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Review

Oral chondroprotection with nutraceuticals made of chondroitin sulphate plus glucosamine sulphate in osteoarthritis

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ABSTRACT

Oral supplementation of chondroitin sulphate plus glucosamine helps repair the articular surface in osteoarthritis. Chondroitin-S reduces the concentration of the pro-inflammatory cytokines and transcription factor involved in inflammation. GlcN.S enhances cartilage specific matrix components and prevents collagen degeneration in chondrocytes by inhibiting hydrolytic enzymes, and preventing the oxidation of lipids and proteins. Chondroitin-S plus GlcN.S are slow-acting drugs that alleviate pain and partly restore joint function in OA patients. Orally administered pharmaceutical-grade chondroitin-S plus GlcN.S stabilize the joint space narrowing and significantly decrease the number of patients with new erosive OA. They are safe and no adverse events have ever been reported; they are recommended by EULAR and OARSI. The cost/effectiveness of the oral chondroitin-S plus GlcN.S therapy derives from the reduction of costs for physiotherapy, and for gastroprotective and non-steroidal drugs. The synergistic association of these two world-widely preferred nutraceuticals is a step forward in the management of OA.

Contents

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1. Introduction and scope

Osteoarthritis is essentially a debilitating disease characterized by a gradual loss of articular cartilage in synovial joints, that causes painful impairment. Functional limitation gradually occurs as a result of joint stiffness and progressive loss of joint motion owing to deformities (loss of the cartilage surface and side growth of osteophytes) accompanied by inflammation of the synovial membrane.

The pharmacological therapy is directed to the prevention of pain and the improvement of function. Standard analgesic/anti-inflammatory drugs include NSAIDs, opioids, paracetamol, vitamins, COX-2 inhibitors, capsaicin and glucocorticoids, that however might exert various gastrointestinal and cardiovascular side effects; most importantly they do not correct the degenerative disorder of the connective tissue. Ideally, the therapy would be expected to preserve the articular structures, improve the quality of life, and guarantee safety.

Chondroitin-S and GlcN in various forms (scaffolds, hydrogels, injectables, dietary supplements) represent valid therapeutic alternatives because they are ubiquitous in the human body, being two components of the cartilage (Busilacchi, Gigante, Mattioli-Belmonte, Manzotti, & Muzzarelli, 2013; Goldberg, Von Feldt, & Lonner, 2002; Henrotin, Mathy, Sanchez, & Lambert, 2010; Henrotin and Lambert, 2013; Luttosch & Baerwald, 2012; Muzzarelli, 2009; Muzzarelli et al., 2012a; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). In fact chondroitin-S and GlcN are amply available as dietary supplements for the prevention of cartilage loss *via* increase of their concentration in the joint: they help preserve, or even repair, the damaged articular surface in OA (Volpi, 2011).

Glucosamine in the form of GlcN.S is one of the most commonly used complementary medicines in Western countries: moreover, a considerable proportion of the Australian population aged 45 and over consumes GlcN.S. The same is reported for Korea, Russia and other countries (Noskov, Lavrukhina, Shirokova, & Pryanichnikova, 2013; Seo et al., 2013; Sibbritt, Adams, Lui, Broom, & Wardle, 2012).

The scope of this review article, the first devoted exclusively to oral chondroprotection with the aid of the association of chondroitin-S (a polysaccharide ester) with GlcN.S (an aminosugar salt), is therefore the evaluation of recent data on said dietary supplement as a slow-acting modifier of osteoarthritis for (i) oral administration of components of the cartilage, and (ii) prevention and treatment of the degeneration of the joint cartilage in the elderly. Aspects related to sources of the raw materials, analytical chemistry, pharmacokinetics, enhancement of assimilation *in vivo*, economics and recommendations made by Societies are also considered. Understanding the synergy of the two ingredients of this dietary supplement in providing safety of use and efficacy in the cartilage regeneration on the medium/long term is a further object of this work.

2. Biochemical data on chondroitin sulphate

A few extensive reviews have related the biomedical effectiveness of chondroitin-S to its origin, quality and formulation, in the light of its structural diversity and biochemical behavior (Fosang, 1999; Fosang & Beier, 2011;Fosang & Rogerson, 2010; Hochberg, 2010; Kamarul, Ab-Rahim, Tumin, Selvaratnam, & Ahmad, 2011; Vangsness, Spiker, & Erickson, 2009, among others).

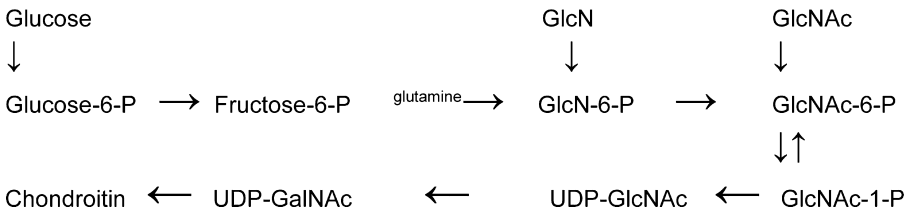
A conspicuous feature of OA is the gradual loss of aggrecan from cartilage. Aggrecan, the main space-filling compound in the ECM of the articular cartilage, is a large proteoglycan made of a central protein core to which numerous chondroitin-S and keratan sulphate chains are covalently attached. Aggregate formation is through the N terminus globular domain (G1) of the aggrecan core protein which binds non-covalently to decameric units of HA and to the link protein to form stable complexes. The release of aggrecan from the complex involves enzymatic hydrolysis of the core protein. The inter-globular domain between G1 and G2 was found to be sensitive to proteolysis by MMP and aggrecanase. The C terminus G3 domain is missing from one half of the cartilage aggrecan, as a result of extracellular processing. The GAG attachment region between G2 and G3 comprises a keratan-S domain and two chondroitin-S subdomains; aggrecanase can truncate aggrecan molecules at this position. The overall carbohydrate content is therefore variable and can amount to 95%.

Although it is clear that MMP-13 (collagenase-3) is the major cartilage collagenase, and that a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-4 and/or -5) are the main aggrecanases in human cartilage, it is not clear how their zymogens are activated, nor how their activities are modulated *in vivo*. Based on their activities *in vitro*, these serine proteinases are thought to act indirectly, by activating MMP-3 (stromelysin) and MMP-2 (gelatinase A), which can subsequently activate collagenases. At the moment it can be said that 1 = Aggrecan is degraded by many proteinases under physiological conditions; 2 = Aggrecanases cleave at multiple sites in the aggrecan core protein; as a point of difference, the collagenase cleavage is at a single site in the collagen-II triple helix. 3 = IL-1 stimulation induces active aggrecanase in cartilage; 4 = Aggrecanases are constitutively active in synovium. 5 = Extensive aggrecanolysis is reversible *in vivo*. 6 = Aggrecan half-life in cartilage is *ca.* 3.4 years.

The anionic nature of aggrecan makes it osmotically active, thereby imparting resilience and allowing the cartilage to deform reversibly under load. It has been confirmed by Bali, Cousse, and Neuzil (2001) that the anti-inflammatory and chondroprotective properties of chondroitin-S result from enhanced biosynthesis of connective tissue components like hyaluronan, and higher viscosity of the synovial fluid at the afflicted sites. Aggrecan, as well as collagen-II, is a typical chondrogenic marker that reveals the cartilage regeneration (Remya & Nair, 2013).

Chondroitin-S is formed by a repeated disaccharide unit consisting of D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc) with MW in the range 20–50 kDa (Collins, 1987). The structure of chondroitin is written in shorthand either as [GlcA β(1–3)-GalNAc β(1–4)] or as -4)-[βGlcA-(1–3)-βGalNAc]-(1-. When the linear chain of these disaccharide structural units is sulphated either in the 4 or the 6 position of GalNAc, the polysaccharides are called chondroitin-4-sulphate (C4S) and chondroitin-6-sulphate (C6S) respectively. Besides the micro-heterogeneity mentioned above, further micro-heterogeneity occurs when differently sulphated dimeric units are located within the polymeric chains: this inherent characteristic and the number of dimeric units forming the polymer exert influence on the pharmacological activity.

Human proteoglycans having chondroitin-S chains are abundant in cartilage, aorta, skeletal muscle, eye, lung and brain (David G et al., 1989; David CL et al., 1998; Kimata et al.,1974; Oohira et al.,1994; Zako, Shinomura, Miyaishi, Iwaki, & Kimata, 1997). They are synthesized intracellularly starting from glucose or glucosamine precursors (Scheme 1) and are secreted by chondrocytes as a macromolecular complex to be released to the extracellular matrix or to remain localized at the cell surface. The biosynthesis of the chondroitin-S chain of proteoglycans occurs in the Golgi apparatus simultaneously with the sulphate ester formation catalyzed by sulfotransferases at the positions of their respective competence (Habuchi, 2000; Prydz & Dalen, 2000). A need for detailed structural information on cartilage extracellular matrix molecules has been generated by interest in their pathological degradation. Posttranslational modifications are believed to play a role in determining the susceptibility to proteolytic degradation during the development



Scheme 1. The natural connection between glucosamine and chondroitin: biosynthetic incorporation of GalN into chondroitin from glucose or glucosamine precursors.

of osteoarthritis. For example, Zaia, Liu, Boynton, and Barry (2000) showed how the application of MALDI-TOF mass spectrometry to extracellular matrix protein and proteoglycan helps elucidate mechanisms in extracellular matrix biochemistry.

Although chondrocytes within the cartilage account only for 1–5% of the cartilage volume, they are responsible for maintaining the correct composition and organization of collagen, elastin fibers, and proteoglycans within the ECM. However, defects larger than 6 mm rarely if ever heal, and progressively degenerate (Khan, Gilbert, Singhrao, Duance, & Archer, 2008). In fact, owing to the lack of blood vessels, the chondrocytes within the cartilage receive nutrients only by diffusion from the surrounding tissue. To avoid this limitation, basic components should be available for biosynthesis in large concentrations. Glucosamine production/supply is the rate-limiting step in GAG synthesis, and GlcN.S supplementation is mandatory to overcome this bottleneck. Expectations for GlcN to exert therapeutic effect are based on *in vitro* plasma concentrations of at least 50 μmol/L (GlcN = $C_6H_{13}NO_5$, MW 179.17). However, after the administration of 1500 mg of GlcN.S the peak GlcN concentration in serum was in the range 2–12 μmol/L (control values 0.3–3.5 μmol/L (Biggee, Blinn, McAlindon, Nuite, & Silbert, 2006; Block, Oegema, Sandy, & Plaas, 2010).

The extraction of chondroitin-S from animal tissues is made with the aid of proteases that hydrolyze the protein core to which the chondroitin-S chains are attached (Dumitriu, 1996) and requires complex purification procedures owing to risks of viral and prionic contaminations (*e.g.* from pigs). High costs are related to the paucity of raw materials due to protection of animal species (*e.g.* sharks), but other animal species produce chondroitin-S, *in primis* squids and crabs. Chondroitin-S can be efficiently isolated from the broiler chicken by-product generated by mechanical deboning, which is a mixture of crushed bone, cartilage, skin, adipose tissue and muscle. Chondroitin-S was liberated by using proteolytic enzymes like papain, pancreatin, kiwi fruits and flavourzyme: pancreatin is an economical source of protease for this purpose. The data in Fig. 1 are indicative of the differences existing between isolates from various tissues of the same animal, owing to accompanying hyaluronan and dermatan-S, and hence of the complexity of the purification process of chondroitin-S. Electrophoresis on cellulose acetate (although still recommended by US and European Pharmacopeias) is an obsolete and inadequate technique, being unable to reveal undesirable residual keratan sulfate in biomedical preparations of chondroitin-S since these two GAG migrate together as shown in Fig. 1 (Nakano, Pietrasik, Ozimek, & Betti, 2012). In contrast, strong anion exchange HPLC (SAX-HPCL) is the most appropriate method for detection of the eventual presence of keratan-S in chondroitin-S. Therefore, anion exchange-based methods should be implemented by manufacturers during large-scale production of chondroitin-S (Pomin, Piquet, Pereira, & Mourão, 2012).

The purified sample of chondrotin-S extracted from cheese was subjected to chondroitinase ABC, and the disaccharides, once analyzed by SAX-HPLC, resulted to be a mixture of one non-sulphated disaccharide [UA1→3GalNAc], and two mono-sulphated: [UA1→3GalNAc6(SO_4)] and [UA1→3GalNAc4(SO_4)]. Disulphated and trisulphated disaccharides were not detected, thus confirming their absence from cow milk chondrotin-S. Their composition resulted to be: 9% of nonsulphated disaccharide, 26% of disaccharide-6-S, and 65% of disaccharide-4-S; charge density *ca.* 0.9.

Inspection of the 50–70 and 100–110 ppm regions of the ^{13}C NMR spectrum revealed an amount of chondroitin-4S higher than -6S in agreement with the 4S/6S ratio obtained by HPLC. The signals at 107.3 and 104.6 were assigned to the C1 of the GlcA and to the C1 of GalNAc-6SO_4, respectively, and signals at 106.3 and 103.6 were assigned to the C1 of the GlcA and to the C1 of GalNAc-4SO_4. The signal at 70.5 was related to C6 of GalNAc-6SO_4 while the one at 64.1 was assigned to C6 of GalNAc-4SO_4.

The PAGE analysis showed an average molecular mass of 15.4 kDa calculated from a calibration curve of chondroitin-S fractions of known molecular masses (Coppa et al., 2012).

Product processing and purity determination of chondroitin-S are important owing to their effects on human osteoarthritic cartilage and chondrocytes (Sahoo, Tharalekshmy, Ng, & Naumov, 2012; Tat, Pelletier, Mineau, Duval, & Martel-Pelletier, 2010). Chondroitin-S extracted and purified from various animal sources, shows different properties and effects (Volpi, 2007, 2009): therefore, clear analytical protocols and advanced equipment are essential for the quality assessment of food grade chondroitin-S. Size exclusion chromatography allows clear discrimination of chondroitin-S of bovine, whale and shark origin.

These instrumental analytical methods are convenient for obtaining data of interest in the field of food chemistry. Likewise, capillary isotachophoresis was proved by Vaclavikova and Kvasnincka (2013) to be a suitable method for the routine analysis of GlcN and chondroitin-S in the dietary supplements considered in this review.

3. Pre-clinical studies on chondroitin sulphate

Chondroitin-S exhibits proven pharmaceutical properties and efficacy: a number of articles on the activity of orally administered exogenous chondroitin-S have been reviewed by Lauder (2009). With aging, cartilage gradually loses matrix, hydration water, and cells. The chondroitin-S concentration in osteoarthritic knees is inversely proportional to the severity of the illness (Bollet & Nance, 1966; Matthews, 1953). The mechanism responsible for this includes age-related changes in biosynthetic and catabolic events, where the chondroitin-S content of aggrecan decreases (Temple et al., 2007). The mean synovial concentration of chondroitin-S (103.1 nmol/ml for C6S and 18.4 nmol/ml for C4S) declines with age in healthy persons. The concentration of C6S in the 70–79 year old persons is approximately half that of the 20–29 years group; the ratio C6S: C4S also steadily decreases with advancing age (Nakayama et al., 2002).

Chondroitin-S exerts a beneficial effect in slowing down the development of arthritis and in reducing disease markers, findings which support its beneficial activity in humans as a chondroprotective drug. Experimental evidence confirms the hypothesis that chondroitin-S may reduce inflammatory processes by acting on the nuclear translocation of NFkB, which is closely associated with the blood biomarkers of inflammation, primarily IL-1, IL-6 and

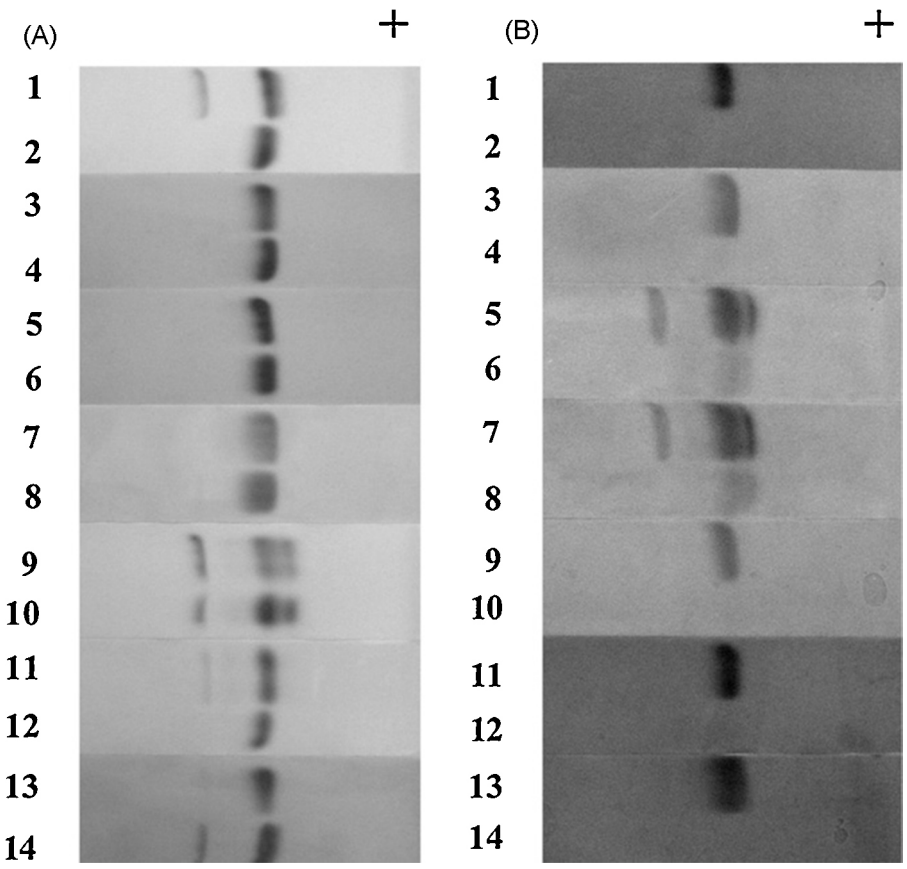


Fig. 1. Electrophoresis of papain digests of broiler chicken tissues and by-product from mechanical deboning on cellulose acetate strips in 0.1 M potassium phosphate, pH 7.0 (as approved by US Pharmacopeia). (A) Lanes 1 and 14, mixture of standard GAGs, hyaluronan, chondroitin-S and DS, in that the latter two GAGs have the same electrophoretic mobility, which is faster than the mobility of hyaluronan. Lanes 2, 3, 4, 5 and 6, GAGs from keel, anterior sternum, distal femoral, proximal tibial and proximal humeral cartilages, respectively; lanes 7 and 8, GAGs from coracoid and sternum bones, respectively; lanes 9, 10, 11 and 12, GAGs from skin, adipose tissue, muscle and keel cartilage perichondrium, respectively; lane 13, GAGs from the by-product from mechanical deboning. (B) GAGs from keel cartilage, coracoid bone, skin, adipose tissue, muscle, keel cartilage perichondrium and by-product from mechanical deboning, treated with testicular hyaluronidase (lanes 2, 4, 6, 8 10, 12 and 14, respectively) and without (odd lanes, respectively).

From Nakano et al. (2012).

C-reactive protein. Likewise, there is now a consistent body of knowledge indicating that GlcN.S, given at a daily oral dose of 1500 mg, is able to significantly reduce OA symptoms. This dose of GlcN.S prevents the JSN observed at the femoro-tibial compartment in patients with mild to moderate OA of the knee (Martin, Van Sell, & Danter, 2012). This effect is also reflected by the reduction in the incidence of OA-related surgery (Reginster, Neuprez, Lecart, Sarlet, & Bruyere, 2012).

According to Harlan, Haut, and Orth (2012), GlcN plus chondroitin-S mediate anti-inflammatory effects by decreasing the synthesis of nitric oxide, as a confirmation of the data by Meininger, Kelly, Li, Haynes, and Wu (2000) as well as the release of proteoglycan from the extracellular matrix in equine cartilage explants. Indeed, GlcN.S exhibits antioxidant action and protective effect in human chondrocytes by decreasing nitric oxide synthase expression and activity, and consequently the oxidative stress generated by ROS (Valvason, Musacchio, & Pozzuoli, 2008; Jerosch, 2011). Rabbits supplemented with chondroitin-S had less severe cartilage degeneration, and a combination of chondroitin-S plus GlcN.HCl acted synergistically in stimulating GAG synthesis. Chondroitin-S in admixture with manganese ascorbate is also effective in inhibiting hydrolases (Das & Hammad, 2000; Lippiello et al., 2000).

Taniguchi et al. (2012) demonstrated that long-term administration of GlcN and chondroitin-S reduced cartilage degeneration at 8 months of age in a model of spontaneous OA in guinea pigs. The marked loss of total RNA and the increase in MMP-3 mRNA were also inhibited by GlcN and chondroitin-S. Likewise, ongoing studies further confirm and support the beneficial metabolic synergy of glucosamine and chondroitin sulphate: Calamia et al. (2012) demonstrated that in normal chondrocytes stimulated with IL-1β, chondroitin-S reduces inflammation directly by decreasing several complement components, and indirectly by increasing TNF-α-induced proteins whose overexpression correlates with a decrease in MMP1 and MMP3 activation.

In a model of acute or induced chronic arthritis, the effect of the oral administration to rats for 15 days of either *Echinacea* or Genuphil was investigated. (Genuphil is a mixture of equal parts of chondroitin-S, GlcN.S, and DMSO that provides organic sulfur indispensable for proteoglycan synthesis). Anti-cyclic-citrullinated peptide, C-reactive protein and rheumatoid factor (to be seen as rheumatic markers) decreased significantly in *Echinacea*- and Genuphil groups compared to untreated acute and chronic groups. Histological evidence further demonstrated the advantage of using Genuphil (Arafa, Hamuda, Melek, & Darwish, 2013). Significant reductions in the concentration of C-reactive protein were associated with regular use of GlcN, thus showing the efficacy of the latter on the alleviation of inflammation, a major symptom of OA (Kantor et al., 2012).

4. Pharmacokinetics of chondroitin sulphate in patients

Certain pre-clinical studies have yielded disparate results owing in part to the use of formulations differing from each other in terms of origin of ingredients, and uncertainty about the actual

quantity, quality, purity, and (in the case of chondroitin-S) structure of their components. The daily dosages and dosing regimens employed have been largely empirical owing to scant pharmacologic information, whilst instrumental analytical techniques permit easy determination of endogenous chondroitin-S in plasma.

Orally ingested chondroitin-S is absorbed as a high MW polysaccharide and is detected in plasma in the form of partially depolymerized and/or desulphated derivatives (Volpi, 2002). Following the oral administration of 4 g chondroitin-S (bovine origin) to 20 healthy volunteers the plasma level of chondroitin-S at baseline up to 48 h post ingestion increased significantly, peaking at 2 h: a total absorption of 2.5–5.0% chondroitin-S was observed. Pre-dose plasma levels of chondroitin-S were detectable in all the subjects and averaged 3.80 ± 1.77 μg/ml (1.4–7.6 μg/ml). After administration, the max plasma concentration was 12.73 ± 4.69 μg/ml with t_{max} 2.4 ± 1.4 h; the increase in plasma level was significant from 2 to 6 h. However, while using shark chondroitin-S, Volpi (2003) found similar peak levels, but at 8.7 h, the difference possibly owing to the variety of origins, molecular weights and charge densities (Ronca & Conte, 1993). It should be recalled in fact that shark cartilage is chondroitin-6S that contains (in terms of average molar ratio) more than one sulphate group per disaccharide repeating unit (Kennedy & White, 1983).

The pharmacokinetic examination of a group of patients with symptomatic knee OA who received every day 1.5 g GlcN.HCl, 1.2 g chondroitin-S or a combination of them for more than 3 months (Jackson et al., 2010) showed that the final endogenous chondroitin-S concentration and disaccharide composition were not detectable owing to the experimental conditions used, essentially because the dose was too small, and the mean endogenous pre-dose concentration was erroneously estimated as high as 20 mg/mL with a possible individual variation of 5 μg/mL. Thus, according to Volpi (2010), those conditions prevented Jackson et al. (2010) from drawing acceptable conclusions. Further results obtained by other researchers in man and experimental animals, confirm that macromolecules possessing high molecular mass and charge density (sulfate to disaccharide ratio) are absorbed after oral ingestion (Pecly, Melo-Filho, & Mourao, 2006) and in part can bind to vascular components and escape quantitative determination.

Therapeutic effects definitely confirm the feasibility of the oral administration. The effect of two doses (300 or 900 mg/kg daily) of highly purified chondroitin-S was evaluated in an adjuvant arthritis animal model after oral administration. Chondroitin-S reduced the severity of arthritis along with oxidative stress, a consequence of chronic inflammatory processes. The effects were confirmed by improved total antioxidant status and c-glutamyltransferase activity. Chondroitin-S administered under a pre-treatment regimen reduced the production of pro-inflammatory cytokines, C-reactive protein in plasma, phagocytic activity and the intracellular oxidative burst of neutrophils (Bauerova et al., 2011). Moreover, chondroitin-S reduced the nuclear translocation of NFkB in psoriatic skin cells and LPS-induced inflammation in astrocytes by inhibition of TLR4 (Canas et al., 2010; Möller et al., 2010). Palmieri, Conte, Giovannini, Lualdi, and Ronca (1990) showed that the orally administered chondroitin-S (MW 7.5 kDa) is absorbed as further depolymerized low-MW derivatives. The metabolic fate of partially depolymerized chondroitin-S is similar in man and laboratory animals.

5. Biochemical data on glucosamine salts

The subject of GlcN.S as a nutraceutical has been reviewed by Aghazadeh-Habashi and Jamali (2011), Anderson, Nicolosi, and Borzelleca (2005), Deal and Moskowitz (1999), Herrero-Beaumont and Rovati (2006), Ibrahim, Gilzad-Kohan, Aghazadeh-Habashi, and Jamali (2012), Kean and Thanou (2010), Laverty, Sandy, Celeste, Vachon, Marier, and Plaas (2005), Matheson and Perry (2003), Muzzarelli and Muzzarelli (2006), Noak et al. (1994), Tamai et al. (2002), Towheed (2003), and Welch et al. (2012), among others.

GlcN (2-amino-2-deoxy-β-D-glucopyranose) is an endogenous aminomonosaccharide biosynthesized from glucose, as already noted in Scheme 1 (Leffler, Philippi, Leffler, Mosure, & Kim, 1999; Runkel & Cupp, 1999). Glucosamine free base is usually produced from chitosan of marine origin, that is the cationic (1–4)-2-amino-2-deoxy-β-D-glucan with degree of acetylation in the range 0.10–0.30 (Kim, SK, 2011; Kim, SK, 2013; Muzzarelli, 2012; Sanches-Silva et al., 2012; Vazquez et al., 2013). The hydrolysis protocols leading to the monomeric GlcN are of chemical or enzymatic nature (Das, Sen, & Roy, 2012; Muzzarelli, Stanic, & Ramos, 1999). Multi-functional roles of chitosan and its oligomers as protective agents for the human body have been reported (Muzzarelli et al., 2006; Walsh, Sweeney, Bahar, & O'Doherty, 2013).

However, there are alternative sources based on process engineering and metabolic strategies (for example the control over dissolved oxygen) capable to enhance the GlcN production by recombinant *Escherichia coli*, to the point that data on production, applications, and marketing of GlcN have been published (Chen, Liu, Li, Du, & Chen, 2012; Liu et al., 2013). As far as fungi are concerned, a wild-type *Aspergillus* sp. cultivated under submerged fermentation and stimulated with methanol yielded the considerable GlcN concentration of 7.48 g/L (Sitanggang, Wu, Wang, & Ho, 2010). Further research on the same *Aspergillus* sp. led to the highest specific GlcN production rate during 12 and 60 h at dissolved oxygen of 30% and 60%, respectively; thus a strategy useful for the scaling-up of the GlcN production was envisaged (Zhang JX et al., 2012). The technology for the isolation and purification of glucosamine of high purity from the hydrolysed chitin-glucan complex of higher fungi (Muzzarelli et al., 2012b) was developed by Artamonova and Sharnina (2013).

Crystalline GlcN.S (Dona®, Viartil®, Arthryl®, Xicil® and other trademarks of Rottapharma, Italy) is a pure substance in which the monosaccharide is present in stoichiometric ratios of 2:1:2:2 with sulphate, chloride and sodium ions respectively; this patented combination confers stability to the preparation (Grant & Gracy, 2000; Herrero-Beaumont & Rovati, 2006). GlcN.S is considered a specific drug for osteoarthritis, effective for relief of symptoms as NSAIDs, but much better tolerated (Qiu, Gao, Giacovelli, Rovati, & Setnikar, 1998). Owing to these properties, it seems particularly indicated in the long-term management of OA, although the pharmacokinetic properties and bioavailability of dispersible tablets of GlcN.HCl in healthy volunteers are well assessed too (Wu et al., 2012). Isolated chondrocytes from healthy subjects and OA patients provided data regarding the role of GlcN in supporting joint health. Primary bovine chondrocytes were incubated with various amounts of GlcN in monolayers (2D) and in cell-laden hydrogels (3D constructs). Notably, GlcN enhanced cartilage specific matrix components, aggrecan and collagen-II, in a dose-dependent manner up to 2 mM but the effect was lost by 15 mM. GlcN up-regulated TGF-β1 mRNA levels (David-Raoudi et al., 2009; Varghese, Theprungsirikul, & Sahani, 2007). In another preclinical study, human osteoarthritic synovium explants were cultured and hyaluronan production was measured in the culture medium supernatant by using an enzyme-linked binding protein assay. Real time reverse transcription-polymerase chain reaction was performed for hyaluronan synthases on RNA isolated from the explants. GlcN.HCl (0.5–5 mM) significantly increased hyaluronan production compared to control, whereas GlcNAc had no significant effect. Addition of 5 mM glucose also increased hyaluronan production. Gene expression of HAS was not altered (Uitterlinden et al., 2008).

GlcN is effective in the treatment of adjuvant arthritis in Sprague–Dawley male rats. All rats that received *Mycobacterium butyricum* without GlcN developed arthritis; this fact suggests that GlcN prevents arthritis and alleviates signs and symptoms (Gilzad-Kohan & Jamali, 2012). Likewise, GlcN modified the progression of experimental knee osteoarthritis in dogs (10 mg/kg of oral GlcN daily for four weeks); the efficacy was assessed by changes in plasma IL-6 and MMP-3, and no adverse reaction was detected (Ajadi, Oladele, Ebenezer, & Olajide, 2013).

GlcN is also able to inhibit MMPs and aggrecanase, the predominant cleavage enzyme in the cartilage (Derfoul, Miyoshi, Freeman, & Tuan, 2007; Sandy, Gamett, Thompson, & Verscharen, 1998). The potent inhibition of MMP-2 and MMP-9 by GlcN.S is due to down-regulation of transcription factor, NFkB in human fibrosarcoma cells (Kim, MM et al., 2007; Rajapakse, Mendis, Kim, & Kim, 2007). In the rat articular chondrocyte, GlcN prevented, in a dose-dependent manner, the deleterious effect of IL-1β on the anabolism of proteoglycan involving the repression of GlcAT-I, a key enzyme in the biosynthesis of GAG. In the same way, the amino sugar reduced nitric oxide, prostaglandin E2 and MMP-3, that are inflammatory mediators induced by IL-1β (Gouze, Bordji, & Gulberti, 2001). In fact, in cultured chondrocytes from mouse knee cartilage stimulated by lipopolysaccharide (LPS), C4S and C6S reduced the inflammatory response involving NFkB by acting on toll-like receptor 4, thus showing anti-apoptotic effect at 0.5–1.0 mg/ml, and inhibited caspase-3 and -9 at higher concentrations (Campo et al., 2008; Campo et al., 2009).

GlcN.S significantly inhibited (**1**) the NFkB activity in a dose-dependent manner; (**2**) the nuclear translocation of p50 and p65 proteins; (**3**) the gene expression and the synthesis of COX-2 induced by IL-1β, whereas no effect on COX-1 was proved; (**4**) the release of PGE2 to conditioned media of human osteoarthritic chondrocytes stimulated with IL-1β; (**5**) the production of proteases (aggrecanase, MMP-1. MMP-3, MMP-13). It can be stated therefore that GlcN.S inhibits the synthesis of pro-inflammatory mediators in chondrocytes stimulated with IL-1β, *via* a NFkB-dependent mechanism, thus retarding the wear of cartilage, and preventing the progression of OA. In the last analysis, it has the role of a symptom-relieving and structure-modifying drug in OA (Largo et al., 2003; Nakamura, 2011). To make a further step forward, Yamagishi et al. (2012) induced adjuvant arthritis by injecting Freund complete adjuvant (FCA) in the right hind paws of rats. The combined administration of methionine and GlcN suppressed synovial hyperplasia and the destruction of the cartilage surface and articular meniscus of the knee joints of FCA-injected rats. Moreover, this combined administration prevented the levels of nitric oxide, prostaglandin E-2 and hyaluronan from rising in the plasma of said arthritic rats.

By enhancing the protective metabolic response of chondrocytes to stress, GlcN.S and chondroitin-S may improve their capacity for repair and regeneration. These compounds function as biological response modifiers that boost natural protective responses of tissue under adverse environmental conditions (Lippiello, 2003). Chondrocytes consume glucose as a primary substrate for ATP production in glycolysis and utilize GlcN.S and other sulphated sugars as structural components for extracellular matrix synthesis; they are dependent on hexose uptake and delivery to metabolic and biosynthetic pools. Data from several laboratories suggest that chondrocytes express multiple isoforms of the GLUT/SLC2A family of glucose/polyol transporters. GlcN (**a**) inhibited IL-1β and TNF-α-induced NO production in normal human articular chondrocytes *via* inhibition of the inducible NO synthase expression; (**b**) suppressed the production of IL-1β-induced COX-2 and IL-6; (**c**) reversed the decrease in proteoglycan synthesis induced by IL-1β, and (**d**) affected both synthesis and translocation of NFκB to the nucleus. When chondrocytes undergo a phenotypic change leading to aberrant expression of various catabolic genes including *IL1β*, GlcN behaves as a NFκB inhibitor and counteracts the cytokine-induced abnormal gene expression (Fig. 2). The inhibitory effect of GlcN on NFκB was first observed in rat and human OA chondrocytes with GlcN.S (10–1000 μg/mL) (Chiusaroli, Piepoli, & Zanelli, 2011; Gouze et al., 2001; Imagawa et al., 2011).

Among the effects on human peripheral blood neutrophils, GlcN.S (0.01–1.00 mM) dose-dependently suppresses the superoxide anion induced by formyl-Met-Leu-Phe (fMLF) or complement-opsonized zymosan, and inhibits the phagocytosis of the latter. Furthermore, GlcN.S inhibits the release of lysozyme from phagocytosing neutrophils and suppresses neutrophil chemotaxis toward zymosan-activated serum. GlcN.S inhibited the fMLF-induced up-regulation of CD11b, the polymerization of actin, and the phosphorylation of p38 mitogen-activated protein kinase, MAPK (Hua, Sakamoto, & Nagaoka, 2002). In contrast, GlcNAc, does not affect said neutrophil functions. These observations support the view that glucosamine suppresses the neutrophil functions, thereby exhibiting anti-inflammatory actions in arthritis.

In volunteers, C_{max} was determined to be between 1 and 4 h after ingestion of a dose of 20 mg GlcN.S per kg body weight. Free GlcN was detected in synovial fluid where its concentrations remained elevated in most animals even 12 h after administration, as opposite to the nearly complete clearance of GlcN in serum 6 h after dosing (Laverty et al., 2005); actually these authors were the first to demonstrate that free GlcN can be quantitatively determined in synovial fluid before and after administration.

6. Intestinal absorption of glucosamine and selenium

In synovial fluids of patients with rheumatoid or osteo-arthritis, the human chitinase glycoprotein 39 (HC-gp39) is secreted by articular chondrocytes, synoviocytes and macrophages at elevated levels (Boot, Renkema, Strijland, Zonneveld, & Aerts, 1995; Boot, Blommaart, & Swart, 2001; Suzuki, Fujimoto, Goto, Morimatsu, Syuto, & Iwanaga, 2002). Recklies, White, and Ling (2002) identified a second mammalian chitinase, abundant in the gastro-intestinal tract, that is extremely acid stable and exhibits a pH optimum around pH 2. It is referred to as acidic mammalian chitinase and is capable of hydrolyzing artificial chitin-like substrates as well as animal and fungal chitin. Thus, it is now clear that there are enzymes in the gut promoting the degradation of chitin/chitosan to its oligomers and monomers and their absorption into the bloodstream.

The presence of a gut chitinase may account for previous observations that the oral ingestion of poly(N-acetyl-D-glucosamine) results in serum levels of glucosamine and N-acetyl-D-glucosamine. Thus, the use of chitosan and poly-GlcNAc for the sustained release of glucosamine and its derivatives is of growing interest. The oral ingestion of Poly-NAG® (Lescardeen, USA) was followed by the appearance of oligomers and monomers of glucosamine and N-acetyl glucosamine in the blood (Grant & Gracy, 2000; Talent & Gracy, 1996). Significant clinical improvement was found in the symptom assessment of the subjects taking oral PolyNAG®. It was concluded that oral ingestion of the polymer resulted in depolymerization to monomers of glucosamine and its derivatives, which were absorbed and functioned in the chondroprotective processes like free GlcN. According to Qian et al. (2013), GlcN.HCl oral formulations (solutions and tablets with the addition of chitosan) increased the plasma concentration and bioavailability of GlcN in rats and dogs, particularly at C_{max}. Chitosan enhanced the intestinal permeability of GlcN by reversibly opening the tight junctions of the intestinal epithelial cells, thus acting as an effective absorption enhancer for GlcN. Chitosan, a dietary supplement itself, reaches the low intestine also in combination with bile salts and adsorbed lipids. The use of an

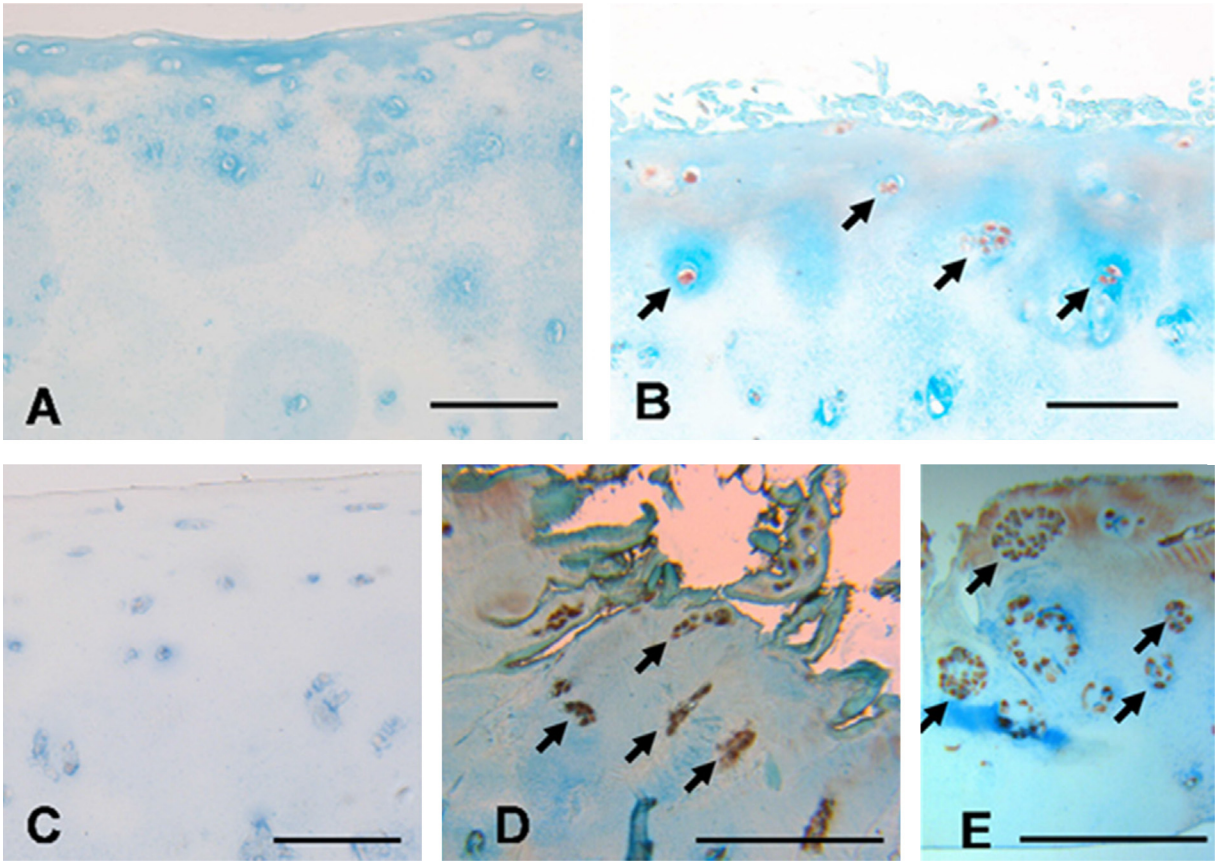


Fig. 2. Immunocytochemical analysis demonstrating that the majority of articular chondrocytes from femoral neck fracture patients do not produce IL-1β (A) or MMP-13 (C). In contrast, chondrocytes in the superficial zone of cartilage from OA patients show positive staining of IL-1β (B) and MMP-13 (D) indicating the presence of chondrocytes with a degradative phenotype, that form clusters and produce MMP-13 as illustrated in (E).
From Imagawa et al. (2011).

enhancer should no longer be considered an option because alternative absorption routes result in lower GlcN concentration at the synovial destination.

OA patients also have reduced concentration of the antioxidants vitamins C and E and thus undergo oxidative stress that is measurable in terms of malondialdehyde concentration (Surapaneni & Venkataramana, 2007). Ascorbic acid stimulates collagen synthesis and promotes the ordered deposition of its fibers, and to a lesser extent the synthesis of aggrecan. It is worth pointing out that the chitosan ascorbate salt can be safely administered orally and therefore one may speculate that in future experiments it will be combined with chondroitin-S for enhanced synergy.

Likewise, α-tocopherol is known for its strong antioxidant effects, protection against ROS, and enhancement of chondrocyte growth; moreover, zinc, copper and selenium are part of antioxidant metallo-enzymes (Wang Y et al., 2004). A novel selenium-bearing chondroitin that formed nanoparticles in distilled water *via* a self-aggregation progress was synthesized by Han, Guo, Lei, Dennis, Wu, and Wu (2012). The documented antitoxin capacity of Se-chondroitin-S nanoparticles demonstrated that the Se-chondroitin-S was less cytotoxic to chondrocytes than sodium selenite. This fact may support potential applications of Se-chondroitin-S nanoparticles for the therapy of Kashin-Beck disease and osteoarthritis.

7. Clinical evidence

Symptomatic, slow-acting drugs for the treatment of osteoarthritis (SYSADOA) have long been prescribed in European countries (Pavelka et al., 2002). Their clinical efficacy can be demonstrated only after a relatively long period of regular intake, but once administration is terminated, they show carryover effects of various durations, depending on the formulations. Chondroitin-S and GlcN can be assimilated to the oral SYSADOA that provide pain relief and increased mobility in OA patients (Uebelhart, 2008). In fact, both chondroitin-S and GlcN.S are currently recommended by EULAR and OARSI in Europe for the care of knee, hip and hand OA (Erickson and Messer, 2013; Jordan et al., 2003; Zhang W et al., 2007). The significance of their clinical efficacy is debated: they are sold as “generally recognized as safe” dietary supplements in North America, whereas they are registered drugs in Europe.

The rationale behind the use of the SYSADOA is the reduction of NSAID use in the overall drug management of OA disease thereby limiting the very significant risks of upper gastro-intestinal erosions, bleeding ulcers and deleterious renal effects in the elderly. Oral chondroitin-S plus GlcN.S modify the course of OA disease, and may stop its progression, besides positively acting on the mobility of the OA patients. OARSI guidelines recommend GlcN and chondroitin-S as DMOADS, and NICE is recommending GlcN.S (Davies et al., 2013).

Contrary to the disappointing lack of positive results in a paper by Wildi et al. (2011) on patients with primary OA of the knee, the data of Gabay, Medinger-Sadowski, Gascon, Kolo, and Finckh (2011) were quite encouraging: 80 patients received a single 800-mg tablet of chondroitin-S and 82 patients received an identical placebo each day for a total period of 6 consecutive months. The results of this clinical trial showed that the 6-month treatment with chondroitin-S is significantly superior to placebo with regard to

improvements in global hand pain and hand function. Most importantly, chondroitin-S was associated with a significant decrease in the duration of morning stiffness (Gabay et al., 2011).

A randomized, double-blind, placebo-controlled study about the effect of a dietary supplement containing 1200 mg of GlcN.HCl, 60 mg of chondroitin-S and 45 mg of quercetin glycosides involved patients with symptomatic knee OA. The results of symptomatic efficacy assessment based on Japanese Orthopedic Association criteria showed that scores for two of the four symptom/function subscales, as well as the aggregate scores, were significantly improved at the end of the study (week 16) or earlier in the supplements group compared to the placebo group. Moreover, analyses of cartilage metabolism biomarkers demonstrated a trend of improvement in collagen-II synthesis/degradation balance in the supplements group during the follow-up (Kanzaki et al., 2012; Tiku, Narla, Jain, & Yalamanchili, 2007).

Zegels, Crozes, Uebelhart, Bruyere, and Reginster (2013) made a comparative, double-blind, randomized, multicenter study to evaluate the efficacy and safety of a single oral dose of a 1200 mg sachet of chondroitin-S *vs* 3 daily capsules of 400 mg and *vs* placebo during 3 months, in 353 patients over 45 years old, with knee OA. In the last analysis, a daily administration of an oral 1200 mg sachet of chondroitin-S allowed a significant clinical improvement compared to placebo; the 2 regimens led to the same results. This work is an extension of the one by Uebelhart, Knols, de Bruin, and Verbruggen (2006) also dealing with patients with knee OA, whose data strongly supported the validity of the oral administration of 800 mg of chondroitin-S daily *versus* placebo during a 2-year survey. It confirmed that oral chondroitin-S was able to significantly stabilize the medial femoro-tibial JSN measured with a digitalized analysis of high-quality knee X-rays, whereas placebo did not.

Geraci et al. (2012) evaluated the effectiveness of a food supplement sachet containing GlcN.S, chondroitin-S, hydrolyzed collagen-II, hyaluronan and L-carnitine fumarate (a compound with a role in cellular energy metabolism) in a multicenter randomized double blind controlled clinical trial involving 120 patients suffering from knee OA. Likewise Stoppoloni et al. (2013) found that L-carnitine was very effective in stimulating cell proliferation and to induce ATP synthesis, mainly in 3D cultures. In conclusion, L-carnitine enhances cartilage matrix glycosaminoglycan component production and cell proliferation; the combination of said substances is beneficial to patients with mild to moderate OA.

Selvan, Rajiah, Nainar, and Mathew (2012) performed a clinical study on GlcN.S *vs* a combination of GlcN.S and NSAIDs in mild to moderate knee OA. The conclusions drawn from this study suggest that the GlcN.S has a carryover effect, and that long-term therapy with GlcN sulphate may reduce the dependence upon NSAIDs and delay disease progression. Durmus, Alaly, Aliyazicioglu, Buyukakincak, and Canturk (2013) studied the effects of GlcN sulphate and exercise therapy on serum level of leptin in 37 female patients with knee OA in a randomized controlled clinical trial. One group received an exercise program for 12 weeks and the other one received GlcN.S in addition to the exercise program for the same period. Both groups showed significant improvement in serum leptin levels, pain, disability, muscle strength and functional performance with no statistically significant difference between the two groups after the therapy. Evidently the exercise alone was adequate to prevent structural changes, to relieve the OA symptoms, and also to affect serum plasma levels of the leptin, that is an important mediator of cartilage metabolism.

GlcN and chondroitin-S produced beneficial effects not only in OA: Greenlee et al. (2013) demonstrated that the combination of GlcN.S and chondroitin-S may reduce aromatase inhibitor-induced arthralgia, with minimal side effects and no changes in estradiol levels. Yue et al. (2012) carried out a cluster-randomized, placebo-controlled study about the use of said combination, which they found to be more effective than placebo in the management of the Kashin-Beck disease. Bell, Kantor, Lampe, Shen, and White (2012) demonstrated that current use of GlcN with or without chondroitin was associated with a significant decrease of risk of death from cancer and with a large risk reduction for death from respiratory diseases.

Indeed the oral administration of the title compounds is beneficial to healthy subjects too: Momomura et al. (2013) investigated the effect of GlcN administration (1.5 or 3 g/day) on cartilage and bone metabolism of bicycle racers. They demonstrated that CPII, a marker of collagen-II synthesis, was not substantially changed, but CTX-II, a marker of collagen-II degradation, was reduced by consumption of 3 g/day GlcN. Accordingly, the ratio of CTX-II/CPII was reduced by GlcN and the effect of GlcN was dose-dependent. By contrast, NTx levels, a bone resorption marker, and BAP levels, a bone formation marker, were not altered by GlcN. Therefore, GlcN exerts chondroprotective action by preventing collagen-II degradation in athletes.

8. Safety

Chondroitin-S and GlcN salts are considered to be safe and effective by ACR, EULAR, NICE, OARSI and other Societies, and according to *in vitro* and *in vivo* evidence collected using MEDLINE (1950 to NOV 2012) and EMBASE (1980 to NOV 2012) databases (Davies et al., 2013); however five trials over the last 20 years reported on generic side effects such as nausea.

Moreover, the NaCl salt present in the GlcN.S formulation is approximately 21% by weight, thus the administration of 1500 mg of product carries 315 mg of NaCl that, although it increases the daily intake of salt, does not seem to be a quantity capable to influence blood pressure and renal function (Herrero-Beaumont & Rovati, 2006).

The claim that GlcN could provoke allergic reaction in subjects prone to shellfish allergy, since it is extracted from shellfish chitin, was found to be wrong, the allergenic substance being tropomyosine instead, *i.e.* a protein from crustacean flesh that might be present in trace amounts in raw chitin, but certainly not in chitosan (Muzzarelli, 2010, 2012). Human and animal studies have suggested that GlcN can affect glucose metabolism and induce insulin resistance. Chien et al. (2009) showed that *in vitro* GlcN inhibited glucose uptake in a concentration-dependent manner by a competition for the glucose transporter GLUT4. Moreover, in fructose-fed rats, GlcN accelerated the development of insulin resistance. Thus GlcN should be applied with caution in type-2 diabetic patients, although it had no effect on fasting blood glucose levels, glucose metabolism, or insulin sensitivity at any oral dose level in healthy subjects, diabetic individuals, and patients with impaired glucose tolerance (Simon, Marks, Leeds, & Anderson, 2011).

9. Reviews and meta-analyses

9.1. Glucosamine sulphate

Due to their basic role in cartilage and synovial fluid synthesis, GlcN.S and GlcN.HCl have been tested in numerous clinical OA trials and the effects have been summarized in reviews and meta-analyses (Block et al., 2010; Bruyere & Reginster, 2007; Bruyere et al., 2008; Dahmer & Schiller, 2008; Huskisson, 2008; Jerosch, 2011; Kirkham & Samarasinghe, 2009; Kubo, Ando, Mimura, Matsusue, & Mori, 2009; Mazieres et al., 2007; McAlindon, LaValley, Gulin, & Felson, 2000; Poolsup, Suthisisang, Channark, & Kittikulsuth, 2005; Ragle & Sawitzke, 2012; Reginster, Deroisy, Rovati, Lee, Lejeune, & Bruyere, 2001; Reginster, Bruyere, & Neuprez, 2007; Richy et al., 2003; Towheed et al., 2006).

The quality of evidence was evaluated by comparing data from clinical studies, meta-analyses, and reviews dated 1950–2007 on the effect of SYSADOA, including glucosamine sulphate. GlcN.S demonstrated pain reduction and physical function improvement with very low toxicity, with moderate to high quality evidence (Bruyere et al., 2008). The results of the Cochrane review by Towheed et al. (2006) were included in their evaluation. It can be concluded that long-term treatment with glucosamine: **(I)** reduces pain, **(II)** improves function/mobility of the joint, **(III)** reduces OA progression, **(IV)** reduces risk of total joint replacement (Jerosch, 2011). EULAR came to similar conclusions and rated GlcN.S in their guidelines for knee OA with the highest level of evidence, thus recommending its use (Jordan et al., 2003). The results of all these studies demonstrate that GlcN.S has many favorable effects on cartilage: **(A)** it has anabolic stimulating effect on cartilage synthesis; **(B)** it inhibits catabolic degenerative reactions; **(C)** as a consequence it alleviates pain and swelling and improves the mobility of the affected joint.

9.2. Chondroitin sulphate

EULAR awarded chondroitin-S both the highest evidence grade and the highest recommendation strength concerning knee OA. Chondroitin-S is one of the SYSADOA, the first effects of which (with the exception of analgesics and NSAIDs) become noticeable after 2–3 weeks of regular intake. Chondroitin-S has a prolonged effect that lasts for several months. There is ample agreement on the following points: **(i)** Chondroitin-S exerts *in vitro* a beneficial effect on the metabolism of chondrocytes, synoviocytes and cells from subchondral bone. **(ii)** It increases the synthesis of collagen-II and proteoglycan in human articular chondrocytes, and reduces certain pro-inflammatory factors and proteases: as a consequence apoptosis declines, whereas the anabolic/catabolic balance of the ECM ameliorates. **(iii)** Chondroitin-S exerts beneficial actions on pain and function according to clinical trials. Its structure-modifying capacity has been reported in recent meta-analyses. The results in knee osteoarthritis indicate a small yet significant reduction in the JSN. **(iv)** Because declared quality and quantity of chondroitin-S in some nutraceuticals have been found to be unreliable, pharmaceutical-grade chondroitin-S is recommended by international societies because effective and fully safe for patients with OA. The positive impact of chondroitin-S on OA was also confirmed by meta-analyses, which unanimously showed a significant favorable effect of chondroitin-S over placebo, such as statistically significant improvement in joint swelling (Bruyere et al., 2008; Das & Hammad, 2000; Hathcock & Shao, 2007; Hochberg and Clegg, 2008; Hochberg, Chevalier, Henrotin, Hunter, & Uebelhart, 2013; Lee, Woo, Choi, Ji, & Song, 2010, Leeb, Schweitzer, Montag, & Smolen, 2000; Natural Standard, 2007; Reginster & Tajana, 2011; Reichenbach et al., 2007; Sawitzke, Shi, & Finco, 2008; Uebelhart, 2008).

9.3. Synergy between chondroitin sulphate and glucosamine sulphate

The combination of GlcN.S plus chondroitin-S **(i)** increases hyaluronan production by human synovial cells, which has a beneficial effect on maintaining viscosity in the synovial fluid **(ii)** stimulates chondrocyte metabolism leading to the synthesis of collagen and proteoglycan, **(iii)** inhibits leukocyte elastase and hyaluronidase present in deleterious concentration in the synovial fluid of patients; **(iv)** suppresses IL-1-induced gene expression of iNOS, COX- 2, mPGEs, and NF-κB in cartilage explants; as a consequence **(v)** reduces production of NO and PGE2, two mediators responsible for the death of chondrocytes, and for inflammatory reactions, under the prevalent action of chondroitin-S; **(vi)** diminishes the nuclear translocation of NF-κB, which depresses the formation of pro-inflammatory cytokines IL-1β and TNF-α, and pro-inflammatory enzymes COX-2 besides nitric oxide synthase-2.

10. Economic evaluations

Economic analysis examined the cost/effectiveness of the inclusion of chondroitin-S and GlcN treatments to current care (Bruyere et al., 2009). A medico-economic search of the files of 11,000 OA patients was conducted to assess the beneficial effect of chondroitin-S on the quantity of NSAIDs prescriptions in France and to determine whether the drug was used correctly in terms of adequate dose and treatment duration. The cost of NSAIDs prescriptions by general practitioners was reduced by an estimated 67% in patients treated with chondroitin-S. The quantity of NSAIDs prescribed was reduced by two-thirds when chondroitin-S was re-prescribed. The cost related to chondroitin-S treatment was compensated by the reduction in physiotherapy costs and by fewer co-prescriptions for gastroprotective drugs. The authors concluded that a regimen of chondroitin-S for several weeks at the dose of 1200 mg/d lowers prescriptions of NSAIDs which can be completely avoided in nearly half the cases (Conrozier, 1998).

Economic analyses were conducted by Bruyère et al. (2009) in a randomized, double-blind, placebo-controlled trial of 2-year duration where the effect of the chondroitin-S on health-related quality of life in patients with knee OA was assessed. The quality of life evaluated by using the WOMAC index was translated into health utility index (HUI) and incremental cost effectiveness ratio (ICER) was calculated taking into account the cost of chondroitin-S and its effect on HUI scores, compared to placebo. Using the price bracket of chondroitin-S in Europe, ICER assessment always resulted in a cost below € 30,000 per quality-adjusted life-year (QALY) gained, after 6, 12 and 24 months of treatment. The authors concluded that chondroitin-S treatment could be considered as cost-effective in patients with knee OA up to a period of 24 months. However, the study was limited by the absence of direct utility assessment as well as the absence of effective treatment as comparator.

The clinical effectiveness and cost/effectiveness of GlcN.S or GlcN.HCl plus chondroitin-S was assessed in relation to the progression of OA of the knee. Searching in electronic databases from 1950 to 2008, the authors identified RCTs of at least 12-month duration and updated with searches for primary studies. Five systematic reviews and one clinical guideline met the inclusion criteria. A cost-effectiveness model was constructed using cohort simulation and drawing on available evidence. Sensitivity analysis was undertaken and value of information analysis conducted. Cost-effectiveness modeling was restricted to GlcN.S. The incremental cost per QALY gain for adding GlcN.S to current care was estimated to be 21,335 Great Britain pounds (£). Deterministic sensitivity analysis suggested that the cost-effectiveness of GlcN.S therapy was particularly dependent on the magnitude of the quality of life gain, the change in knee arthroplasty probability with therapy and the discount rate. At a cost per QALY gained threshold of £ 20,000, the likelihood that GlcN.S was more cost-effective than current care was 0.43, while at a threshold of £ 30,000, the probability rises to 0.73. The authors (Black et al., 2009) concluded that GlcN.S showed some clinical effectiveness in the treatment of OA of the knee and there was evidence to support its potential clinical impact, but cost-effectiveness was not conclusively demonstrated.

More recently, Rubio-Terrés (2010) investigated the average cost per patient with OA treated with chondroitin-S compared with NSAIDs for 6 months and the possible impact that the reduction NSAIDS use due to monotherapy with or combined administration of chondroitin-S treatment could have on the budget of the

Spanish National Health System. A cost-minimization model compared both treatments (efficacy equivalence assumption) using the recommended doses and regimens during a 6-month period. To determine the consumption of health care resources, the authors used data obtained from a retrospective study of 530 patients with OA treated with chondroitin-S or NSAIDs. The results showed that the overall 6-month cost per patient given chondroitin-S was € 141 compared with € 182 when treated with NSAIDs and the author's conclusions were that the expected savings for the Health System during the next 3 years would be over € 38,700,000 if patients currently treated with NSAIDs would gradually adopt chondroitin-S.

11. Conclusion

At present the scientific community through several RCTs agrees on the fact that oral chondroprotective chondroitin-S plus GlcN.S has proven efficacy in modulating OA and an excellent safety profile in long-term use. These substances are essential components of the cartilage metabolism and can stimulate important cartilage regeneration processes in OA. Chondroitin-S and GlcN are able to inhibit inflammation and oxidative-stress, thus protecting chondrocites against apoptosis, catabolic processes of extracellular matrix components and cartilage degeneration. The consequence is a reduction of OA symptoms and progression, justifying their SYSADOA and SMOAD appellatives. Recent economic evaluations demonstrated the cost/effectiveness of oral chondroitin-S plus GlcN.S therapy by the reduction in physiotherapy costs and by fewer co-prescriptions for NSAIDs and gastro-protective drugs. Further pre-clinical and clinical studies, in conjunction with advanced chemical and biochemical research on chondroitin and its sulphated esters, will surely clarify the role and the indication of these substances in OA therapy. Inconsistency in the chemical/biochemical potency of certain products used, under-dosing of patients as well as unpredictable or erratic bioavailability indices for the lack of GlcN efficacy observed in some studies should be overcome: clinical trials using pharmaceutical grade GlcN or formulations with certified bioavailability will yield positive results. More trustworthy and robust procedures should be implemented not only for analysis and regulation of chondroitin-S formulations, but also in the course of the industrial production.

Conflict of interests

No conflict of interests exists.

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Glossary

ACR: American College of Rheumatology
Chondroitin-S: Chondroitin sulphate ester
COX: cyclo-oxigenase
EULAR: European League Against Rheumatism
GAG: Glycosaminoglycan
GlcN: either glucosamine or unspecified GlcN salt
GlcN.S: Glucosamine sulphate salt
GlcN.HCl: Glucosamine hydrochloride
GRADE: Grading of Recommendations Assessment, Development and Evaluation system
IL: Interleukin
JSN: Joint space narrowing
LR: Leptin receptor
MMP: Matrix metalloproteinase
NF-κB: Nuclear transcription factor-κB that initiates pro-inflammatory genes
NICE: National Institute of Health and Clinical Excellence
iNOS: Inducible nitric oxide synthase
NSAIDs: Nonsteroidal anti-inflammatory drugs
OA: Osteoarthritis
OARSI: Osteoarthitis Research Society International
PGE2: Prostaglandin E2
RCT: Randomized controlled trials
ROS: Reactive oxygen species
SMOAD: Structure-modifying OA drug
SYSADOA: Symptomatic slow-acting drug in osteoarthritis
TGF-β1: Transforming growth factor-β1
WHO: World Health Organization
WOMAC: Western Ontario & McMaster Univ.