Symptom and Structure Modifying Properties of Chondroitin Sulfate in Osteoarthritis

Jean-Yves Reginster[1,*], Florence Heraud[2], Brigitte Zegels[1] and Olivier Bruyere[1]

[1]*Department of Public Health Economics, University of Liège, Liège, Belgium;* [2]*Laboratoires Génévrier, BP 47, F-06901 Sophia Antipolis, France*

Abstract: Chondroitin sulfate (CS) is a complex carbohydrate polymer with variable sulfation which impacts function. CS exhibits a wide range of biological activities. Many experimental and clinical data are available, affirming that CS represents an effective and safe symptomatic treatment of osteoarthritis (OA) with delayed and sustained effects.

Key Words: Chondroitin sulfate, sulfation patterns, cartilage, glycosaminoglycans, osteoarthritis, SYSADOAs.

INTRODUCTION

The articular cartilage is a very specialized tissue, consisting of only one type of cells, the chondrocytes, dispersed in an extracellular matrix mainly made up of water, fibers of collagen, proteoglycans (PGs) and noncollagenic proteins. The cartilage is a tissue with a low cell density, where the chondrocytes represent only 1 to 10% of the total mass of cartilage. The chondron, the functional unit of cartilage, is composed of one or several chondrocytes surrounded by a matrix poor in collagen [1]. The chondrocytes have at the same time capacities of synthesis and degradation, and thus ensure the homeostasis of the extracellular matrix by the renewal and the degradation of its components. For the maintenance of the integrity of the cartilage structure, it is essential that catabolism does not exceed the anabolism.

The principal type of collagen in the articular cartilage is the type II collagen (known as major collagen), which roughly accounts for 90 to 95% of total collagen and give it its basic architectural structure. The monomers of PG (Fig. **1**) consist of a central polypeptide chain called core protein, bound in a covalent way to molecules of glycosaminoglycans (GAGs). The GAGs are polymers of disaccharidic units that consist of 3 main forms: hyaluronic acid, chondroitin sulfate (CS) and keratan sulfate (KS) [2].

The Cartilage Contains 2 Main Classes of PGs

- Aggrecans which form aggregates of PGs while joining the hyaluronic acid by non covalent connections. The connection between hyaluronic acid and aggrecan is stabilized by a link protein. These 3 components are all synthesized by chondrocytes. Up to hundred monomers of PGs can be related to only one chain of hyaluronic acid being able to constitute a complex of a molecular weight of 100 million daltons (Da).

The GAGs have negative charges, which attract water molecules in cartilage, and thus create a strong osmotic pressure. This interaction increases the volume of collagenous fibers network and distends the cartilage structure. It is the balance between the osmotic pressure due to the PGs and the tension of collagen fibers, which ensures the elasticity and resilient cartilage properties. Within the matrix, PGs mainly ensure the compressive strength.

- The non aggregans small PGs such as decorin, biglycan and fibromodulin, which proteins present analogies, but which are coded by different genes. These small PGs are supposed to take part in the stabilization of cartilage matrix.

Osteoarthritis (OA) is the most common musculoskeletal disease and it reaches 10% of the world's population over 60 years [3]. OA is a disease affecting the entire joint, not only characterized by a loss of cartilage associated with changes in the subchondral bone. OA has long been considered as being the results of age or trauma, today the etiology of OA is known as multiple and includes various mechanical, biochemical and genetic factors [4]. According to the World Health Organization (WHO) definition, OA is the resultant of mechanical and biological phenomena, which destabilize the balance between the synthesis and degradation of cartilage and the subchondral bone [5]. The progressive cartilage destruction observed during OA results from morphological, biochemical, molecular and biomechanical modifications of the chondrocytes and the extracellular matrix. During OA, a deregulation of anabolic and catabolic activities of chondrocytes is observed with a prevalence of catabolic activity. The collagen and aggrecan synthesis is increased at the early stage of the disease and is decreased at the late stage. At the early stage of the disease, the overproduction of the PGs involves a hyperhydratation responsible for the softening of the cartilage. The synthesis of these molecules becomes then very quickly down-regulated. The OA cartilage is also the center of structural modifications of PGs. These modifications affect the chains, whose length is reduced. Other functions are affected during OA, in particular, the synthesis and degradation of growth factors and cytokines are increased or reduced. The cytokines, such as interleukin-1β, serve to increase the catabolic activity of the chondrocyte, which results in the release of proteolytic enzymes, including aggrecanases and matrix metalloproteinases (MMPs) , that cause destruction of the cartilage matrix [6].

*Address correspondence to this author at Head, Department of Public Health, Epidemiology and Health Economics, University of Liège, Liège, Belgium; Tel: +32 4 2703257; Fax: + 32 4 2703253;
E-mail: jyreginster@ulg.ac.be

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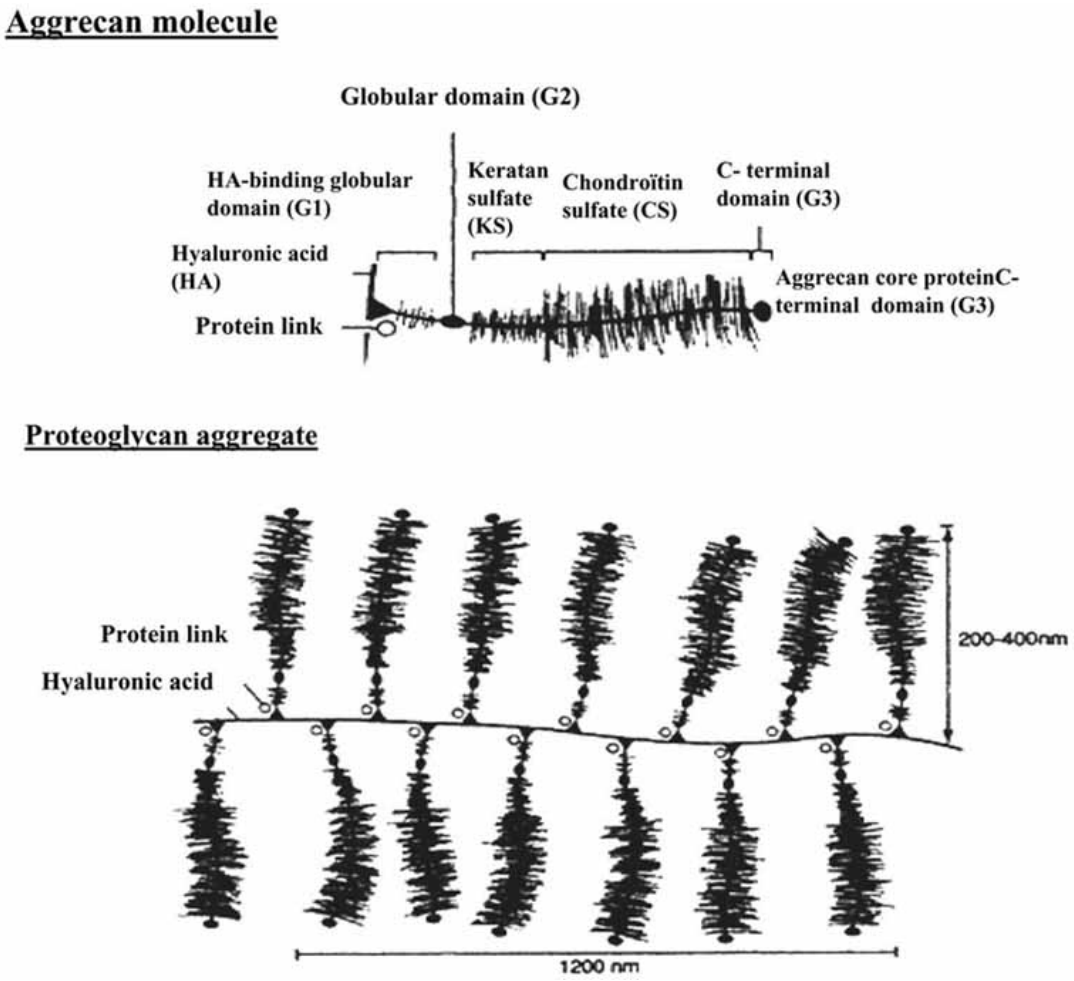

Fig. (1). Top: Aggrecan molecule consisting of a core protein with several domains: hyaluronan-binding G1 domain, G2 domain, KS-rich region, CS region, and C-terminal domain.

Bottom: Macromolecular aggregates are formed through aggrecan molecules binding to a chain of hyaluronan, this binding is stabilized by link protein.

(From Hasler *et al*. [80]; reproduced with permission from Beggel House, Inc.).

OA is not only a cartilage disease but disorders are also detected at the levels of the subchondral bone and synovial membrane.

The pathology is generally subdivided in 4 stages corresponding to the importance of the lesions. Stage I corresponds to a partial rupture of the collagen network and a swelling of PG gel. In stage II surface cracks appear as well as a release of breakdown products into the synovial fluid. At the time of stage III, cracks are deep and associated with an inflammatory reaction in the synovial membrane. Stage IV is defined by the cartilage disappearance. This erosion extends to the subchondral bone which is stripped [4].

Conventional pharmacological treatment of OA consists of nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics. However, many of these agents can cause serious side effects. One of the strategies to prevent or slow down the aggravation of the disease and its consequences, to relieve pain, improve functional capacities and decrease the handicap lies in the use of CS. CS is one of the components of PGs that contributes to the structural and functional properties of articular cartilage. CS is widely used by patients suffering from OA; a deeper understanding of CS structure / function relationships, and how these ones impact on therapeutic outcomes, is required. CS is high molecular mass (between 10 000 and 60 000 Da) and charge density polysaccharide [7,8]. CS is naturally produced by the organism; they have been reported to be present in various tissues such as connective and structural tissues and so are they mainly found in cartilage [9-11]. The CS chain comprises a linkage region, a repeat region and finally a chain cap. The repeat region of CS is a repeating disaccharide of D-glucuronic acid (GlcA) linked *via* β(1-3) bonds to N-acetyl-D-galactosamine (GalNAc), which may be O-sulfated on the C-4 and/or C-6 position of GalNAc and C-2 or C-3 position of GlcA [12]. These disaccharides units are linearly associated *via* β(1-4) linkages: $[-4)GlcA(\beta 1\text{-}3)GalNAc(\beta 1\text{-}]_n$. There are two main structural categories of CS, traditionally known as CS-A and CS-C (Fig. **2**). The term CS-A and CS-C has been used to describe respectively CS sulfated on the C-4 and on the C-6 of GalNAc. Several types of galactosaminoglycan disaccharides (non-, mono-, di- and, more rarely, trisulfated) have

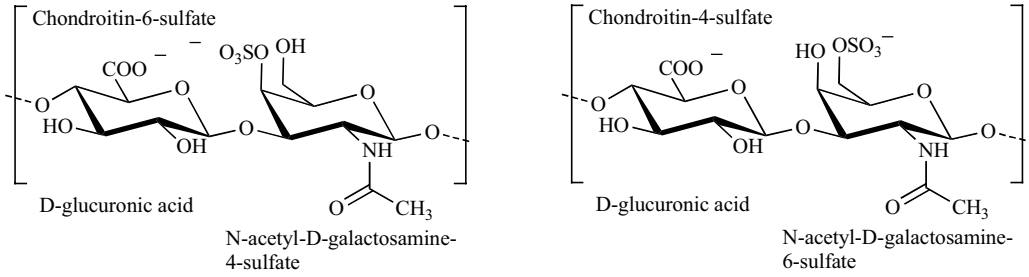

Fig. (2). Chemical structure of the repeated disaccharide unit for chondroitin-4-sulfate molecules and chondroitin-6-sulfate molecules.

been described in the literature [13,14]. Interestingly, the sulfation type (4- versus 6-sulfation of GalNAc in CS), sulfation pattern (statistical distribution of sulfates in CS), the molecular mass of CS, and the spacing between CS branch points on the core protein of aggrecan vary significantly with the disease (OA or rheumatoid arthritis), age, depth within the cartilage layer, and anatomical site [12,15,16]. CS, used as drugs, is currently manufactured from various natural sources. It is usually extracted from bovine, porcine or avian cartilaginous material and also from shark cartilage. For these reasons of direct biological and pharmacological relevance, it is of interest to characterize and understand the chemical composition of this biopolymer from various origins.

CS is classified like symptomatic slow acting drugs in osteoarthritis (SYSADOAs), acting after a few weeks time, hence the definition of slow action in opposition to the use of analgesics and NSAIDs, which act a few hours.

This mini review focuses on the properties and efficacy of CS in the treatment of OA.

PART I: CHEMICAL AND BIOCHEMICAL ASPECTS OF CS

I.1. Purification and Isolation of CS

GAGs are not primary gene products and therefore their analysis cannot rely upon genomic approaches; structural analysis requires their isolation followed by a complex characterization process. Over 10 species of CS have been extracted and isolated from various tissues. Because of the diversity of tissues containing CS, no single set of procedures will be suitable for their specific isolation. Moreover, CS can be divided into 2 groups: secretory CS in the extracellular matrix and membrane-bound CS. Therefore, 2 steps of isolation and subsequent purification of CS are roughly identified. The first primarily step isolates polysaccharides from other components: mainly hyaluronic acid, sometimes dermatan sulfates (DS) and PGs are extracted from tissues with a saline usually containing denaturing agents such as guanidine-chlorhydric acid and/or detergents such as phosphate buffered saline [17,18]. The second one separates polysaccharides from impurities by a combination of separation methods including solvent fractionation with ethanol or acetone for example, ultracentrifugation, ion-exchange chromatography, enzymatic degradation employing CS lyases such as chondroitinases ABC or glycosidases such as O-glycosidase and gel chromatography [17,19-21]. Antibodies are also useful tools to purify the corresponding PGs and many antibodies are now available for particular domains of CS and core proteins of individual CS, for example a monoclonal antibody named CS-56, which reacted with CS-A and CS-C [22].

I.2. Sequencing of CS

Different chemical strategies, sometimes similar to those employed for the purification and isolation techniques, have been developed that permit investigations into the structure and the sequencing of CS.

In most of the cases, analysis of CS is performed after its specific depolymerisation with chondroitinases with the aim of generating oligosaccharides. These enzymes were isolated from bacteria and belong to the class of lyases (EC 4.2.2.-) which specifically degrade CS according to its fine chemical structure. Chondroitin ABC lyase acted endolytically on C6S and DS at nearly identical initial rates, and acted on C4S at a reduced rate, while chondroitin AC lyases act on CS alone [23]. Chondroitin AC and ABC lyases generate di- and tetrasaccharides [24]. Chondroitin C lyase, another CS degrading enzyme, is reported to cleave the CS chain only at bonds involving a 6-sulfated GalNAc, leaving intact blocks of 4-sulfated residues. Thus, the standard method for characterizing GAGs involves chemical (*i.e.*, through the use of nitrous acid) or enzymatic digestion of the polysaccharide chains, followed by separation and quantification by high-performance liquid chromatography (HPLC) analysis to determine the percent composition of various sulfated disaccharides [25,26].

Advances in analytical separational techniques, including agarose-gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), strong anion exchange-high-performance liquid chromatography (SAX-HPLC), capillary electrophoresis (CE), and fluorophore-assisted carbohydrate electrophoresis (FACE) have enabled structural analysis and quantification of CS. These techniques provided information on charge density, polydispersity and molecular size of the chains [27-29]. Mao *et al*. described methods and general principles of PAGE and its applications to several aspects on GAGs; this review shows that CS may be separated for rapid analysis by continuous isocratic PAGE and visualized by combined Alcian blue and silver staining and for qualitative molecular mass analysis, the direct visual comparison with "counting ladder" proves to be sufficient [27]. Yang *et al.* performed the isolation of 8 oligosaccharides by SAX-HPLC and determined the size of each by gel permeation chromatography

high-performance liquid chromatography (GPC-HPLC), which varied between 498 and 2687 Da [29]. Furthermore, recent developments in the electrophoretic separation and detection of unsaturated disaccharides by enzymatic or chemical degradation have made it possible to examine alterations of GAGs with respect to their amounts and fine structural features in various pathological conditions, thus becoming applicable for diagnosis in the future [30]. Other analytical methods have been developed to quantify CS in biological samples and/or pharmaceuticals such as the carbazole assay [31], dye binding analysis [32,33], photometric titration [34] and high-performance size exclusion chromatography (HPSEC) [35]. Carbazole assay and dye binding method are the most commonly used methods for the quality control of CS as a pharmaceutical ingredient, since these reactions are not specific for CS and highly affected by salts and pH. Thereafter, the analysis of CS can be performed by using the various immunochemical techniques already established: immunoblotting, enzyme linked immunosolvent assay (ELISA) and ELISA-based procedures. Numerous works have directed their efforts to prepare monoclonal antibodies against GAGs [36-38]. The main advantage of the application of immunochemical techniques is that they do not require purified samples in contrast to chromatography and electromigration procedures and they may also give information on the distribution of different types of CS within a tissue.

Advances in mass spectrometry analysis have also enabled significant developments in the study of GAGs. However, the linear sequence of the polysaccharide is lost in this process. By contrast, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), fast-atom bombardment (FAB-MS) and electrospray ionization mass spectrometry (ESI-MS) can provide important structural information on CS oligosaccharides, including elemental composition, molecular weight and sequence information until the total structure elucidation [29,39,40]. The complex fragmentations that arise upon tandem mass spectrometry can be used in certain cases to determine the position of sulfate groups and distinguish between iduronic acid (IdoA) (corresponding to the unit of DS) and GlcA epimers [40].

These methods for digestion, processing and analysis are robust and reliable and demonstrate the great heterogeneity of structural organization of CS chains from vertebrate and especially mammalian species (*e.g.* human, bovine and porcine). The 4-sulfated GalNAc is the major sulfation type in tracheal cartilage CS bovine and porcine and in sternum cartilage CS avian with the balance being mainly GalNAc 6-sulfation. In fact, CS from trachea is mainly GalNAc 4-sulfated while that from load bearing cartilages has higher levels of 6-sulfation [24]. Studies show that, in both avian and porcine CS, the 4-sulfation level is between 78% - 81% and the level of 4-sulfation in the bovine CS is 64% - 69%. In addition, in shark cartilage, higher levels of 6-sulfation are found (about 70%), with 4-sulfation making up the balance along with about 25% 2-sulfation of the uronic acid residues [41,42]. 25 different non-, mono-, di-, and trisulfated CS have been described. Non-, mono-, di-, and trisulfalted disaccharides are generally minor components of CS molecules and the great majority is cpmposed of monosulfated units. A recent study[1] aiming at the determination of the ratio 4-sulfation to 6-sulfation suggests that with the increase in the length of chains, the bovine and porcine CS show increasing levels of 4-sulfation. On the contrary, the CS from avian have an invariant level of 4-sulfation along the chain and no apparent changes amongst chains of differing lengths. Moreover, available tools permit acquisition of compositional, but not sequence data, from CS, and it is likely that CS functional units will be embedded within specific sequences. Indeed, the CS sulfation is non-random and occurs in domains, differences in CS composition and sequence exist between species. So, CS could be found as a pure polymer or a mixed copolymer, in which GalNAc sulfation isoforms may be located in large blocks or distributed throughout the chain.

Using a 2 step enzymatic digestion/quantification by mass spectroscopy protocol, Desaire *et al*. showed that CS from bovine trachea has randomly distributed 4-sulfated and 6-sulfated disaccharides throughout the repeat region of the polysaccharides, whereas in CS from shark cartilage the 6-sulfated disaccharides form "blocks" of repeating disaccharides with the same sulfation pattern [43]. On the contrary, recent study, which data have not yet been published, has shown that CS chains from bovine trachea are not random and that the incidence of 4-sulfation is higher at the linkage region while 6-sulfation is concentrated towards the chain cap. Though, further studies to establish the sulfation profiles of CS in the size and distribution of blocks of 4-sulfated GalNAc residues will enhance our understanding of CS and inform also on the CS compositional changes between different species. In addition, these levels are dynamic, changing during ageing [12,44] and pathology [28]. These well-described structural modifications involve changes in ratios of 4-sulfated to 6-sulfated disaccharides. For example, the extensive level of GalNAc 6-sulfation (about 95%) reported for human articular cartilage applies only to the adult [24]; at birth this level is close to zero but rises significantly during the first 20 years of life [12,15].

Not only does CS structure change with tissue source and age, but also is there variability within a single CS chain. The chain cap of CS is a GalNAc or GlcA residue; a 4,6-disulfated GalNAc residue, rare in the repeat region of human articular cartilage CS, represents over 50% of the chain caps for a "normal" adult, but only about 30% at the termini of CS chains from OA cartilage [16]. While the CS chain caps may be oversulfated, the linkage regions have been shown to exhibit undersulfation relative to residues within the repeat region with preferential localization of unsulfated and 4-sulfated GalNAc residues at linkage regions [12,45]. The overall structure of CS chains is thus highly complex. Hence, the source tissue used for CS preparation can have a significant impact upon the composition, sequence and hence functionality of the material isolated.

I.3. Conformational Analysis of CS

The correlation between chemical structure and conformations of CS is carried out according to two approaches:

[1]Lauder, R.; Ellis, T.; Huckerby, T.; Morris, H.; Burger, F. Abstract#152, OARSI, **2006**.

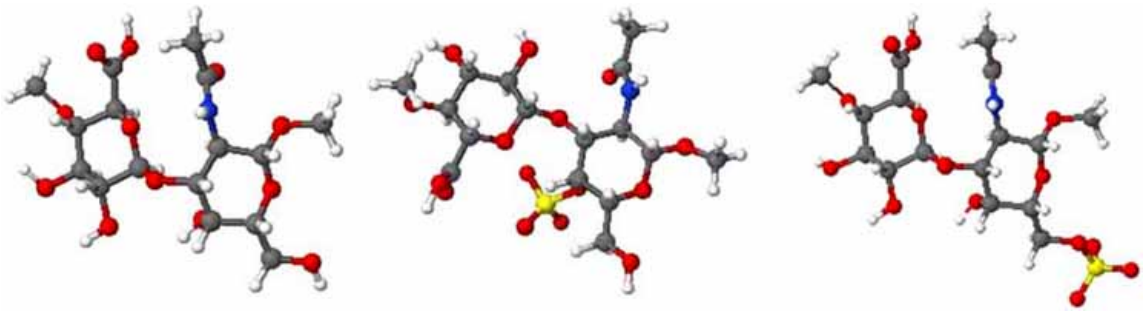

Fig. (3). X-ray diffraction structures of C0S, C4S and C6S, in side, shown in stick and ball representation with carbon in gray, oxygen in red, nitrogen in blue, sulfate in yellow and hydrogen in white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

experimental (nuclear magnetic resonance, radio crystallography, X-ray fiber diffraction) and "in silico" (molecular modelling). These various techniques have several objectives: to reveal, on the one hand, the structure of the molecule itself, its behaviour with solvents and, on the other hand, the mechanism of interaction with others molecules like proteins.

The three-dimensional structure of CS was thus studied by X-ray fiber diffraction [46-48], by nuclear magnetic resonance (NMR) [29,49] and also by molecular modeling [50]. The analysis of the X-ray diffraction pattern from CS has shown that GAGs adopt helical structures, whose pitch can vary with the associated counterion, sodium or calcium [48]. CS structures were examined by high-field ^{1}H and ^{13}C NMR spectroscopy. Data of the full assignment of signals in the NMR spectra permit the accurate determination of the chemical fine structure of small CS oligosaccharides and their non reducing capping structure. The structure for heterogeneous polysaccharides preparations were also determined [29,49].

The general molecular mechanics program MM3 [51] has been applied to calculate the adiabatic energy maps for each disaccharide: C0S, C4S, and C6S and thus to predict which conformations of chondroitin are energetically preferred in aqueous solution (Fig. **3**). Based on these maps, higher levels of structural organization have been simulated. The resulting chains present behaviour of semi-rigid polymers, with the following order of stiffness: C4S > C0S > C6S. Moreover, the exploration of the stable ordered forms leads to numerous helical conformations of comparable energies. Several of these conformations correspond to the experimentally observed ones. The ability of coordination with cations has also been explored, resulting in a preferential stereospecificity for calcium ions over sodium ions [50]. Currently, no conformational analysis of fragments having sulfation pattern heterogeneity has yet been carried out.In addition, the coarse-grained molecular modelling system of Bathe *et al*. was developed to predict the effects of C0S, C4S and C6S structure on osmotic pressure in aqueous solution [52,53].

Neither the position nor the extent of sulfation of the CS chains was predicted to affect osmotic pressure at physiological ionic strength. 6-sulfation is found not to affect the intrinsic stiffness of CS chain, whereas 4-sulfation is found to have a considerable effect. This finding is attributed to the proximity of the sulfate group in C4S to the β1,3 linkage, where it sterically hinders linkage flexibility by interacting with the ring oxygen of GlcA residue. In contrast, the sulfate group in C6S is distal from both the β1,3 and β1,4 linkages so that it does not significantly affect glycosidic linkage flexibility with respect to CS [52]. This finding for C4S is consistent with the study by Rodriguez-Carvajal *et al*. [50] but the result for C6S is in contradiction with that work, in which C6S was found to be more flexible than C0S. Additional theoretical investigations using an explicit solvent model should be pursued to attempt to resolve this discrepancy [52].

The more interesting results which modelize the CS interaction with proteins are the crystal structures of complexes between proteins and oligosaccharides. But only few structures have been determined and are limited to a few bacterial enzymes that degrade CS such as chondroitin AC lyases. Studies in progress of CS binding sites on proteins like growth factors or enzymes implied in the cartilage degradation and the specificity, with which they recognize their ligands, is an important consideration in their structural biology. A recent study[2] on the interaction between CS and interleukin-1β (IL-1β), an important mediator of the inflammation in OA, has been currently performed. The docking of oligosaccharides to human recombinant IL-1β (hrIL-1β) show that several CS fragments with different sulfate repartitions and different lengths all bind to the same face of hrIL-1β, thus blocking the formation of a complexe between hrIL-1 and its type 1 IL-1β receptor.

I.4. Biosynthesis of CS

The biosynthesis of GAGs is of prime importance in biology due to the role of these macromolecules in the mechanisms of extracellular regulation. The chemical complexity of GAG structures necessitates a multistep process and the development of sophisticated protecting group strategies and stereo- and regiocontrolled glycosylation reactions. In response to these challenges, several groups have co-opted enzymes for the CS biosynthesis [54-56].

CS chain is synthesized both in chondrocytes and bone cells by the action of specific glycosyl-transferases; the CS catabolism occurs in the matrix and involves numerous

[2]Heraud, F.; Burger, F.; Rebuffet, E.; Amigues, S.; Soler, C.; Imberty, A. Abstract.# 192, OARSI, **2006**.

MMPs and lysosomal enzymes. The CS glycosyltransferases transfer GlcA and/or GalNAc residues from each UDP-sugar to the nonreducing terminus of the CS chain [55,56]. CS chain is covalently attached by a common tetrasaccharide sequence (GlcA1, 3Gal1, 3Gal1, 4Xyl-O-Ser) to the serine residues of core proteins while they are adherent to the inner surface of endoplasmic reticulum and Golgi vesicles. Addition of the first sugar residue, xylose, to core proteins begins in the endoplasmic reticulum, followed by the addition of two galactose residues by two distinct glycosyl transferases in the early cis/medial regions of the Golgi (Fig. **4**). The linkage tetrasaccharide is completed in the medial/trans Golgi by addition of the first GlcA residue, followed by transfer of GalNAc to initiate the formation of a galactosaminoglycan rather than a glucosaminoglycan (Fig. **5**). This specific *N*-acetylgalactosaminyl transferase is different from the chondroitin synthase involved in generating the repeating disaccharide units to form the chondroitin polymer.

Sulfation of the chondroitin polymer to particular positions by specific sulfotransferases occurs as the polymer is being formed. The sugar residues are sulfated to varying degrees and positions depending upon the tissue sources and conditions of formation [56]. Chondroitin 4-sulphotransferase-1 (C4ST-1) and chondroitin 6-sulphotransferase-1 (C6ST-1) transfer sulfate from adenosine 3'-phosphate 5'-phosphosulfate (PAPS) to positions 4 and 6 respectively of the GalNAc residues of chondroitin [57].

All the enzymes in the pathway for CS biosynthesis have been cloned and expressed and, so, may help to understand the molecular basis of several biological events compromised by alterations in this pathway [54]. Recently, Kitagawa *et al*. demonstrated an "*in vitro*" polymerization reaction to generate unmodified CS using a recombinant chondroitin synthase enzyme in conjunction with a chondroitin polymerizing factor protein [58]. Although many methods

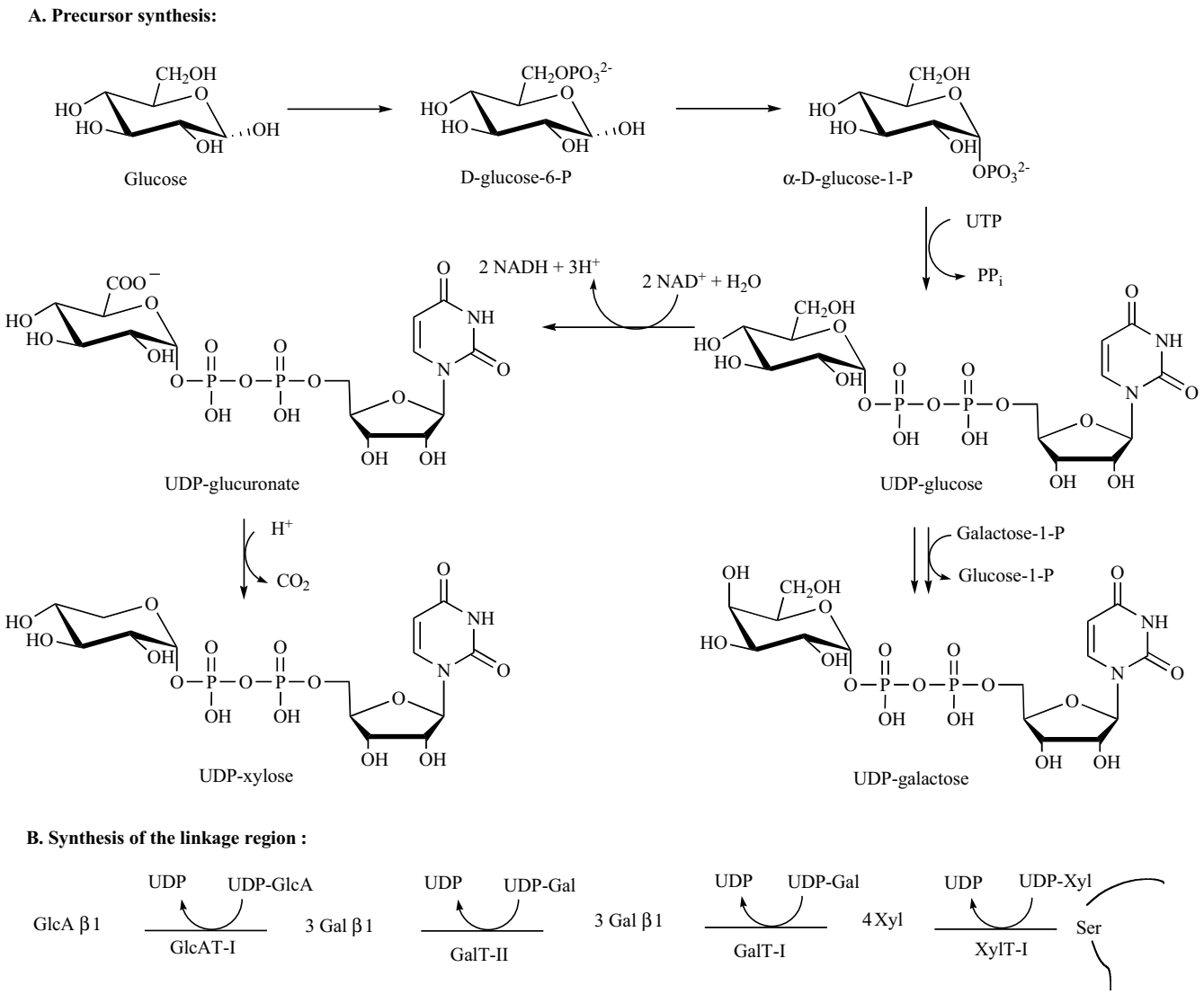

Fig. (4). Biosynthesis of UDP-Galactose, UDP-Glucuronate, and UDP-Xylose: precursor and linkage region synthesis. (A) Formation of sugars necessary for the linkage region biosynthesis of CS. Glucose is the main source of all GalAG precursors. (B) Synthesis of the linkage region. The linkage region is formed by sequential addition of xylose, two galactoses, and glucuronic acid catalyzed by xylosyltransferase I (XylT-I), galactosyltransferase I (GalT-I), galactosyltransferase II (GalT-II), and glucuronyltransferase I (GlcAT-I), respectively.

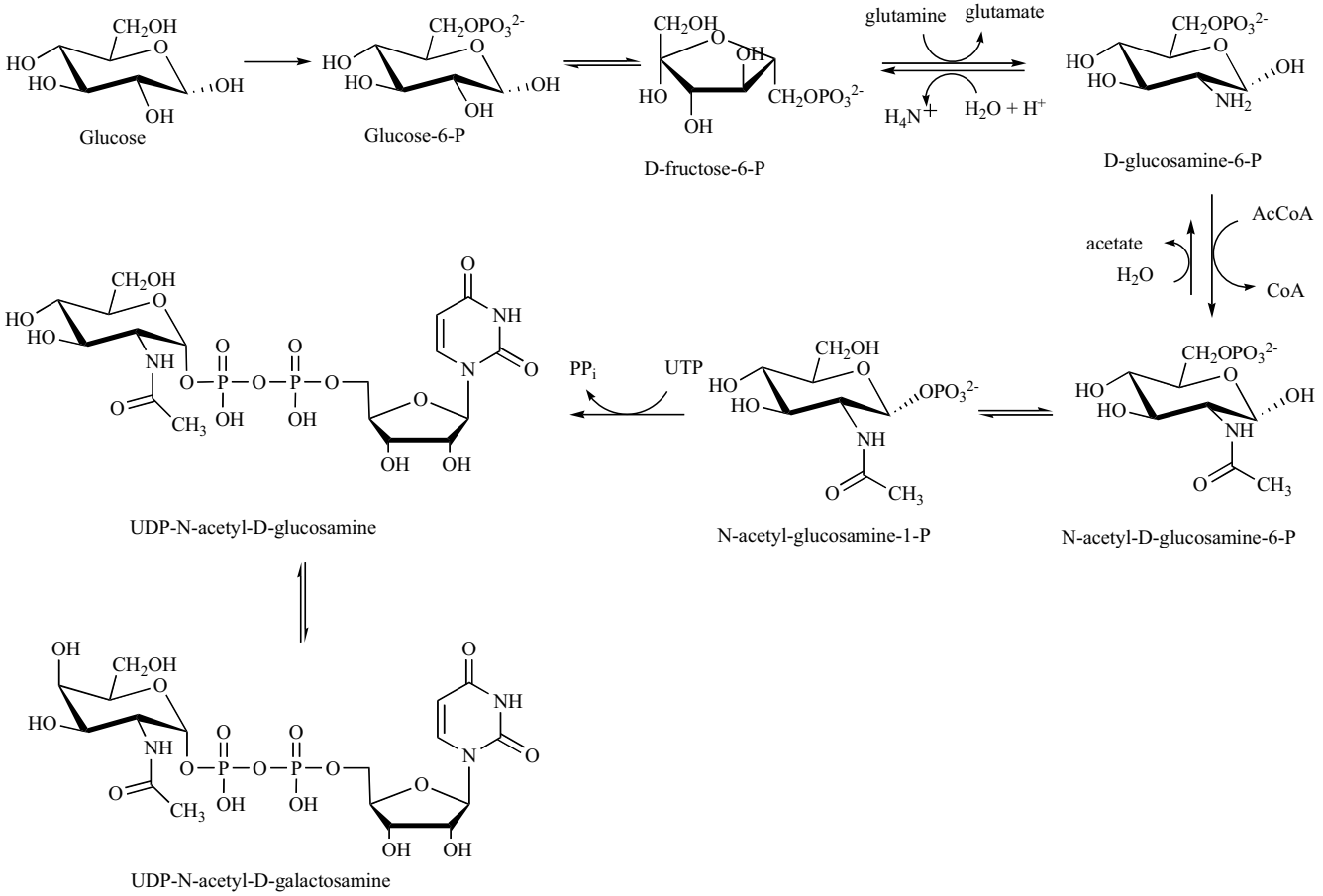

Fig. (5). Biosynthesis of UDP-N-Acetyl-D-galactosamine. Formation of one of the sugar necessary for the CS chain polymerization.

for analysis of CS or oligosaccharides are available, methodologies for complete structure elucidation are not so mature yet and thus hinder knowledge on sequences. Progress in this field and in identification of the enzymes involved in CS biosynthesis, determination of their specificities, and regulation mechanisms will undoubtedly allow the elucidation of CS structural organization according to the PG, tissue, organism, and developmental status.

CONCLUSION

Analysis of CS is used for quantitative purposes or its characterization. Very simple assays of high efficiency are developed for analytical determinations, with or without prior separation or degradation, such as chromogenic, electrophoretic, and solid phase assays. In addition, more sophisticated techniques are proposed for CS structural characterization in highly purified samples, such as HPLC and CE. Structural and conformational investigations of CS, including crystallographic, spatial modeling, and RMN studies, have shed considerable light on CS sequence and sulfation pattern. The proportion of 4 and 6 sulfated disaccharides, the sulfation degree and the molecular mass depend on CS tissue and animal origins and are responsible for the CS properties. Therefore, this important structural heterogeneity causes notable variations concerning the biological and pharmacological properties of the different molecules of CS according to their origins.

PART II: PHARMACOLOGICAL, PHARMACOKINETICS ACTIVITIES AND CLINICAL USE OF CS

II.1. Pharmacological Aspects of CS

CS exhibits a wide range of biological activities and numerous "*in vitro*" studies have been performed to determine the mode of action of CS. Even if other cellular types of the articulation, like synoviocytes or osseous cells, have an undeniable physiopathological role in OA, chondrocytes remain the most important target cells since they ensure the "turn-over" matrix. It is one of the reasons why many works evaluated the effect of CS on articular chondrocytes in culture.

CS stimulates the PG synthesis and inhibits the synthesis of molecules with inflammatory pro activity which will support the degradation of the matrix and thus the OA process [59].

Bassleer *et al*. observed the effects of CS on differentiated human articular chondrocytes cultured in clusters and underlined that CS increases the PG synthesis but do not affect basal prostaglandin E2 (PGE2) production by human chondrocytes. Furthermore, CS inhibits the collagenolytic activity [60].

The effect of CS on the aggregability of neo-synthesized sulfated PG of cultured chondrocytes treated with IL-1β was studied. It was shown that CS increases the amount of func-

tional sulfated PGs in the direct environment of chondrocytes "*in vitro",* mainly by exhibiting aggregating properties on PG. This beneficial effect is associated with a decreased expression of a disintegrin and metalloproteinase with thrombospondin type 1 motifs (ADAMTS-5). These data suggest that CS may have beneficial effects on PG aggregation by 2 different pathways, including stimulation of aggrecan rate synthesis, and/or inhibition of ADAMTS-5 production or activity by the cells[3].

Moreover, a study investigated the "*in vitro*" effects of CS on human articular chondrocytes cultivated in the presence or the absence of IL-1β during 10 days of tridimensionnal culture with and without pressurization cycles and these results confirm that the addition of CS confers a protection in counteracting the IL-1β induced effects [61]. Stromelysin-1 (metalloprotease-3, MMP-3) is a cartilage proteolytic enzyme which induces cartilage destruction. Monfort *et al.* revealed that, at various concentrations, CS reduced MMP-3 expression levels induced by IL-1β in human OA chondrocytes from patients with primary OA [62].

The nuclear factor kappa B (NF-κB) proteins are a family of ubiquitously expressed transcription factors (TFs) that play an essential role in most immune and inflammatory responses. NF-κB signaling pathways mediate critical events in the inflammatory response by chondrocytes, leading to progressive extracellular matrix damage and cartilage destruction [63]. The study of Vergès *et al.* suggests that the clinical effects of CS could partially be due to a decrease in the nuclear translocation of NF-κB in rabbit chondrocytes stimulated with IL-1β [64].

Nitric oxide (NO) belongs to the many mediators implied in the OA patophysiology. It is known that chondrocytes are a major source of intraarticular NO after an oxidative stress and that NO is able to induce chondrocyte apoptosis. Whereas apoptotic chondrocytes constitute 1 to 2% of the cells in the normal human articular cartilage, their proportion would reach approximately 20% in the osteoarthritic human articular cartilage [65]. A preventive treatment by CS made it possible to reduce the number of apoptotic cells. In 70% of the cases, preventive treatment with CS (100μg/mL) induced a decrease in the number of apoptotic chondrocytes (28%) incubated with NO donors. CS protects articular chondrocytes stimulated by the IL-1β from the cytotoxic action of NO [66].

CS inhibits the activity of the human elastase what could partly explain their chondroprotective action. This proteolytic enzyme presents in the azurophil granules of polymorphomonuclear leukocyte plays a part in the degradation of the type II collagen and the PG of articular cartilage and thus takes part in the development of the tissue lesions [67]. The interaction of CS with elastase is explained by the formation of electrostatic connections between the sulfates groups negatively charged with the GAGs and the groups positively charged with the leukocyte human elastase. CS acts by a noncompetitive inhibition [68].

[3]Tahiri, K.; Richette, P.; Korwin-Zmijowska, C.; Burger, F.; Chevalier, X.; Corvol, M.T. Abstract.#168, OARSI, **2006**.

The therapeutical effect of CS could be the consequence of native CS and its products of depolymerization. "*In vitro*" studies, on human leucocytes, indicate that anti-inflammatory drug activity of the CS depolymerization products is due to the inhibition of human leucocytes chemotactism, the lysozyme secretion, phagocytosis and membrane protection against the effects of oxygen free radicals [69].

Moreover, the efficacy of CS as chondroprotective agents has been examined in many animal models. Uebelhart *et al*. highlighted that CS proved to be more efficient in acute forms of cartilage degradation in the rabbits induced by chymopapain. While no difference to controls in cartilage PG content loss could be observed at the end given for a total of 31 days, the PG content was less reduced at 3 months in the treated group [70]. This suggests that the action of CS is delayed, which is consistent with the long-lasting symptomatic effect observed in humans.

CS could associate 3 possible modes of action: an anti-inflammatory activity, a metabolic activity in particular by restoring the properties of PG aggregation and a chondroprotection activity by inhibiting the factors of degradation of the cartilage. CS could promote the processes of articular tissue repair and interfere with the degradation of the extracellular matrix.

II.2. Pharmacokinetic Aspect of CS

From a pharmacokinetic point of view, only small amounts of CS may cross the upper intestine in the intact form probably by a mechanism of endocytosis, but in the distal gastrointestinal tract the molecule is effectively degraded to di- and monosaccharides, presumably by the enzymes in the intestinal flora. The information on CS pharmacokinetics obtained from experimental animals and humans studies has already been reviewed [71]. The relationship between the dynamics and kinetics of CS is not well established. The bioavailability of CS is about 12%. Thanks to a tritiated, fluoroscinated or iodinized marking of CS, an accumulation takes place on thighs and calves levels during the first 40 minutes after the administration [71]. Then its concentration increases gradually on knee level. Conte *et al.* showed that CS, given orally to dogs and absorbed, accumulates preferentially in the kidneys, the liver, the intestine, the eyes, the synovial fluid and the articular cartilage 24 hours after administration. Although CS and its metabolites are eliminated from the organism in a maximum delay of 7 days, its effects persist for more than 3 months [72]. According to this study, the estimated half time of CS oscillates between 10 and 20 hours and is independent of the administration route (endovenous, intramuscular or oral). On the contrary, a study led by Ronca *et al.* showed that CS and its depolymerised derivatives distribution depend on the administration route [69]. Human intestinal absorption is fast, from to 3 at 8 hours [71]. Moreover, several pharmacokinetic studies, carried out in humans, showed an increase in the serum rate of CS following a single oral absorption of CS [72-74]**.** These data confirm in humans the results already obtained in animals, giving a report of an accumulation of CS in articular tissue. However, it should be stated that there are pharmacokinetic differences according to the CS origin used.

A study performed by Volpi, carried out on blood samples after a single oral administration of 4g CS to 20 healthy adults volunteers, has shown an increase in the CS molecular mass in blood [73]. Volpi estimates an increase of more than 200% of the plasmatic chondroitins with the oral administration of bovine CS and a lower increase of 120% of the plasmatic chondroitins with the oral administration of ichthyic CS. Thus, ichthyic CS is absorbed less quickly and in a smaller quantity than the CS of bovine origin [74]. Furthermore, the agarose-gel electrophoretic method used by Volpi [73,74] permits a detection of high molecular mass polysaccharides, over than 2000 Da which correspond to 3 to 4 disaccharides units. These results provide evidences that the exogenous orally administered and absorbed CS is high molecular weight material. However, the current problem lies in the fact that these results do not differentiate the endogenous CS of lower molecular mass from the exogenous ones. According to the local cartilage improvement, the therapeutic effects of exogenous CS may be related to the inhibition of cartilage degradative enzymes, to an anti-inflammatory and anti-oxidant action and to the stimulation on PG aggregation.

II.3. Clinical Efficacy of CS in The Management of OA

The aim of this paragraph is to summarize the main clinical trials performed with CS (Chondrosulf®) in the treatment of OA while being focused mainly on the efficacy and tolerance aspects. Several randomized, multicentric, controlled trials showed the relevant and significant clinical effectiveness of CS in reducing pain and improving joint function in patients with OA.

In a double-blind, placebo controlled clinical study [75], 85 patients with knee OA were randomly assigned to receive 400mg twice a day of CS for 6 months. Lequesne's Index and spontaneous joint pain (VAS) decreased constantly in the CS group. The walking time (defined as the minimum time to perform a 20-meter walk) showed a statistically significant constant reduction only in the CS group. All these results suggest that CS acts as a symptomatic slow-acting drug in knee OA.

The Bourgeois *et al.* clinical study is a double-blind, randomized, controlled versus placebo study of the effectiveness of 1200mg of CS in single dose and CS 1200mg in 3 doses of 400 mg every day for 3 months. The overall results show an efficacy and a tolerance in all points comparable between one daily dose and 3 doses per day of CS in the functional symptomatology treatment of the knee OA [76].

The aim of the Morreale *et al.* study was to assess the clinical efficacy of CS in a 3 month- treatment, in comparison with one month treatment with the NSAID diclofenac sodium, and six months of placebo treatment, in patients suffering from knee OA. The diclofenac sodium shows, in this study, its analgesic and anti-inflammatory effects for the period of administration, however, as soon as this treatment is suspended, one can observe an increase in the symptoms, confirming that the NSAIDs do not affect the progress of the OA disease. It also showed that the treatment with diclofenac sodium was not superior in terms of reduction of algofunctional indexes [77].

In addition, CS presents global therapeutic activity with sustained effect on pain, on the joint function and on paracetamol consumption, comparable in intensity with that of diclofenac sodium, but in a delayed way compared with diclofenac sodium, hence the advantage of the relay with CS. Moreover, these therapeutic effects persist in time after the suspension of the treatment (remanent effect of CS).

Considering the efficacy of CS as SYSADOAs; CS has been also tested for its structure-modifying properties in several clinical trials (potential structure/disease modifying anti-osteoarthritis drug).

In a randomized, double-blind, controlled trial, 46 patients with symptomatic knee OA received 800mg per day of CS or a placebo for one year [70]. This study confirms the positive symptomatic effect of CS in decreasing pain and increasing overall mobility capacity. One also observes a positive effect of CS on the cartilage degradation and subchondral bone markers. The overall results concerning symptomatology, serum markers and radiological measurements of the joint space narrowing suggest that CS has not only a symptomatic efficacy, but also reduces the cartilage degradation. It is the first study suggesting that an anti-OA with slow action has an effect on the evolution of the cartilage.

In another study, 120 patients with knee OA were randomly assigned in a 1 year double-blind placebo controlled multicenter study to receive a 3-month intermittent treatment twice a year of CS (800mg per day) [78]. The differences observed between CS and the placebo group are statistically significant and clinically relevant. The difference observed on the radiological criteria is also statistically significant, which constitutes an additional proof of the chondroprotective properties of CS in knee OA. The 3-month intermittent administration of CS does support the prolonged effect known with symptom-modifying agents for OA. Moreover, the reduction in the symptoms is observed only during the treatment periods by CS, hence the necessity to take the treatment uninterrupted. In conclusion, and in line with the recent international recommendations, this study confirms that CS has a validated position in the knee OA therapeutic strategy.

A recent randomized, double-blind, placebo controlled study has been performed on 300 patients suffering from symptomatic knee OA during 2 years [79]. The primary study end point criterion is radiological and evaluation is carried out by quantitative measurements ofthe minimum and mean joint space width of the most severely affected compartment of the target knee. After 2 years of treatment, the analysis of the results shows a significant reduction in the joint space width (mean joint space width and minimum joint space width) in the placebo group whereas no degradation is observed in the CS group. Moreover, the differences in joint space narrowing between the 2 treatment groups are statistically significant. This study indicates that CS, already known for the symptomatic action, is also described as a chondroprotective treatment since CS slows down the process of cartilage degradation.

In addition, all clinical trials concluded on good and excellent safety and tolerability of CS and few associated side effects were reported in some of the studies with CS.

Thus, CS appears to be effective and safe and may have a chondroprotective action by modifying the cartilage structure in OA patients.

CONCLUSION

A great heterogeneity in the degree of sulfation as well as in the distribution of sulfate groups is being observed within the CS chain, mostly depending on the animal sources. Fine structure determines the specificity of functions and interactions of CS. Progress in analytical techniques will undoubtedly allow the characterization of each CS structural organization. Moreover, the mechanisms of action of CS are not totally elucidated, however many "*in vitro*" studies confirm the clinical data supporting the idea that CS acts as symptom and structure modifying medication in the treatment of OA.

ABBREVIATIONS

CS	=	Chondroitin sulfate
OA	=	Osteoarthritis
GAG	=	Glycosaminoglycan
PG	=	Proteoglycan
KS	=	Keratan sulfate
MMP	=	Metalloproteinase
NSAIDs	=	Nonsteroidal anti-inflammatory drugs
Da	=	Dalton
GlcA	=	D-glucuronic acid
GalNAc	=	N-acetyl-galactosamine
CS-A	=	Chondroitin-4-sulfate
CS-C	=	Chondroitin-6-sulfate
DS	=	Dermatan sulphate
SYSADOAs	=	Symptomatic slow acting drugs in osteoarthritis
C0S	=	Chondroitin non sulfated
C4S	=	Chondroitin-4-sulfate
C6S	=	Chondroitin-6-sulfate
HPLC	=	High-performance liquid chromatography
PAGE	=	Polyacrylamide gel-electrophoresis
SAX-HPLC	=	Strong anion exchange high-performance liquid chromatography
CE	=	Capillary electrophoresis
FACE	=	Fluorophore-assisted carbohydrate electrophoresis
GPC-HPLC	=	Gel permeation chromatography high-performance liquid chromatography
HPSEC	=	High-performance size exclusion chromatography
ELISA	=	Enzyme-linked immunosorbent assay
MALDI-MS	=	Matrix-assisted laser desorption/ionization mass spectrometry
FAB-MS	=	Fast-atom bombardment mass spectrometry
ESI-MS	=	Electrospray ionization mass spectrometry
IdoA	=	Iduronic acid
NMR	=	Nuclear magnetic resonance
IL-1β	=	Interleukin-1β
hrIL-1β	=	Human recombinant interleukin-1β
C4ST-1	=	Chondroitin 4-sulphotransferase-1
C6ST-1	=	Chondroitin 6-sulphotransferase-1
PAPS	=	Adenosine 3'-phosphate 5'-phosphosulfate
PGE2	=	Prostaglandin E2
ADAMTS-5	=	Disintegrin and metalloproteinase with thrombospondin type 1 motifs
MMP-3	=	Stromelysin-1/metalloprotease-3
NF-κB	=	Nuclear factor Kappa B
TFs	=	Transcription Factors
NO	=	Nitric oxide

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