dietary supplements, owing to pharmacodynamic and pharmacokinetic reasons.

KEYWORDS: clinical trials ■ disease modification ■ glucosamine sulfate ■ IL-1 ■ joint-space narrowing ■ osteoarthritis ■ pharmacokinetics ■ safety ■ structure modification ■ symptom modification

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Osteoarthritis (OA) is the most common form of arthritis and the most prevalent among the rheumatic diseases. It is a degenerative joint disorder affecting the articular cartilage and the underlying subchondral bone with low-grade synovial inflammation. OA may affect all diarthrodial joints and the most appropriate definition of OA combines the description of the disease pathology with the presence of pain that occurs with joint use [1]. OA is particularly frequent at the large, weight-bearing joints of the lower limbs. Radiographic osteoarthritic changes of the knee tibiofemoral compartment occur in 5–15% of the general population aged 35–74 years in the western world [1]. Symptomatic knee disease occurs in approximately 6% of US adults over 30 years of age [2], with general incidence and prevalence increasing two- to ten-fold from 30 to 65 years of age [3]. The impact on disability attributable to knee OA is similar to that caused by cardiovascular disease and greater than that caused by any other medical condition in the elderly [4].

Treatment guidelines for knee and hip OA have been developed by both the American College of Rheumatology [5] and the European League Against Rheumatism [6,7]. The two guidance documents have been developed by different procedures and, although they share some basic principles, they differ with respect to the level of recommendation of specific classes of drugs. This is particularly evident for symptomatic slow-acting drugs in OA, the class of agents in which glucosamine sulfate is generally included, and might be due to the differences in the regulatory status between the USA and Europe. For this and other reasons, the guidelines might need further updates soon. Indeed, a revision of the American College of Rheumatology guidelines is due for publication by the end of 2010.

More recent guidelines have been published by national bodies, for example the UK NICE [201] and global scientific organizations such as the OA Research Society International (OARSI) [8–10]. While the NICE guidelines suffer from local regulatory and budget constraint limitations, the OARSI guidelines provide an objective and comprehensive update of the available therapeutic options.

Given the limitations in terms of both efficacy and safety of the available nonspecific symptom-relieving drugs, such as pure analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), all guidelines acknowledge the need for medications that not only offer acceptable short-term symptom control, but also have a role in the medium- and long-term management of the disease (symptom-modifying effect). In addition, the role would include the possibility to delay the progression of joint structure changes (structure-modifying effect), thereby modifying the evolution of the disease and thus preventing clinically significant disease outcomes (disease-modifying

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effect). These aims may be achieved by drugs that might exert specific effects on OA pathogenesis factors in contrast to the nonspecific symptomatic agents. To date, glucosamine sulfate is probably the drug with the most extensive evidence in this regard.

Glucosamine sulfate

Glucosamine is a naturally occurring monosaccharide, and a normal constituent of glycosaminoglycans in the cartilage matrix and synovial fluid, which exerts specific pharmacologic effects on osteoarthritic cartilage and chondrocytes [11,12]. The glucosamine base needs to be salified for pharmaceutical use, and glucosamine sulfate is the salt originally developed as a prescription drug and has shown benefit in different OA clinical trials [6,9,13]. However, some recent generic products and a number of dietary supplements that contain glucosamine hydrochloride have not demonstrated effectiveness in clinical trials [8–10,201]. In addition, several glucosamine sulfate formulations marketed as dietary supplements could present some pitfalls in the amount and quality of the active ingredient differing from the prescription product. A recent Cochrane Review identified major differences between the positive results of clinical trials conducted with the glucosamine sulfate prescription formulation and those with other glucosamine preparations that failed to show a similar efficacy [13]. Therefore, this article will concentrate on the crystalline glucosamine sulfate formulation that has been originally approved as a prescription drug in Europe and elsewhere, and is available as a branded dietary supplement in the USA. Nevertheless, high-quality clinical trials conducted with glucosamine hydrochloride or with other glucosamine sulfate formulations will also be included, and their results analyzed in order to assess the overall evidence available for the use of glucosamine in OA.

Chemistry

Crystalline glucosamine sulfate is also known as glucosamine sulfate sodium chloride. It is a pure substance (molecular weight = 573.31) synthesized from chitin of sea origin, and in which glucosamine (molecular weight = 179.17), sulfate, chloride and sodium ions are present in stoichiometric ratios of 2:1:2:2 (Figure 1). In fact, it seems that glucosamine sulfate (molecular weight = 456.43) would not be stable unless prepared as crystalline glucosamine sulfate according to the patented process [101]. The dose is expressed as the net content in glucosamine sulfate and the prescription drug is most widely available as sachets of powder for oral solution of 1500 mg glucosamine sulfate to be administered once daily.

It is still unclear how comparable other preparations of glucosamine sulfate – mainly available as generics or as a dietary supplement in countries where the substance is regulated – are with this prescription formulation in terms of active ingredient content, purity and stability, since this information is generally not available.

Pharmacodynamics

Glucosamine is preferentially incorporated by chondrocytes into the components of glycosaminoglycan chains in the intact cartilage [14], stimulating the synthesis of physiological proteoglycans [15–17] and decreasing the activity of catabolic enzymes in the cartilage, including matrix metalloproteinases [16–18]. *In vivo*, the compound is effective in experimental animal models of OA [19,20]. For several years, the mechanism of action of glucosamine sulfate was considered the mere incorporation of glucosamine into glycosaminoglycans as a simple building block, thereby stimulating their synthesis. However, this hypothesis seems to be oversimplified. In fact, while metabolic effects exerted at the level of articular cartilage might support a long-term joint structure-modifying activity, they hardly explain the relatively short-term symptomatic benefit outlined in clinical trials.

However, recent studies have shown that high glucosamine concentrations stimulated glycosaminoglycan synthesis *in vitro* [21] and they may largely exceed the concentrations achieved in biological fluids after oral administration to humans [22]. Nevertheless, selected *in vitro* models also demonstrated that glucosamine was effective in increasing proteoglycan levels by upregulating the expression of corresponding genes at concentrations 100-fold lower and compatible with those found in biological fluids during treatment in humans [16]. Furthermore, the compound may selectively accumulate in the cartilage after repeated dosing [23], thus likely providing higher local concentrations. An alternative hypothesis has been proposed, suggesting the existence of relevant glucosamine metabolic activities in tissues where extracellular glucosamine concentrations might be higher, including the intestine, liver and kidney, which might modulate the compound antiarthritic effects [24]; however, this hypothesis seems at least premature at this time. A potentially better alternative suggests that the increase in the production of cartilage extracellular matrix is mediated by

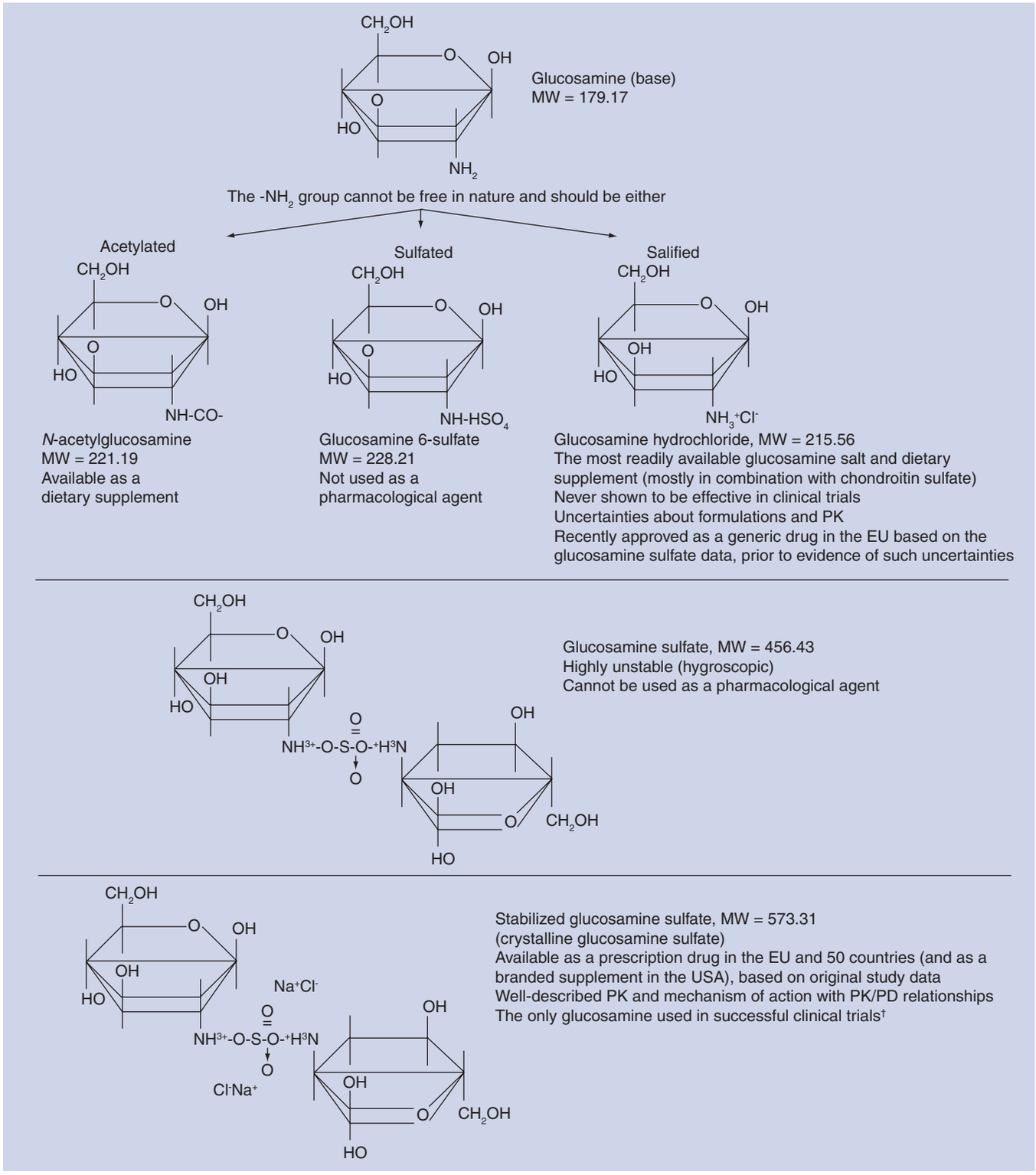

Figure 1. Glucosamine and its salts.
†A glucosamine sulfate salt stabilized with KCl instead of NaCl is available as a supplement, but has never been tested in PK studies or shown to be effective in clinical trials.
MW: Molecular weight; PD: Pharmacodynamic; PK: Pharmacokinetic.

glucosamine-induced upregulation of TGF-β, which has been observed at clinically relevant concentrations in the low micromolar range [25].

A unifying hypothesis for glucosamine sulfate's mechanism of action in OA has recently been proposed, and supports the role of this

compound as both a symptom-modifying and a structure-modifying agent in OA. This mechanism refers to glucosamine-induced reversal of the proinflammatory and joint-degenerating effects of IL-1β [26,27] through the blockade of the cytokine intracellular signaling cascade, by inhibiting the activation of the nuclear factor (NF)-κB pathway [28]. Indeed, glucosamine sulfate has been shown to inhibit the IL-1β-induced activation and nuclear translocation of NF-κB in human osteoarthritic chondrocytes [29]. Moreover, glucosamine sulfate was able to inhibit both gene expression and protein synthesis of cyclooxygenase (COX)-2 selectively over COX-1, via the inhibition of NF-κB activation. Thus, glucosamine sulfate further prevented the release of prostaglandin E_2 into the culture medium [28].

Several new lines of evidence are progressively appearing to further substantiate this mechanism of inhibition of IL-1β-induced expression of genes involved in the pathophysiology of joint inflammation and tissue destruction. Indeed, NF-κB activity has been found to be inhibited by glucosamine sulfate in both human chondrocytes and synoviocytes, with subsequent decrease in COX-2 protein synthesis, prostaglandin E_2 and nitric oxide release, showing a pattern that differs from that of other potential antiosteoarthritic agents and NSAIDs [30]. Moreover, glucosamine sulfate consistently decreased IL-1β-induced metalloproteinase synthesis in both types of cell [30].

Most of these *in vitro* experiments used glucosamine concentrations higher than those found in human plasma after therapeutic doses (see 'Pharmacokinetics' section later). However, recent studies pointed out that this mechanism is operative at glucosamine concentrations at approximately 10 μM or lower [31], which have been found in human plasma or synovial fluid after therapeutic doses of crystalline glucosamine sulfate. Indeed, effective glucosamine concentrations (expressed as IC_{50}) that inhibit IL-1-stimulated gene expression of different proinflammatory or prodegenerative transcripts, including IL-1β itself, matrix metalloproteinase-3, COX-2 and inducible nitric oxide synthase, have been reported to range between 6.2 and 13.8 μM [30].

In another *in vitro* study that confirmed the suppressive effect of glucosamine on both anabolic and catabolic gene expression in the osteoarthritic cartilage [32], the authors speculate that the effect of glucosamine sulfate as a potential disease-modifying agent might be due to anticatabolic activities, rather than anabolic activities. Interestingly, glucosamine sulfate has been found to be a stronger inhibitor [31]. This and other recent human pharmacokinetic findings might help to explain the different findings of recent clinical trials between these two glucosamine salts.

In addition, the increase of sulfate concentrations following the administration of glucosamine sulfate might also contribute to the differences between glucosamine sulfate and glucosamine hydrochloride [33,34]. Thus, the sulfate increase might overcome a deficiency in inorganic sulfur caused by low levels of dietary proteins (containing sulfur-rich amino acids) in the elderly. Sulfur is an essential mineral ion for the synthesis of proteoglycans and other sulfur-containing metabolic intermediates (e.g., coenzyme A and glutathione), which are important for chondrocyte metabolism [33,34].

Pharmacokinetics & metabolism

The pharmacokinetics and metabolism of glucosamine sulfate have recently been extensively reviewed [23,35]. For a long time, limited knowledge regarding the pharmacokinetics of glucosamine (including oral bioavailability, peak plasma levels and tissue distribution) hampered the full understanding of the relationship between the compound's clinical effects and its mechanism of action. The great difficulty in establishing the compound's pharmacokinetics and thus performing bioequivalence studies against the patented formulation of crystalline glucosamine sulfate led to the appearance of other undocumented glucosamine salts (e.g., hydrochloride), improperly stabilized glucosamine sulfate substances, and different dosage forms or regimens on the market, which have obtained negative results in different clinical trials (as reviewed later) [12].

The main limitation of the description of the pharmacokinetics of glucosamine has been the lack of suitable bioanalytical methods with sufficient sensitivity and specificity for the detection of the compound in biological fluids. Early studies tried to elucidate the pharmacokinetics and metabolism of oral glucosamine sulfate in rats, dogs and humans, using ^{14}C-labeled glucosamine [22]. Although these studies provided compelling information regarding the absorption, distribution and elimination of radioactivity, they were not fully 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

concentrations of the unchanged compound following oral administration of therapeutic doses [22].

Specific and sensitive bioanalytical methods, such as liquid chromatography with mass spectrometry detection for the determination of unchanged glucosamine in human plasma, urine and synovial fluid, have recently become available [36,37], thus allowing the collection of more detailed information on the pharmacokinetic profile of unchanged glucosamine in humans, including its bioavailability and distribution at the site of action in the joint.

The complete pharmacokinetic profile of glucosamine has been described for the first time by Persiani *et al.*, who used repeated administrations of the standard crystalline glucosamine sulfate formulation at 1500 mg once daily in healthy volunteers in a rigorously designed pharmacokinetics study [36]. They found that glucosamine is rapidly bioavailable from orally administered crystalline glucosamine sulfate with maximum average plasma concentrations up to 100-fold higher than endogenous levels and in the 10 µM range after approximately 3 h. Steady-state pharmacokinetic parameters indicate that glucosamine is distributed to both vascular and extravascular compartments and is eliminated with a half-life estimated to be approximately 15 h, thus supporting once-daily dosing. The absolute bioavailability could not be assessed in this study, but was estimated to be approximately 25% with the help of other recent animal data [38]. Finally, the pharmacokinetics of glucosamine were linear in the dose range of 750–1500 mg of glucosamine sulfate, but not at higher doses [36].

Data from knee OA patients, demonstrated by the same group, indicated the presence of endogenous glucosamine levels in both plasma and synovial fluid, with distinct intrasubject variability particularly at the synovial fluid level [39]. The potential pathophysiological significance of this variability should be further investigated. In these patients, repeated once-daily doses of 1500 mg crystalline glucosamine sulfate reached similar peak plasma and synovial fluid concentrations that were in the 10 µM range [36].

Single-dose pharmacokinetic studies with other glucosamine formulations at similar doses, in particular the glucosamine hydrochloride solid preparation used in the Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) found glucosamine peak concentrations in plasma that were only 3 µM [40]. These concentrations are at least threefold lower than the steady-state concentrations reached with 1500 mg of crystalline glucosamine sulfate. Furthermore, in this failed GAIT study, the rate, maximum concentration and extent (area under the curve) of glucosamine bioavailability were even lower when the glucosamine hydrochloride solid preparation was given chronically in fractioned doses of 500 mg three-times daily [40]. Moreover, a comparative study on the pharmacokinetics of glucosamine confirmed these differences in bioavailability observed after repeated administration of crystalline glucosamine sulfate, 1500 mg once daily (as in all successful glucosamine sulfate clinical trials), versus glucosamine hydrochloride, 500 mg three-times daily (as in GAIT) [41]. Such lower concentrations found with glucosamine hydrochloride are less effective *in vitro* at inhibiting IL-1β-mediated effects, the mostly imputed mechanism of action of glucosamine described previously [29,30]. Taken together, these data may explain the conflicting clinical trial results.

Besides the difference in dosing regimen, animal studies have shown that glucosamine hydrochloride may have a lower bioavailability than crystalline glucosamine sulfate [42]. In addition, the bioavailability of glucosamine is decreased in studies where the compound was combined with chondroitin sulfate [40,41], suggesting that the efficacy of this drug combination is uncertain.

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

Glucosamine is mainly metabolized through the hexosamine pathway and, as an amino sugar, it is not a substrate of the cytochrome P450 system, thus metabolic drug–drug interactions are unlikely during the clinical use of glucosamine.

Clinical efficacy

The role of glucosamine sulfate as both a symptom-modifying and a potential structure-modifying drug in OA is supported by the proposed underlying mechanisms of action described earlier. All clinical trials performed until 2009 have been systematically evaluated in a recent re-edition of a Cochrane Review [13] that supersedes two previous high-quality meta-analyses [43,44]. This Cochrane Review examines the results of all 25 eligible randomized controlled trials available with any glucosamine formulation for symptom modification on approximately 5000 patients, and concludes that glucosamine is superior to placebo for improvement in both pain and function, with a moderate effect size. Notably, these results were driven by the

randomized controlled trials that evaluated the prescription formulation of crystalline glucosamine sulfate (labeled 'Rotta preparation' in the Cochrane Review) and that found clinically relevant effect sizes for both pain and function relative to placebo. However, a potential bias might also exist in these studies since most were sponsored by the manufacturer. Pooled results for trials using a different glucosamine preparation failed to reach such statistical significance when compared with placebo. Although similar results had been described in previous meta-analyses, a systematic review did not appropriately manage the high heterogeneity that inevitably occurs when trials of different design and duration are considered in the context of such types of meta-analysis [45]. Conversely, this was appropriately done in the meta-analysis by Reginster, which included only high-quality, pivotal clinical trials of prescription crystalline glucosamine sulfate, 1500 mg once daily, in the analysis [46].

Glucosamine sulfate should not be regarded as a drug for short-term symptom relief, but one for OA disease management. This includes symptom modification over appropriate treatment durations of at least 6 months according to current regulatory guidelines [202], and probable long-term trials for assessing both joint structure modification and symptom modification. Three pivotal trials currently satisfy these requirements for crystalline glucosamine sulfate.

Reginster *et al.* [47] and Pavelka *et al.* [48] were the first to show a potential OA-modifying effect by a pharmacological agent in two long-term studies. In 3-year-long, randomized, placebo-controlled clinical trials, they demonstrated that glucosamine sulfate was not only able to control the symptoms of the disease over such a long-term treatment course, but also significantly decrease the progression of joint structure changes in patients with mild-to-moderate OA of the knee. The data were particularly strong in the subgroup of female postmenopausal patients [49]. Joint structure changes were principally assessed by monitoring radiographic joint-space narrowing. The results are summarized in Table 1 and show a similar quantitative effect in the two trials, as previously [44] and more recently [13] acknowledged in meta-analyses. Some concerns have been expressed regarding the standardization of x-rays in these studies, particularly on the results that were obtained with the full-extension knee radiographic view, a technique that was state-of-the-art at the time of the trials, but that might be less efficient than later widely accepted semiflexed views. In addition, the results might have been biased by the marked symptom improvement caused by glucosamine sulfate. In fact, the improvement in pain might have influenced the degree of knee extension and artificially increased the tibiofemoral joint radiographic space, by changing the medial tibial plateau alignment with the x-ray beam [50]. However, it has been recently demonstrated that pain was not a confounder in joint-space narrowing assessment in these trials [51]. Furthermore, the long-term symptom-modifying efficacy of glucosamine for OA was considered as 'impressive' [52].

In addition, follow-up data of the two patient cohorts suggest that on average 5 years after the end of these long-term trials and drug withdrawal, the patients who had received glucosamine sulfate were less likely to undergo total joint replacement [53]. Thus, these data would indicate that glucosamine sulfate might indeed affect the progression of OA, preventing clinically significant disease outcomes. This finding might be explained by the symptom- and structure-modifying effect achieved during the treatment period in terms of a clinically relevant response. In particular, a recent position paper suggested that 'failure' of glucosamine sulfate treatment in a patient might be a good predictor of joint surgery and a proper surrogate outcome during long-term clinical trials for disease

Table 1. Radiographic joint space width and 3-year joint-space narrowing in millimeters at the narrowest point of the medial tibiofemoral compartment of the knee joint.

Study (sample size)	Enrollment JSW (mean ± standard deviation)		Mean 3-year JSN (95% CI)		Ref.
	Placebo	*GS*	*Placebo*	*GS*	
Reginster (n = 106)	3.95 ± 1.24	3.82 ± 1.32	-0.40 (-0.56 to -0.24)	-0.07* (-0.22 to 0.07)	[47]
Pavelka (n = 101)	3.63 ± 1.57	3.89 ± 1.48	-0.19 (-0.29 to -0.09)	-0.04** (-0.06 to 0.14)	[48]

**p = 0.003; **p = 0.001 versus placebo.*
GS: Glucosamine sulfate; JSN: Joint-space narrowing; JSW: Joint-space width.

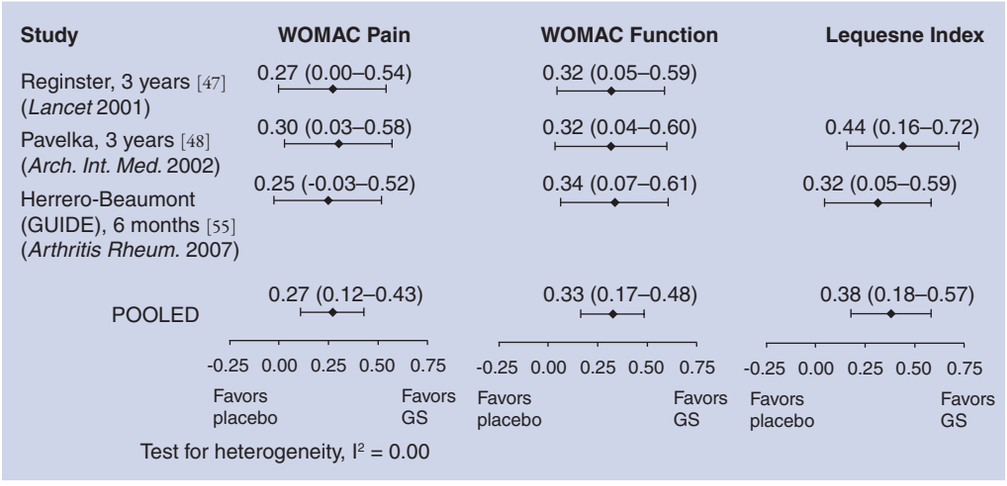

Figure 2. The size of effect of prescription glucosamine sulfate 1500 mg once daily on knee osteoarthritis symptoms in pivotal trials.
GS: Glucosamine sulfate; GUIDE: Glucosamine Unum in Die (once-a-day) Efficacy; WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

modification in OA [54]. When this group of experts assessed the proportion of 'failures' defined as a joint-space narrowing of 0.5 mm or more, and less than 20% improvement in the Western Ontario and McMaster University OA Index (WOMAC) pain subscale in the two long-term studies by Reginster *et al.* [47] and Pavelka *et al.* [48], they found that 41% of patients failed treatment with glucosamine sulfate versus 60% with placebo (p = 0.003) [54].

A recent pivotal study, the Glucosamine Unum in Die (once-a-day) Efficacy (GUIDE) trial [55], assessed the role of glucosamine sulfate in the management of knee OA symptoms over 6 months in comparison with placebo and acetaminophen (paracetamol) as a reference drug. Acetaminophen is the preferred long-term symptomatic medication indicated by most current OA practice guidelines [6–10,201]. The GUIDE trial evaluated 318 patients (88% women) randomized to double-dummy placebo or 1 g acetaminophen tablets three-times a day, or to the standard prescription formulation of glucosamine sulfate soluble powder, 1500 mg once daily. The data demonstrated that the mean improvement in the Lequesne index with glucosamine sulfate was significantly higher than with placebo. Conversely, the improvement with acetaminophen failed to reach statistical significance versus placebo. The clinical relevance of the effect size of glucosamine sulfate on the primary outcome in this trial is supported by the higher proportion of treatment responders to glucosamine sulfate according to the OARSI-A criteria: 39.6% versus 21.2% with placebo (p = 0.007). Acetaminophen also had more responders than placebo, although at a lower degree of significance (33.3%, p = 0.047). These results support the hypothesis that the prescription formulation of glucosamine sulfate might be the preferred symptomatic medication in knee OA, although a concern regarding study blinding has been suggested. The meta-analysis by Reginster also found clinically relevant effect sizes on both pain and function in the three pivotal clinical trials (Figure 2) [46]. These effect sizes are comparable with those of NSAIDs, but while the NSAID-induced effect sizes are usually only observed in short-term clinical studies less than 12 weeks in duration [56], the efficacy of glucosamine sulfate is sustained in high-quality trials of 6 months to 3 years [46].

The positive results with prescription crystalline glucosamine sulfate contradict those from the NIH-supported GAIT study [57], where the glucosamine-treated group showed only a non-significant trend of efficacy. This large 6-month trial compared glucosamine, given as glucosamine hydrochloride 500 mg three-times daily, or chondroitin sulfate, or their combination, with placebo and celecoxib as reference standards. The overall results are shadowed by the huge placebo effect (60%) and by the use of not properly controlled rescue analgesic medication, since even the reference standard medication produced disappointing, but statistically significant, results in efficacy compared with placebo [58]. Conversely, none of the supplements were significantly superior to placebo [57]. Glucosamine hydrochloride at the dose of 500 mg three-times daily had only been used in one previous randomized controlled trial whose results were mostly negative [59]. The accompanying editorial

on the GAIT article states that "the NIH findings for glucosamine is not surprising, given the nonconventional glucosamine formulation used and that all previous favorable trials had been conducted with the crystalline glucosamine sulfate prescription preparation" [60]. As described in the 'Pharmacodynamics' and 'Pharmacokinetics & metabolism' sections earlier, the peak glucosamine plasma levels achieved with the NIH hydrochloride formulation are much lower [40] than with the prescription sulfate formulation, and therefore they might not share the same pharmacological properties [30].

In addition, the presence of sulfates has been suggested as an important glucosamine mechanism of action [33]. Interestingly, the most significant results in the GAIT study were achieved in a subgroup of more severe patients when glucosamine hydrochloride was combined with chondroitin sulfate [57], presumably by increasing the plasma levels of sulfate [33] and even of glucosamine metabolites to concentrations closer to those achieved with the prescription glucosamine sulfate formulation used in GUIDE and the other pivotal trials.

More recently, the 2-year subgroup extension of the GAIT study was published [61,62]. Despite the questionable study design and the poor glucosamine hydrochloride preparation used at the wrong dosing schedule, they observed encouraging long-term symptom-modifying trends with glucosamine similar to those observed with celecoxib [62]. Even more remarkably, structure-modifying effects with this glucosamine preparation were close to statistical significance, especially in the milder patient subgroup [61].

Taken together, current treatment guidelines on OA management, such as those described by OARSI [8–10] and NICE [201], acknowledge the difference between glucosamine sulfate and glucosamine hydrochloride. While the OARSI [8–10] and European League Against Rheumatism [6] guidelines uniformly recommend the use of prescription glucosamine sulfate formulation for knee OA, the NICE guidelines fail to do so since only glucosamine hydrochloride was available on the market in the UK at the time of development of the guidelines [201]. The American College of Rheumatology guidelines for OA management are not expected to recommend the use of glucosamine in their new issue. This could be explained by the fact that glucosamine is marketed as a dietary supplement in USA and it is impossible to ensure an adequate quality of product that cannot show a comparable efficacy and safety demonstrated by crystalline glucosamine sulfate, a prescription product in Europe and elsewhere [HOCHBERG M, PERS. COMM.].

Finally, a recent pharmacoeconomic study demonstrated that prescription crystalline glucosamine sulfate formulation is a cost-effective therapy compared with acetaminophen or placebo for use in patients with OA of the knee [14,63]. Thus, the prescribed glucosamine sulfate formulation represents an attractive alternative for the management of OA, which has a high socioeconomical and personal cost.

Safety & postmarketing surveillance

All studies and meta-analyses carried out have recognized the good overall safety profile of glucosamine sulfate and of glucosamine in general [47,48,55,57,62]. Clinical studies have never shown statistically or clinically significant differences between glucosamine sulfate and placebo in the incidence of adverse events or safety related drop-outs [47,48,55]. On the other hand, in comparative trials, the incidence of adverse events and related drop-outs was always significantly higher in the reference groups receiving conventional NSAIDs than in the glucosamine group, with the majority of adverse events in the NSAID-treated patients affecting the GI tract [57,60].

Although at a significantly lower incidence than that shown by conventional NSAIDs, adverse events observed in the glucosamine sulfate group also affected the gastrointestinal system. They included: mild and transient abdominal pain, nausea, dyspepsia, diarrhea or constipation. Headache, drowsiness and fatigue have also been reported. Uncommon adverse events included depressed mood, vertigo and skin rash. Cross-reactions in patients with seafood allergy are unlikely, owing to the exhaustive purification process that excludes the presence of protein residues in the starting material of sea origin.

Glucosamine is an amino monosaccharide that can enter the hexosamine pathway (one of the alternative routes of glucose metabolism) and thereby may increase insulin resistance, as suggested by animal experimental studies using suprapharmacological intravenous doses of the compound [64]. Although this remains an area of concern, human studies with extremely high intravenous [65] or even intra-arterial [66] doses of glucosamine indicated that the hexosamine pathway activation probably does not operate in humans because insulin sensitivity, secretion or action were not affected in these studies. More recently, a study in healthy volunteers who

received repeated oral doses of a glucosamine sulfate formulation at 1500 mg once daily demonstrated no changes in serum insulin or blood glucose levels with the 3-h glucose tolerance test [67]. Another placebo-controlled study showed that patients with Type 2 diabetes receiving a glucosamine and chondroitin sulfate combination for 3 months had no change in their diabetes management or hemoglobin A1c concentrations [68]. Finally, fasting plasma glucose levels were not modified in short-term studies with crystalline glucosamine sulfate as well as in the long-term trial by Reginster [47]. By contrast, the 3-year study by Pavelka reported four patients who developed diabetes during the study, but three were in the placebo group and only one received glucosamine sulfate treatment [48]. Other laboratory evaluations have never detected significant abnormalities in hematological or other metabolic parameters.

Interactions with other drugs are unlikely, since glucosamine is mainly absorbed via glucose transporters and does not compete for general absorption mechanisms. In addition, it is mainly metabolized independently of the cytochrome P450 enzyme system [70].

Since crystalline glucosamine sulfate is a prescription drug in several countries, it is subject to stringent postmarketing surveillance analysis. The latest Periodic Safety Update Report estimates an exposure of around 20 million patients in the period between 1995 and 2010. Few adverse drug reactions were reported and these did not affect the good safety pattern described earlier.

Regulatory affairs

Crystalline glucosamine sulfate is approved as a prescription medicinal product for the treatment of OA or its symptoms (affecting mainly the knee) in over 60 countries, including the EU and several countries in Eastern Europe, Asia and Latin America.

Based on the results of clinical trials, the most widely approved dosing regimen of the prescription medication is 1500 mg glucosamine sulfate once daily, mainly given as an oral soluble powder of crystalline glucosamine sulfate. In some countries, glucosamine-based medicinal products are available over the counter in variable regimens, often as solid oral formulations of other glucosamine salts (e.g., hydrochloride).

In the USA, glucosamine-containing products are considered dietary supplements due to the provisions of the Dietary Supplement Health and Education Act of 1994 [203]. This has led to the appearance of different glucosamine products on the market that are not subject to any pharmaceutical control by the US FDA. Likewise, the authorities of other countries have adopted a similar approach. In this regard, the active ingredient content of several of these products has been recently questioned [71]. Whether this had an impact on the disappointing results of some recent clinical trials using them [13], together with deficiencies in the study design [46], is unknown. In other trials where the pharmaceutical grade of the dietary supplement formulation was assured, as in the NIH-GAIT study [57], other factors already described in this article, such as the use of glucosamine salts with no previously documented efficacy (i.e., hydrochloride) at a distinct dose regimen and subsequent differences in pharmacokinetic and pharmacological behavior, might have played a role.

Conclusion

Crystalline glucosamine sulfate is a specific symptom-modifying drug in OA for both short- and long-term treatment courses, whose use is largely supported by different high-quality clinical trials. Two 3-year trials also suggested that the drug has joint structure-modifying properties and might be a disease-modifying agent, and therefore should be used as a first-line drug for the management of OA. Furthermore, long-term follow-up data of patients participating in the 3-year trials suggested that previous treatment with glucosamine sulfate might prevent the need for total knee replacement.

The clinical evidence of the efficacy and safety of glucosamine sulfate has been the object of rigorous systematic reviews and meta-analyses, and resulted in the inclusion of the drug in most of the currently available practice guidelines.

The pharmacological events that support the clinical effects of glucosamine sulfate in OA seem to be related to the inhibition of the IL-1β intracellular signaling pathway, and thus of cytokine-induced gene expression. These pharmacological effects are achieved *in vitro* at drug concentrations found in plasma and synovial fluid of knee OA patients after oral administration of the standard therapeutic doses. Differences in the clinical effects with generic or dietary supplement glucosamine hydrochloride formulations may indeed be related to differences in dose regimens and in pharmacokinetics, which may lead to differences in the pharmacological properties. In addition, the presence of sulfates in the prescription drug, stabilized according to a patented process, has also been suggested to be important because it may favor

some of the compound's pharmaco-metabolic characteristics that might not be shared by glucosamine hydrochloride. Conversely, preparations of glucosamine sulfate other than the prescription formulation manifest differences in quality and dose regimens that would require appropriate pharmacokinetic and bioequivalence studies. Since these are not currently available, it is impossible to apply the efficacy and safety results obtained with crystalline glucosamine sulfate to these other preparations and *vice versa*.

Future perspective

Glucosamine sulfate is the first pharmacological agent for which a combined symptom-modifying and potential structure-modifying effect has been shown in long-term clinical trials. While this evidence is still unique within the treatment of OA, future studies of new drugs should take into account this experience and the evolving technology in outcome measurement and clinical trial performances.

Symptoms of the disease will need to be assessed over an appropriate length of treatment (i.e., for at least 6 months and/or the duration necessary to evaluate joint structure changes), if any. Clinical assessment should be carried out using appropriate patient-reported outcomes.

To date, joint structure-modifying activity has been evaluated mainly by radiography, quantitatively monitoring the joint-space narrowing as a surrogate for cartilage loss and adopting a qualitative assessment for bone reactive changes. Newer radiographic techniques are emerging to assess these outcomes with more accuracy and precision, but should be properly validated before being adopted in clinical trials. In the meantime, other imaging techniques may prove to be more powerful in detecting cartilage quantitative and qualitative changes, as well as modifications in other joint structures and tissues: the best candidate technique in this regard is MRI.

Changes in joint structure should be clinically relevant in terms of their association with favorable symptom outcomes or by modifying the natural progression of the disease. In addition, long-term trial outcomes should focus on parameters such as patient's disability, quality of life and disease management. The latter should prospectively assess a possible decrease in the rate of surgical total joint replacement, and focus on appropriate pharmacoeconomic evaluations.

Finally, glucosamine sulfate has been studied mainly in clinical trials of primary knee OA. Efficacy of the prescription preparation at other joints should be thoroughly investigated. In addition, anecdotical evidence of efficacy in secondary OA, including post-traumatic disease or sport injury and rehabilitation has been reported and requires confirmation in appropriately designed studies. Given its putative mechanism of action, the potential of glucosamine sulfate in the prevention of OA might also be investigated.

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Executive summary

Chemistry

- Crystalline glucosamine sulfate is the stabilized form of glucosamine sulfate, which is used as a prescription drug in osteoarthritis (OA).
- Glucosamine, sulfate, chloride and sodium ions are present in stoichiometric ratios of 2:1:2:2.
- Other glucosamine salts (e.g., hydrochloride) and substances are widely used as generics or dietary supplements, and these may not share the same properties.

Mechanisms of action

- Different *in vitro* studies have shown that glucosamine sulfate has both anabolic properties – stimulating the synthesis of proteoglycans – and anticatabolic activities – inhibiting the effects of enzymes such as metalloproteinases.
- While other explanations are possible for the metabolic effects of glucosamine sulfate at clinically relevant concentrations, the main mechanism of action seems to be the inhibition of the proinflammatory and joint-degenerating effects of IL-1β. This anti-IL-1β effect is probably accomplished by suppression of NF-κB activation. Thus, glucosamine sulfate inhibits IL-1β-stimulated gene expression of COX-2, inducible nitric oxide synthase, cytokines and metalloproteinases at glucosamine concentrations (10 μM) found in human plasma and synovial fluid after administration of glucosamine sulfate standard oral doses.
- However, the mere incorporation of the amino sugar in glycosaminoglycans and stimulation of their synthesis as a simple building block is unlikely, owing to the high concentrations needed to achieve this effect.
- Sulfate might also contribute to the mechanism of action of glucosamine sulfate by overcoming a possible deficiency in inorganic sulfur in the elderly, thus providing an additional mechanism compared with other glucosamine salts (e.g., hydrochloride). Furthermore, sulfate might possibly change the different pharmacokinetic behavior of the compound.

Pharmacokinetic properties

- Following oral administration of crystalline glucosamine sulfate 1500 mg once daily in humans, peak plasma concentrations of approximately 10 μM glucosamine are achieved on average after 3 h.
- Similar levels are achieved in the synovial fluid of knee OA patients.
- The terminal elimination half-life from human plasma is estimated to be approximately 15 h.
- Glucosamine absolute bioavailability from crystalline glucosamine sulfate is estimated to be approximately 25% from animal data.
- Glucosamine hydrochloride provides a glucosamine bioavailability in humans that is much lower than 1500 mg once daily glucosamine sulfate and might be less pharmacologically effective.
- Glucosamine is metabolized independently of the cytochrome P450 system.

Clinical efficacy

- All systematic reviews and meta-analyses agree on the efficacy of crystalline glucosamine sulfate in the control of OA symptoms, with an at least comparable magnitude to conventional nonsteroidal anti-inflammatory drugs and with a long-lasting effect after drug withdrawal.
- Three pivotal placebo-controlled trials demonstrated a statistically significant and clinically relevant symptom-modifying effect for glucosamine sulfate treatment ranging from 6 months to 3 years in knee OA patients with mild-to-moderate disease.
- The two pivotal 3-year trials showed a joint structure-modifying effect based on radiological joint space narrowing.
- In the pivotal 6-month trial (Glucosamine Unum in Die Efficacy [GUIDE] study), a comparator group receiving the currently preferred symptomatic medication acetaminophen failed to reach a significant benefit versus placebo, in contrast to the group receiving glucosamine sulfate 1500 mg once daily.
- In the 6-month, NIH-sponsored Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) study, glucosamine hydrochloride 500 mg three-times daily failed to show clinical efficacy compared with placebo. Thus, these results disagree with previous positive results obtained with glucosamine sulfate 1500 mg once daily in GUIDE and other previous trials, highlighting possible differences in pharmacokinetic and pharmacological behavior.
- Similarly, studies performed with dietary supplement or generic glucosamine sulfate preparations did not show the same efficacy of the prescription product, and indeed they have never been shown to possess the same quality and to be bioequivalent with the original prescription preparation.

Safety & tolerability

- In all clinical trials, glucosamine sulfate has had an incidence of adverse events and related drop-outs similar to placebo.
- In comparative trials, glucosamine sulfate safety was significantly better than that of conventional nonsteroidal anti-inflammatory drugs.
- The low proportion of adverse events consists mainly of mild-to-transient episodes of abdominal pain, nausea, dyspepsia, diarrhea, constipation, headache, drowsiness and fatigue. Uncommon adverse events include depressed mood, vertigo and skin rash.
- Cross-reactions in patients with sea food allergy are unlikely, due to the purification process that eliminates protein residues in the starting material of sea origin.
- Human studies failed to show an effect on glucose metabolism, although this remains an area of attention owing to the possible interaction with the hexosamine pathway.
- Glucosamine sulfate is not expected to interact with other drugs, except on rare exceptions with warfarin, antimitotic chemotherapy, acetaminophen or antidiabetes drugs, as it does not interfere with general absorption mechanisms and is not metabolized by the cytochrome P450 system.

Dosage & administration

- The most widely studied and approved prescription formulation is available as sachets of oral soluble powder of crystalline glucosamine sulfate, equivalent to 1500 mg glucosamine sulfate, and administered once daily.

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