Crystalline glucosamine sulfate in the management of knee osteoarthritis: efficacy, safety, and pharmacokinetic properties

Lucio C. Rovati, Federica Girolami and Stefano Persiani

Therapeutic Advances in Musculoskeletal Disease published online 8 March 2012
DOI: 10.1177/1759720X12437753

The online version of this article can be found at:
http://tab.sagepub.com/content/early/2012/03/07/1759720X12437753

Published by:
SAGE
http://www.sagepublications.com

Additional services and information for Therapeutic Advances in Musculoskeletal Disease can be found at:

Email Alerts: http://tab.sagepub.com/cgi/alerts
Subscriptions: http://tab.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav

>> OnlineFirst Version of Record - Mar 8, 2012

What is This?
Crystalline glucosamine sulfate in the management of knee osteoarthritis: efficacy, safety, and pharmacokinetic properties

Lucio C. Rovati, Federica Girolami and Stefano Persiani

Abstract: Glucosamine is an amino monosaccharide and a natural constituent of glycosaminoglycans in articular cartilage. When administered exogenously, it is used for the treatment of osteoarthritis as a prescription drug or a dietary supplement. The latter use is mainly supported by its perception as a cartilage building block, but it actually exerts specific pharmacologic effects, mainly decreasing interleukin 1-induced gene expression by inhibiting the cytokine intracellular signaling cascade in general and nuclear factor-kappa B (NF-κB) activation in particular. As a whole, the use of glucosamine in the management of osteoarthritis is supported by the clinical trials performed with the original prescription product, that is, crystalline glucosamine sulfate. This is the stabilized form of glucosamine sulfate, while other formulations or different glucosamine salts (e.g. hydrochloride) have never been shown to be effective. In particular, long-term pivotal trials of crystalline glucosamine sulfate 1500 mg once daily have shown significant and clinically relevant improvement of pain and function limitation (symptom-modifying effect) in knee osteoarthritis. Continuous administration for up to 3 years resulted in significant reduction in the progression of joint structure changes compared with placebo as assessed by measuring radiologic joint space narrowing (structure-modifying effect). The two effects combined may suggest a disease-modifying effect that was postulated based on an observed decrease in the risk of undergoing total joint replacement in the follow up of patients receiving the product for at least 12 months in the pivotal trials. The safety of the drug was good in clinical trials and in the postmarketing surveillance. Crystalline glucosamine sulfate 1500 mg once daily is therefore recommended in the majority of clinical practice guidelines and was found to be cost effective in pharmacoconomic analyses. Compared with other glucosamine formulations, salts, or dosage forms, the prescription product achieves higher plasma and synovial fluid concentrations that are above the threshold for a pharmacologically relevant effect, and may therefore justify its distinct therapeutic characteristics.

Keywords: efficacy, glucosamine, glucosamine hydrochloride, glucosamine sulfate, knee osteoarthritis, pharmacokinetics, safety

Introduction

Osteoarthritis (OA) is the most common form of arthritis and the most prevalent among rheumatic diseases. OA is a degenerative joint disorder with minimal signs of inflammation, and it is a progressive disease whose clinical manifestations are joint structure abnormalities (visible by imaging modalities) and a symptom complex characterized by pain, function limitation, and disability, with reduced quality of life. The etiopathogenetic trigger is an abnormal intra-articular stress that results in progressive failure of the cartilage extracellular matrix, along with changes in the synovium and subchondral bone. OA is most frequently localized 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 [Pendleton et al. 2000]. Symptomatic knee disease occurs in
approximately 6% of US adults over 30 years of age [Felson and Zhang, 1998], with general incidence and prevalence increasing 2-10-fold from age 30 to 65 years [Oliveira et al. 1995]. The impact on disability attributable to knee OA is similar to that due to cardiovascular disease, and greater than that caused by any other medical condition in the elderly [Guccione et al. 1994].

Given the limitations in terms of efficacy, especially long term, and safety of the available unspecific symptom-relieving drugs, such as pure analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) [Bjordal et al. 2004], there is a growing need for medications that offer acceptable short-term symptom control, but especially have a role in the medium- and long-term symptom management of the disease (symptom-modifying effect), with the possibility of delaying 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 effect). These aims might be achieved by drugs that, unlike nonspecific symptomatic agents, might exert specific effects on OA pathogenetic factors. Glucosamine sulfate is probably so far the drug with the most extensive evidence in this regard, especially due to the clinical studies performed with the formulation known as crystalline glucosamine sulfate.

**Chemistry and pharmacodynamic properties of crystalline glucosamine sulfate**

Glucosamine is a naturally occurring amino monosaccharide and a normal constituent of glycosaminoglycans in the cartilage matrix and synovial fluid [Hamerman, 1989], which when given exogenously, exerts specific pharmacological effects in joint tissues.

Glucosamine is a small molecule (molecular weight [MW] = 179.17) and, chemically, it is a base (Figure 1). Since the –NH₂ group cannot be free in nature, it should be acetylated, sulfated, or salified. Acetylation leads to N-acetylg glucosamine (MW = 221.19), that is seldom used in pharmacologic studies and is available in few countries as a dietary supplement without any description of use in clinical trials. Sulfate conjugation leads, for example, to glucosamine-6-sulfate (MW = 573.31).
which is present in nature but has never been used as a pharmacologic agent. Thus, glucosamine is used in the treatment of OA as one of its salts, namely glucosamine hydrochloride or glucosamine sulfate that, as shown in Figure 1, are different molecules. Glucosamine hydrochloride (MW = 215.16) is the most readily available glucosamine salt and this explains why it is the one most commonly used in dietary supplements and generic glucosamine products. However, it has never been shown to be effective in clinical trials [Zhang et al. 2010], probably because of issues on formulations, doses, and pharmacokinetics, as will be described here. Glucosamine sulfate (MW = 456.43) is hygroscopic and therefore highly unstable. It can be stabilized (Figure 1) with sodium chloride according to a patented process [De Wan and Volpi, 1997] to obtain crystalline glucosamine sulfate, that is, the prescription product (or branded supplement in the USA) object of the present review.

Crystalline glucosamine sulfate (Dona®, Viatril-S®, Arthryl®, Xicil®, Osaflexan®, Glusartel®, or other trademarks by the originator company Rottapharm | Madaus, Monza, Italy), is also known as glucosamine sulfate sodium chloride. It is a pure substance (MW = 573.31) synthesized from chitin of sea origin and in which glucosamine, sulfate, chloride, and sodium ions are present in stoichiometric ratios of 2:1:2:2. The dose is expressed as the net content in glucosamine sulfate and, as a prescription drug, the substance is generally available as sachets of powder for oral solution of 1500 mg administered once daily.

It is unclear at present how other preparations of glucosamine sulfate (including an attempt at stabilization with potassium chloride), mainly available as generics or in countries where the substance is regulated as a dietary supplement, compare with this proprietary formulation in terms of active ingredient content, purity, and stability, since this information is generally not available. In this situation, and especially in view of the absence of appropriate bioequivalence studies, it is not known how the clinical efficacy and safety results obtained with crystalline glucosamine sulfate apply to these uncontrolled nutraceutical or generic preparations, and vice versa. In addition, several dietary supplements claim glucosamine sulfate in content, but they usually do not contain the labeled amount [Russell et al. 2002], nor crystalline glucosamine sulfate.

For several years, the dominant belief supported by the use of the substance as a dietary supplement devoid of pharmacologic activity, has been that most of the activities and the mechanism of action of glucosamine sulfate might be recontexted to the mere incorporation of glucosamine in glycosaminoglycans, and thereby the stimulation of their synthesis as a simple building block. Indeed glucosamine is preferentially incorporated by chondrocytes into the components of glycosaminoglycan chains in the intact cartilage [Noyszewski et al. 2001], and stimulates the synthesis of physiologic proteoglycans [Bassleer et al. 1998; Piperno et al. 2000; Dodge and Jimenez, 2003]. However, this cannot explain the therapeutic effects of the drug in clinical trials and in fact, glucosamine concentrations able to stimulate glycosaminoglycan synthesis in vitro are high [Mroz and Silbert, 2004], and probably largely in excess of those that may be achieved in biological fluids after oral administration to humans [Biggee et al. 2006]. The increased production of cartilage extracellular matrix might be rather better explained by glucosamine-induced upregulation of the transforming growth factor-beta [Varghese et al. 2007], which, even if shown in animals at clinically relevant concentrations [Ali et al. 2011], might be linked to glucosamine-stimulated overactivation of the hexosamine pathway that does not seem to take place in humans as will be described below.

While even recent reviews fail to clearly elucidate it [Block et al. 2010], the generally accepted mechanism of action of the compound in OA relates to glucosamine-induced reversal of the pro-inflammatory and joint-degenerating effects of interleukin-1 (IL-1) [Sandy et al. 1998; Gouze et al. 2001; Shikhman et al. 2001], more specifically inhibiting the cytokine intracellular signaling cascade, namely the activation of the nuclear factor-kappa B (NF-kB) pathway [Gouze et al. 2002]. In particular, glucosamine sulfate has been shown to inhibit IL-1-induced activation and nuclear translocation of active NF-kB family members in human osteoarthritic chondrocytes [Largo et al. 2003].

As is common in mechanistic studies, most of these and other in vitro experiments used glucosamine concentrations higher than those found in human plasma after therapeutic doses and that are in the 10 µM range [Persiani et al. 2005b]. This is further complicated with glucosamine by the high concentrations of glucose in the medium.
Therapeutic Advances in Musculoskeletal Disease 0 (0)

...of most in vitro experiments, which compete with glucosamine for the glucose transporter GLUT1, preventing efficient glucosamine uptake into the cells that can be overcome only by high glucosamine concentrations. Recent studies employing different and more physiologic medium conditions in human chondrocyte cell models have shown that crystalline glucosamine sulfate can inhibit IL-1-stimulated gene expression at glucosamine concentrations similar to, or even lower than those found in plasma or synovial fluid of knee OA patients receiving the drug at the therapeutic dose of 1500 mg once daily [Chiusaroli et al. 2011]. Minimal effective concentrations of glucosamine ranged between 1 µM and 10 µM for inflammatory factors and cytokines such as COX-2, inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha, IL-6 or IL-1 itself, and between 0.1 µM and 1 µM for matrix degradation factors, such as stromelysin-1 and A Disintegrin And Metalloproteinase with Thrombospondin motif (ADAM-TS5) (aggrecanase 2). Interestingly, the gene expression of NF-kB subunits and JunB was inhibited at even lower concentrations, between 1 nM and 0.1 µM [Chiusaroli et al. 2011]. Therefore, while glucosamine modulation of OA-relevant gene expression triggered by IL-1 is probably initiated by a decrease in NF-kB nuclear translocation [Letari et al. 2003], the effect on transcription might be sustained afterwards by the inhibition of NF-kB subunit expression [Chiusaroli et al. 2011]. Notably, a selective epigenetic mechanism has also been recently advocated to explain the inhibitory effect of glucosamine on NF-kB-dependent transcription [Imagawa et al. 2011].

While it is unclear whether different glucosamine salts exert the same pharmacologic effects, studies in osteoarthritic cartilage found that glucosamine sulfate is a stronger inhibitor of gene expression than glucosamine hydrochloride [Uitterlinden et al. 2006]. Actually, the differences between glucosamine sulfate and glucosamine hydrochloride might be important at both the pharmacologic and pharmacokinetic levels. In addition, sulfate concentrations obviously increase after administration of glucosamine sulfate [Hoffer et al. 2001; Cordoba and Nimni, 2003]. This might possibly overcome a deficiency in inorganic sulfur caused by low levels of dietary proteins (containing sulfur amino acids) in the elderly: sufficient sulfur is essential for the synthesis of proteoglycans and other S-containing metabolic intermediates (e.g. coenzyme A, glutathione, etc.) that are important for chondrocyte metabolism [Hoffer et al. 2001; Cordoba and Nimni, 2003]. Overall, these pharmacologic hints and the differences in the pharmacokinetic pattern that will be summarized here may help to explain the different findings of recent clinical trials with different glucosamine salts and formulations.

Animal models of experimental OA may help to characterize the biological plausibility of the use of a drug in the human disease. Similarly to other compounds, glucosamine sulfate has been shown to be effective in prophylactic models of surgically induced OA [Altman and Cheung, 2001]. More recently, the compound was used in a treatment paradigm in rats after anterior cruciate ligament transaction, and it improved OA histological changes including cartilage disorganization, hypocellularity, proteoglycan reduction, denudation of articular surface and deep fissures, together with a 60% reduction in the synovitis score compared with controls [Wen et al. 2010]. This was in parallel with a clinically relevant attenuation of the nociceptive behavior as characterized by a decrease in mechanical allodynia threshold, and in weight-bearing distribution in the injured paw [Wen et al. 2010].

On the other hand, surgically induced experimental OA may not reflect all aspects of spontaneous idiopathic OA in humans. For this reason, crystalline glucosamine sulfate was recently tested in STR/ort mice that spontaneously develop genuine OA with age, in which the whole joint undergoes degenerative changes entirely similar to those described in human OA [Mason et al. 2001]. It was found that when given chronically in a therapeutic fashion after development of the disease, crystalline glucosamine sulfate ameliorated the histological damage (Figure 2), the extent of the lesions, and histomorphometry in this model [Chiusaroli et al. 2011].

**Efficacy**

Glucosamine sulfate is proposed as a specific symptom-modifying and structure-modifying drug in knee OA. This recommendation is based on early proof-of-concept studies and more recent high-quality pivotal trials performed with prescription crystalline glucosamine sulfate 1500 mg once daily. Indeed, glucosamine sulfate scored the highest level of evidence and strength of recommendation for knee OA symptoms in the current European League Against Rheumatism.
and it is recommended by the latest Osteoarthritis Research Society International (OARSI) guidelines [Zhang et al. 2007, 2008; Zhang et al. 2010], because of these latter studies [Reginster et al. 2001; Pavelka et al. 2002; Herrero-Beaumont et al. 2007]. Actually, the market is flooded with different glucosamine formulations, mainly dietary supplements or generics that contain other glucosamine salts (e.g. glucosamine hydrochloride), or undocumented glucosamine sulfate ingredients that may differ from the stabilized crystalline glucosamine sulfate. In fact, they have never been shown to be bioequivalent or therapeutically equivalent with the original prescription product, besides often being used at different daily dosing schedules (e.g. 500 mg three times daily rather than 1500 mg once daily, which may result in a different pharmacokinetic pattern, as will be explained below), if not at even lower daily doses.

All clinical trials of glucosamine in OA reported so far have been systematically assessed in the recent update of a Cochrane Review [Towheed et al. 2009]. This includes data from 4963 patients in 25 clinical trials, the majority of which were performed in knee OA. While the global analysis of placebo-controlled trials do show a moderate effect size on pain, heterogeneity is huge, mainly because of the differences in study design/quality and, especially, in the glucosamine preparation and dosage used. Indeed, the sensitivity analyses in the Cochrane Review conclude that efficacy is driven only by the results of the trials performed with crystalline glucosamine sulfate, while pooled results for trials using different glucosamine preparations failed to show any effect on OA symptoms. The latter negative studies include the National Institutes of Health-supported Glucosamine/chondroitin Arthritis Intervention Trial (GAIT) trial [Clegg et al. 2006], which showed only a modest and nonsignificant trend for symptom improvement using a glucosamine hydrochloride formulation given as 500 mg three times daily. Conversely, the efficacy of crystalline glucosamine sulfate is significant and clinically relevant on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain and WOMAC function subscales, without heterogeneity, in the three high-quality pivotal trials of the 1500 mg once daily prescription formulation [Towheed et al. 2009], as had already been shown in a previous meta-analysis [Reginster, 2007]. These three trials [Reginster et al. 2001; Pavelka et al. 2002; Herrero-Beaumont et al. 2007] have shown a pooled effect size of 0.27 for pain and 0.33 for function, as assessed by the WOMAC index [Reginster, 2007]. The effect size calculated in the Cochrane Review for these three trials is also statistically significant but slightly lower [Towheed et al. 2009]: in fact, Reginster could use the correct and gold-standard method by Hedges that employs the difference in the change from baseline [Hedges and Olkin, 1985]. Conversely, Cochrane Reviews often have to use data from poor-quality articles in which the mean change from baseline is not reported and have therefore established the rule to use the difference in absolute final values, lacking precision and being influenced by even minor imbalance.

Figure 2. Representative histology images of articular cartilage from 8-month-old STR/ort mice that were treated with crystalline glucosamine sulfate 200 mg/kg (b) or vehicle (a) for 3 months. Reproduced with permission from Chiusaroli [2011].
in baseline values: this resulted in slightly lower effect sizes for the three pivotal trials of crystalline glucosamine sulfate compared with the gold-standard data from Reginster [Reginster, 2007]. However calculated, the small-to-medium effect size on pain and function is of major clinical relevance, since, in the three trials, it is sustained over long-term treatment courses of 6 months to 3 years, and it is comparable to that obtained with purely symptomatic medications such as NSAIDs over much shorter treatments, that is, up to 12 weeks in one of the most reputable meta-analyses [Bjordal et al. 2004]. Indeed, the same authors have shown that NSAIDs are much less effective and poorly tolerated when used long term [Bjordal et al. 2004]. Actually, it may be difficult to compare directly glucosamine sulfate and NSAIDs, since the latter are unspecific and fast-acting symptom-relievers: although when all trials, mainly short term, of NSAIDs are considered, the effect size on pain is only 0.32 (and even 0.23 when only the trials that did not exclude nonresponders are analyzed), and 0.29 for function [Bjordal et al. 2004], (i.e. comparable to that found by Reginster for crystalline glucosamine sulfate in the pivotal long-term trials [Reginster, 2007]), the short-term effect size of NSAIDs may be as high as 0.39 if only high-quality trials are considered [Zhang et al. 2010]. The two medications may therefore have different uses: short term for fast-acting NSAIDs, which are then penalized by poor safety and efficacy in the long term where, conversely, crystalline glucosamine sulfate might have its preferred application.

In this context, it is surprising that a questionable but widely publicized meta-analysis was recently published and claimed only minor clinical relevance in the results of glucosamine trials [Wandel et al. 2010]. These authors performed a very complex ‘network’ meta-analysis using a Bayesian approach and in which apparently they only selected large high-quality, placebo-controlled trials, but in reality they pooled extremely different trials [Clegg et al. 2006; Reginster et al. 2001; Pavelka et al. 2002; Herrero-Beaumont et al. 2007; Noack et al. 1994; McAlindon et al. 2004; Rozendaal et al. 2008] in terms of study design, from which they derived odd results. Such differences in trial design include the glucosamine substance used, dose regimen, trial duration, and the studied joint, as will be explained below. In such a confusion, the pain effect size was statistically significant but only 0.17 and, in addition, they back transformed it into a decrease on a 10-cm visual analog scale (VAS), which was again statistically significant, but only 0.4 cm over baseline, that is, lower than the 0.9 cm currently believed to be the minimum perceptible clinical improvement, thus questioning the clinical relevance. Obviously, a flurry of criticism was published by the scientific community based on inappropriate trial selection, odd methodology, and conclusions not supported by the data [Reginster et al. 2010; Giacovelli and Rovati, 2010; Pelletier et al. 2010]. Indeed, the questionable trial selection resulted in high heterogeneity (I²= 63% by the standard methodology) that was not appropriately controlled in the analysis (that used a ‘prior distribution’ with strong emphasis on high heterogeneity). In this respect, the most obvious criticism was that the authors pooled two trials of glucosamine hydrochloride [Clegg et al. 2006; McAlindon et al. 2004] with those of glucosamine sulfate: this is a major problem, since glucosamine hydrochloride has never been found effective in any study or meta-analysis and, in fact, all practice guidelines discourage its use, as well exemplified by the OARSI guidelines update [Zhang et al. 2010]. To make things worse, these authors [Wandel et al. 2010] performed a sensitivity analysis in which they wrongly assigned one of the failed glucosamine hydrochloride trials [McAlindon et al. 2004] to glucosamine sulfate.

Inappropriate trial selection was further magnified by including a trial of an acknowledged poor-quality control formulation of glucosamine sulfate in hip OA [Rozendaal et al. 2008], an indication that is difficult to pool with the knee studies since it requires a different experimental approach. Moreover, two short-term studies of 1–3 months [Noack et al. 1994; McAlindon et al. 2004] were pooled with long-term studies ranging from 6 months to 3 years. Finally, three studies used a glucosamine daily dosage of 500 mg three times daily [Clegg et al. 2006; Noack et al. 1994; McAlindon et al. 2004], which has a different pharmacokinetic profile that may jeopardize the efficacy of the active ingredient, as will be discussed below.

When the three long-term pivotal trials of crystalline glucosamine sulfate 1500 mg once daily in knee OA [Reginster et al. 2001; Pavelka et al. 2002; Herrero-Beaumont et al. 2007], already reviewed by Reginster and the Cochrane Review [Reginster, 2007; Towheed et al. 2009], are left, the effect size on pain relief, calculated by the same Bayesian approach, is 0.34 [Reginster et al. 2010], that is,
even higher than with the standard methodology [Towheed et al. 2009; Reginster, 2007].

Further criticism on the employed methodology [Reginster et al. 2010; Giacovelli and Rovati, 2010] included questioning the clinical relevance derived from artificial back transformation from the effect size to a 10-cm VAS, that surprisingly raised the bar of clinical relevance from the standard 0.20 effect size to 0.37, knowing that NSAIDs have a short-term effect size on OA pain of 0.29 and paracetamol of 0.14 [Zhang et al. 2010]. Finally, ‘network’ meta-analyses usually serve for indirect comparisons of different drugs, an aim that was not even attempted by these authors who indeed have been previously criticized by methodologists when using this approach because ‘their methods are so complex that many are mystified by whether the conclusions make sense’ [Pocock, 2007]. All the above criticisms were so strong and detailed that the BMJ Senior Editor reported in an editorial board postpublication note that the authors’ conclusions were not directly supported by their data [Groves, 2011].

Crystalline glucosamine sulfate pivotal trials not only support the use of the drug for symptom modification, but they also suggest a joint structure-modifying effect in knee OA as documented by a decrease in radiologic joint space narrowing (JSN) in the medial compartment of the tibiofemoral joint. This is significant in the two individual 3-year trials [Reginster et al. 2001; Pavelka et al. 2002], and when they are pooled in the Cochrane Review [Towheed et al. 2009], or in a recent UK Health Technology Assessment (HTA) [Black et al. 2009]. Both trials indicated that in mild disease with an initial joint space width of approximately 4 mm, the placebo group loses around 0.1 mm/year and this is prevented by crystalline glucosamine sulfate 1500 mg once daily.

A third long-term clinical trial of glucosamine for disease modification was deliberately excluded from analysis in the HTA report because it was considered of poor quality. This was actually the 2-year extension of the GAIT study [Sawitzke et al. 2008], the poor quality of which is explained in the HTA report by the fact that it included only a subset of patients from the original 6-month study [Clegg et al. 2006], several patients (over 40%) were excluded from the analysis, there was no intention-to-treat (ITT) analysis with failure to describe appropriately withdrawn patients and, finally, baseline characteristics were unbalanced between groups [Black et al. 2009]. This extension of GAIT was indeed a small study in which, despite use of the wrong salt (glucosamine hydrochloride instead of sulfate), at the wrong dose (500 mg three times daily versus 1500 mg once daily), the only results close to a statistically significant difference with placebo were indeed the JSN sparing effect in the glucosamine group, while celecoxib, chondroitin, or a combination of the latter with glucosamine, were completely inactive [Sawitzke et al. 2008]. In addition, the GAIT authors failed to describe appropriately that in the milder patient subgroup representing the majority of the patient population, that is, those with Kellgren–Lawrence radiologic grade 2, the glucosamine-treated group was at the very limit of a statistically significant favorable effect over placebo [Sawitzke et al. 2008].

We decided therefore to include the GAIT study in a meta-analysis conducted according to standard methods. In this meta-analysis, the two 3-year pivotal trials of crystalline glucosamine sulfate [Reginster et al. 2001; Pavelka et al. 2002] were considered together with the 2-year GAIT extension [Sawitzke et al. 2008], since all were randomized placebo-controlled trials. The outcome measure was the difference in means between glucosamine and placebo in changes between the end of study and baseline, on minimum JSN as reported in the original articles. Heterogeneity was tested with a chi-square test and the $I^2$ statistic: in the absence of significant heterogeneity (despite the major differences in study design and the glucosamine preparations used) a fixed effect model was adopted. The Hedges’ $g$ and the difference in means were used to pool across the studies. A total of 561 patients could be included from the three studies [Reginster et al. 2001; Pavelka et al. 2002; Sawitzke et al. 2008], that is, 106, 101, and 77 patients from the glucosamine groups, and 106, 101, and 70 from the placebo groups, respectively. The overall Hedges’ $g$ was 0.38 (95% confidence interval 0.21–0.55), while the overall difference in means (fixed-effect model) is shown in Figure 3. The structure-modifying data from the two pivotal trials of crystalline glucosamine sulfate were so strong that a statistically significant and clinically relevant effect size of 0.24 on JSN persists despite inclusion of the glucosamine hydrochloride GAIT study.

Clinical relevance of the structure-modifying effect of crystalline glucosamine sulfate was
studies in the observational extension of the two 3-year pivotal trials [Bruyere et al. 2008]. The data indeed suggest that administration of crystalline glucosamine sulfate for at least 12 months might affect the progression of knee OA since there were significantly fewer patients undergoing total joint replacement in the average 5-year follow up after drug withdrawal, with a risk reduction equal to 57% compared with placebo [Bruyere et al. 2008].

Finally, at least three reports described the cost-effectiveness of crystalline glucosamine sulfate 1500 mg once daily in knee OA [Black et al. 2009; Scholtissen et al. 2010; National Collaborating Centre for Chronic Conditions, 2008]. Their results are summarized in Table 1. One of these reports is actually represented by the UK National Institute for Health and Clinical Excellence (NICE) guidelines [National Collaborating Centre for Chronic Conditions, 2008]: at the time of publication NICE was not able to recommend glucosamine because the only licensed product available in UK was glucosamine hydrochloride and the evidence to support its efficacy was poor: conversely the panel concurred that those trials in knee OA that used glucosamine sulfate 1500 mg once daily showed the small benefit we have also outlined here (see the discussion above on the effect size). In addition, they found that the drug is potentially cost effective, but the product was not licensed in UK at that time [National Collaborating Centre for Chronic Conditions, 2008]. Based on input from stakeholders, including the British Society of Rheumatology pointing out that crystalline glucosamine sulfate is now a licensed agent for knee OA in the UK, is effective and cost effective in their model (see http://www.nice.org.uk/nicemedia/live/11926/54907/54907.pdf), the NICE guideline development group decided to review the guideline for possible update in this and other respects (see http://www.nice.org.uk/nicemedia/live/11926/54906/54906.pdf), and the process is in progress. On the other hand, international clinical practice guidelines that go beyond peculiar national situations, uniformly recommend glucosamine sulfate for knee OA, as is the case for the EULAR guidelines [Jordan et al. 2003] and, as mentioned at the beginning of this section, the OARSI guidelines [Zhang et al. 2008]. Actually, in their recent update [Zhang et al. 2010], OARSI proposes a detailed review employing several different models and showing that when the analysis is restricted to high-quality trials, the pain effect size is 0.29, that is in line with the 0.27 found by Reginster [Reginster, 2007]. However, heterogeneity in their analysis is high since all trials, together with major differences, are combined; heterogeneity is decreased when only studies published after 1998 are considered, but the number of trials is increased since low-quality studies are also included, in particular those performed with uncontrolled formulations of ‘glucosamine sulfate’, which brings the effect size down to only 0.13, which is, on the other hand, in line with the effect size of paracetamol in the OARSI meta-analysis [Zhang et al. 2010]. Again, when only the three pivotal, high-quality and long-term trials of crystalline glucosamine sulfate 1500 mg once daily published after 1998 are analyzed [Reginster et al. 2001; Pavelka et al. 2002; Herrero-Beaumont et al. 2007], the effect size is 0.27 for pain and 0.33 for function, thus clearly differentiating this formulation from all other glucosamine preparations.

Safety
In clinical trials, crystalline glucosamine sulfate (and glucosamine in general) has shown an
incidence of adverse events and safety-related drop-outs similar to that of placebo, as was also described in long-term studies up to 3 years. Conversely, all comparative studies with traditional NSAIDs have shown that crystalline glucosamine sulfate has a significantly better tolerability [Towheed et al. 2009], especially at the gastrointestinal level. Although with a similar incidence to placebo in clinical trials, mild and transient gastrointestinal disorders are commonly reported during treatment and include diarrhea, constipation, flatulence, nausea, dyspepsia, and abdominal pain. Headache, somnolence, and tiredness are also described, together with uncommon reports of erythema, pruritus, or skin rash. Indeed, allergic reactions are also rarely described, although cross-reactions in patients with seafood allergy are unlikely, due to a purification process that excludes the presence of protein residues in the starting material of marine origin: this might happen therefore only with other glucosamine formulations of lower quality.

Early pharmacologic studies suggested that, being an amino sugar, glucosamine might overactivate the hexosamine pathway and thus induce insulin resistance by decreasing glucose uptake [Baron et al. 1995; Shankar et al. 1998]. However, experimental studies using a euglycemic hyperinsulinemic clamp excluded a role of oral, intravenous, or intra-arterial glucosamine in the regulation of insulin sensitivity in humans [Muniyappa et al. 2006; Monauni et al. 2000; Pouwels et al. 2001]. Even recent reviews of clinical studies agree that the effects of glucosamine on glucose metabolism are questionable, if any [Dostrovsky et al. 2011; Simon et al. 2011]. However, since longitudinal data in diabetics are limited, caution might be advisable when treating patients with impaired glucose tolerance, and monitoring of blood glucose levels may be necessary in diabetics at the start or end of therapy.

As described above, crystalline glucosamine sulfate composition includes a small amount of sodium chloride, namely 384 mg as an addition to the standard dose of 1500 mg. To exclude this small amount might induce even a slight increase in blood pressure, a retrospective analysis of cardiovascular safety, and other cardiometabolic parameters, namely blood pressure, blood glucose, and blood lipids patterns, has been performed.

### Table 1. Cost-effectiveness of crystalline glucosamine sulfate

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Horizon</th>
<th>Incremental cost-effectiveness ratio</th>
<th>Probability of cost-effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scholtissen et al. 2010</td>
<td>Herrero-Beaumont 2007</td>
<td>6 months</td>
<td>€10,491 versus placebo €13,835 versus paracetamol</td>
<td>71% at €20,000</td>
</tr>
<tr>
<td>National Collaborating Centre for Chronic Conditions, Royal College of Physicians/NICE 2008</td>
<td>Reginster 2001</td>
<td>3 years</td>
<td>£2427 versus placebo</td>
<td>Glucosamine sulfate is potentially cost effective [NICE guideline text]</td>
</tr>
<tr>
<td>National Collaborating Centre for Chronic Conditions, Royal College of Physicians/NICE 2008</td>
<td>Pavelka 2002</td>
<td>3 years</td>
<td>£10,880 versus placebo</td>
<td>Glucosamine sulfate is potentially cost effective [NICE guideline text]</td>
</tr>
<tr>
<td>Black et al. 2009</td>
<td>Pavelka 2002 (symptoms) Bruyere 2008 (TJR)</td>
<td>Remaining lifetime</td>
<td>£21,335 versus current care</td>
<td>43% at £20,000 73% at £30,000</td>
</tr>
</tbody>
</table>

NICE, National Institute for Health and Clinical Excellence.
recently from two of the long-term pivotal trials of crystalline glucosamine sulfate on a total of 428 knee OA patients [Palma dos Reis et al. 2011]. There were no changes compared with placebo in mean systolic and diastolic blood pressure after 6 months in the Glucosamine Unum In Die [once a day] Efficacy (GUIDE) study in a population with average high-normal values, as well as in a subgroup of patients with hypertension [Herrero-Beaumont et al. 2007]. Similarly, blood glucose levels did not change, even in hyperglycemic patients, while total and low density lipoprotein cholesterol did not increase after 3 years in the study by Reginster and colleagues [Reginster et al. 2001], suggesting good long-term safety on these cardiovascular and metabolic parameters [Palma dos Reis et al. 2011].

The physicochemical and pharmacokinetic properties of crystalline glucosamine sulfate suggest a low potential for drug–drug interactions and the compound does not inhibit or induce any enzymes of the CYP450 system [Persiani et al. 2007]. It was only more recently from two of the long-term pivotal trials of crystalline glucosamine sulfate 1500 mg once daily [Persiani et al. 2005b]. Rapid oral bioavailability of the compound was found, with average maximum concentrations ($C_{\text{max}}$) in plasma in the 10 µM range after about 3 h, distribution to both vascular and extravascular compartments, and an elimination half-life of approximately 15 h that largely justifies the once daily dosing regimen. The pharmacokinetics of glucosamine are linear in the 750–1500 mg dose range while higher doses deviate from linearity [Persiani et al. 2005b]. The absolute bioavailability is about 25% [Persiani et al. 2005a].

Glucosamine plasma and synovial fluid levels were also studied in knee OA patients, before and after administration of oral crystalline glucosamine sulfate 1500 mg once daily [Persiani et al. 2007]. Endogenous levels of the compound were detected with significant intrasubject variability that might deserve further investigation regarding its possible pathophysiologic relevance. After repeated administration of oral crystalline glucosamine sulfate 1500 mg once daily, glucosamine concentrations increased in both compartments in an almost 1:1 ratio and reached average peaks in the 10 µM range [Persiani et al. 2007], as in healthy volunteers [Persiani et al. 2005b], and are therefore pharmacologically relevant as described in above [Chiusaroli et al. 2011].

Lower concentrations in the low micromolar range, that might therefore exert lower pharmacologically effects, were described after a single 1500 mg dose of the glucosamine hydrochloride formulation used in the failed GAIT study [Jackson et al. 2010]. Peak concentrations and area-under-curve were even lower when this unit dose was fractioned into 500 mg three times daily repeated administrations [Jackson et al. 2010]. Indeed, when a direct pharmacokinetic comparison was performed between repeated administration of crystalline glucosamine sulfate 1500 mg once daily or glucosamine hydrochloride 500 mg three times daily, the change in glucosamine salt, dose regimen, and formulation resulted in a decrease in glucosamine bioavailability by 75% and peak plasma concentrations by 50% with the hydrochloride, to achieve probably ineffective levels [Altman, 2009]. This might explain the poor clinical results obtained with glucosamine hydrochloride in the GAIT study, but probably also the similarly poor data with all other glucosamine formulations compared with crystalline

**Pharmacokinetics**

Most of the original studies on glucosamine pharmacokinetics have been recently reviewed [Altman, 2009].

In the absence of sensitive and specific bioanalytical methods to detect glucosamine in biological fluids in the past, preliminary indications on glucosamine pharmacokinetics and metabolism were obtained after oral administration to rats, dogs, and humans of 14C-labeled glucosamine sulfate [Setnikar and Rovati, 2001]. It was only more recently that it was possible to develop specific and sensitive assays using liquid chromatography with mass spectrometry detection for the determination of unchanged glucosamine in human plasma, urine, and synovial fluid [Roda et al. 2006], and to describe the pharmacokinetics of glucosamine at steady state after repeated doses of crystalline glucosamine sulfate 1500 mg once daily [Persiani et al. 2005b]. Rapid oral bioavailability of the compound was found, with average maximum concentrations ($C_{\text{max}}$) in plasma in the 10 µM range after about 3 h, distribution to both vascular and extravascular compartments, and an elimination half-life of approximately 15 h that largely justifies the once daily dosing regimen. The pharmacokinetics of glucosamine are linear in the 750–1500 mg dose range while higher doses deviate from linearity [Persiani et al. 2005b]. The absolute bioavailability is about 25% [Persiani et al. 2005a].

Glucosamine plasma and synovial fluid levels were also studied in knee OA patients, before and after administration of oral crystalline glucosamine sulfate 1500 mg once daily [Persiani et al. 2007]. Endogenous levels of the compound were detected with significant intrasubject variability that might deserve further investigation regarding its possible pathophysiologic relevance. After repeated administration of oral crystalline glucosamine sulfate 1500 mg once daily, glucosamine concentrations increased in both compartments in an almost 1:1 ratio and reached average peaks in the 10 µM range [Persiani et al. 2007], as in healthy volunteers [Persiani et al. 2005b], and are therefore pharmacologically relevant as described in above [Chiusaroli et al. 2011].

Lower concentrations in the low micromolar range, that might therefore exert lower pharmacologically effects, were described after a single 1500 mg dose of the glucosamine hydrochloride formulation used in the failed GAIT study [Jackson et al. 2010]. Peak concentrations and area-under-curve were even lower when this unit dose was fractioned into 500 mg three times daily repeated administrations [Jackson et al. 2010]. Indeed, when a direct pharmacokinetic comparison was performed between repeated administration of crystalline glucosamine sulfate 1500 mg once daily or glucosamine hydrochloride 500 mg three times daily, the change in glucosamine salt, dose regimen, and formulation resulted in a decrease in glucosamine bioavailability by 75% and peak plasma concentrations by 50% with the hydrochloride, to achieve probably ineffective levels [Altman, 2009]. This might explain the poor clinical results obtained with glucosamine hydrochloride in the GAIT study, but probably also the similarly poor data with all other glucosamine formulations compared with crystalline
glucosamine sulfate, as described in the Cochrane Review [Towheed et al. 2009]. Crystalline glucosamine sulfate has also been shown to produce higher plasma and synovial fluid levels after oral (nasogastric) administration in animals (horses) after identical administration modalities compared with the hydrochloride salt [Meulyzer et al. 2008], suggesting a superior pharmacokinetic profile independent of the dose regimen or pharmaceutical form.

Conclusion
Crystalline glucosamine sulfate is the original glucosamine prescription product that has shown efficacy and safety in clinical trials in knee OA when used at a dose of 1500 mg once daily. In addition to the clinical data, its use is supported by a distinct pharmacologic profile and, most importantly, well described pharmacokinetics. Other glucosamine products are available in different formulations, or as other salts, or with varied dosing regimens: they have never been shown to be effective in clinical trials nor bioequivalent with crystalline glucosamine sulfate. The latter is the only glucosamine product recommended by current clinical practice guidelines.

Acknowledgements
We wish to thank Giampaolo Giacovelli, PhD and Beatrice Barbetta, BStat from the Clinical Research Unit, Department of Biostatistics of Rottapharm/Madaus, for their valuable input on the statistics used in this review.

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement
The authors are scientists from the Clinical Research Unit of Rottapharm/Madaus, that is the originator of crystalline glucosamine sulfate and markets the product in different countries of the world. The authors agreed individually to be listed as authors in this invited review due to their direct involvement in most of the studies described, and in the continued development of crystalline glucosamine sulfate for the treatment of OA. There was no involvement of Rottapharm/Madaus as a corporate entity in the article preparation and submission process.

References


Groves T. (2011) Report from BMJ post-publication review meeting [accessible at http://www.bmj.com/content/341/bmj.c6475.full/reply#bmj_el_242904].


and synovial fluid levels following administration of glucosamine sulphate or glucosamine hydrochloride. *Osteoarthritis Cartilage* 16: 973–979.


Reginster, J.Y., Altman, R.D and Hochberg, M.C. (2010) Prescription glucosamine sulphate is effective...
in knee osteoarthritis. BMJ 341: c6335 [full text available at http://www.bmj.com/content/341/bmj.c6675.full#reply#bmj_el_242366].


