



Chondroitin sulphate: A complex molecule with potential impacts on a wide range of biological systems

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KEYWORDS

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Summary Chondroitin sulphate (CS) is widely consumed orally by humans, and non-humans as it is believed to be beneficial for those with joint-related pathologies. Data concerning the functions of chondroitin sulphate in this, and other, biological systems are being actively extended. However, it is important to appreciate that chondroitin sulphate molecules represent a heterogeneous population the structure of which varies with source. As commercially available chondroitin sulphate is derived from a range of sources, and the molecular functions of chondroitin sulphate depend upon the structure, there are a range of structures available with differing potential for therapeutic impacts on a range of pathologies.

While the safety of CS is not presently in doubt, poor quality finished products have the potential to compromise clinical and lab-based studies and will fail to give consumers all of the benefits available. Major parameters including bioavailability and uptake have been studied but it is clear that significant challenges remain in the identification of composition, sequence and size impacts on function, understanding how the consumed material is altered during uptake and travels to a site of action and how it exerts an influence on biological processes. If we understand these factors it may be possible to predict impacts upon biological processes and identify specific chondroitin sulphate structures which may target specific pathologies.

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Introduction

The glycosaminoglycan (GAG) chondroitin sulphate (CS) is taken orally in capsules and tablets,^{1,2} as an additive to food and drink, it is used in creams,³ eye drops,⁴ cosmetics and medical applications.⁵ Data are emerging which show the potential of CS as a therapeutic, at the same time as our understanding of its complex structure^{6–9} and functions^{10,11}

is still developing. Chondroitin sulphate does not have a unique structure, it is a molecular type with a very wide range of structures⁶ and the impact of this diversity upon function is significant.¹⁰

Chondroitin sulphate is a polymeric carbohydrate which comprises a repeating disaccharide motif of glucuronic acid (GlcA) and N-acetyl-galactosamine (GalNAc) (Fig. 1), often modified by sulphate groups replacing one, or more, of the OH groups on C4 and C6 of GalNAc⁶ and C2 and C3 of GlcA (Table 1).^{6,10,12} These four modifications can generate 16 isomers of the repeating disaccharide, if sulphation was random an n-mer could have any of 16ⁿ forms.

Abbreviations: CS, chondroitin sulphate; GAG, glycosaminoglycan; GlcA, D-glucuronic acid; GalNAc, N-acetyl-galactosamine.

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Table 1 The range of CS types.

ID		Structure	Ref.
O	Di-O5	-4)GlcA(β 1-3)GalNAc(β 1-	85
A	Di-A	-4)GlcA(β 1-3)GalNAc4S(β 1-	86
C	Di-C	-4)GlcA(β 1-3)GalNAc6S(β 1-	86
D	Di-diS _D	-4)GlcA2S(β 1-3)GalNAc6S(β 1-	86
E	Di-diS _E	-4)GlcA(β 1-3)GalNAc4S,6S(β 1-	87
	Di-triS	-4)GlcA2S(β 1-3)GalNAc4S,6S(β 1-	85
M		-4)GlcA3S(β 1-3)GalNAc4S,6S(β 1-	88
K		-4)GlcA3S(β 1-3)GalNAc4S(β 1-	88
L		-4)GlcA3S(β 1-3)GalNAc6S(β 1-	88
	Di-tetraS	-4)GlcA2S,3S(β 1-3)GalNAc4S,6S(β 1-	38

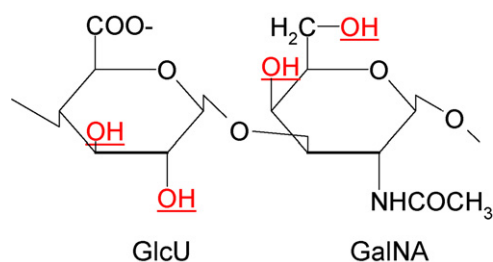
The size of CS chains found *in vivo* is challenging to establish as many techniques for determining this parameter are calibrated for proteins which behave in a very different way to CS. However, chain size is known to vary with source, and available data suggest that tracheal CS is *ca.* 20–25 kDa while shark CS is larger at *ca.* 50–80 kDa.¹³ However, any population of CS chains, even from a single tissue source, is heterogeneous with respect to size.

Thus, while the diversity of CS polymers found *in vivo* is large,⁶ it is less than that theoretically possible. This shows CS chain structure is likely to be non-random, and that this non-random structure may be what allows CS to contain biological information, and impact biological processes in a manner which is modulated by the presence of specific structural motifs.

Sources of CS

CS is abundant and widely distributed in humans,⁷ other mammals⁶ and invertebrates,^{14,15} reflecting its central role in biological processes.^{11,16} Isolation and analysis has been reported for many sources⁶ although commercial CS is mainly derived from trachea,⁶ nasal septa,¹⁷ chicken keel¹⁸ shark cartilage⁶ and fish.¹⁹

CS sulphation varies with source organism, tissue,⁶ location within a tissue²⁰ and age.^{7,20} Tracheal cartilage CS is mostly sulphated at GalNAc C4 with some C6 sulphation as an alternative⁶ (Fig. 1), while in shark cartilage CS sulphation at C6 dominates over C4 sulphation and GlcA C2 sulphation is observed⁶ (Fig. 1). Thus, it is important that studies of CS function, whether *in vitro* or *in vivo* studies, report data showing the structure of the CS used. This information is essential for comparisons between studies, to understand

**Figure 1** Structure of chondroitin sulphate.

differing, possibly conflicting, outcomes and to understand structure/function relationships, thereby moving our focus to the features within CS chains which impact biological processes. Some studies are already reporting in detail the structural characteristics of the CS used.^{21–23}

Quality and identity of CS

One of the major issues relating to the success and appropriateness of CS as a complementary therapy is quality and identity. Potential contaminants include protein from the source tissue, water which is bound tightly by CS, organic solvents used in some purification processes²⁴ and small organic molecules from the tissue or purification process. Some products contain less than the claimed amount of CS, in some cases as little as 10%,¹ and tracheal CS may be substituted for higher priced shark-derived materials.²

CS structure varies with source and function varies with structure,^{6,10} therefore it is essential that quality and identity issues be resolved by determination of CS abundance and source.⁶ In the absence of this quality assurance, interpretation of experimental studies may be compromised.

Exogenously consumed CS

CS is usually ingested as an intact polymer and determining the bioavailability, and fate, of exogenously consumed CS is challenging. Some animal studies employ radio-labelled CS while human studies have determined changes in plasma CS levels above background using HPLC methods.^{23,25}

Several studies have examined changes in the levels of CS in blood following oral administration^{23,25–27} of CS in humans, horses and dogs. For tracheal CS these studies point to a rapid increase in plasma CS levels with a peak within 1–5 h following administration, returning to baseline after *ca.* 10 h. Shark CS is reported to have a slower uptake, with the peak levels not occurring until *ca.* 8.7 h after administration, but the level remained above baseline until *ca.* 16 h following administration.²³ Analysis of the uptake of desulphated chondroitin suggested a very rapid uptake, with peak levels occurring after 15 min, followed by very rapid clearance, returning to baseline after 3 h.²⁸ Bioavailability data for CS are challenging to acquire²⁹ and a range of values have been reported. Overall these point to exogenous CS having a bioavailability of around 10–20%.

These studies can tell us about changes in CS abundance but not about the size of the CS found in the blood, as analysis involves depolymerising the CS. *In vitro* studies suggest that CS polymers cross the gut wall at a rate varying inversely with polymer size,^{27,30} and these differences in peak CS levels are consistent with slower uptake of the larger shark derived material. However, Barthe et al.³¹ have shown that while low levels of intact CS polymer were taken up in the intestine, the majority of uptake was of small CS oligosaccharides generated by the flora of the colon. Thus, differences in CS uptake may reflect differences in their breakdown in the colon and subsequent uptake.

If the predominant form of CS taken up is small oligosaccharides³¹ then these may mediate biological processes rather than intact polymers; it has already been established that they can do so.^{30,32,33} However, with a few exceptions^{10,32,34} the size and structure of moieties in CS which impact biological processes, and the mechanisms by which they exert influence, are not known.

Safety of CS

Meta-analyses of data from clinical studies of commercial CS from natural sources all conclude that no safety issues are associated with CS,³⁵ that CS is well tolerated and adverse reactions are rare. Data also suggest that there is no adverse impact upon glycemic control in diabetics.³⁶ However, as CS is often found along with glucosamine in supplements it is noteworthy that adverse events have been associated with glucosamine in some patients who are also taking warfarin.³⁷

The CS found in nutritional supplements has one or two sulphates per disaccharide unit, however, highly sulphated CS(E) (see Table 1) and CS with four sulphates per disaccharide unit³⁸ has been shown to be capable of activating the contact system and inducing production of anaphylactic mediators, including C3a and C5a.^{39,40} Oversulphated CS has also been used in the drug Arterparon to treat joint pathology.⁴¹ However, association with adverse allergic responses⁴² lead to its withdrawal. Overall, these data highlight the potential for highly sulphated CS to induce unwanted, and potentially pathological, responses.

Naturally occurring CS of a sort found in nutritional supplements has also been shown to be capable of causing complement activation leading to the production of C3a and C5a.⁴³ Importantly, this effect is induced by the intact polymer and not small oligosaccharides, the dominant form taken up following consumption. However, some CS polymer does appear to be taken up³¹ and so biological impacts such as this must not be overlooked as potential side effects.

Other concerns focus upon the possible presence of co-isolated harmful agents. The threat of transmissible spongiform encephalopathies (TSE) has been raised, although no evidence exists that any TSE agents have ever contaminated a CS preparation, and many purification protocols use a protein degrading enzyme, or alkali hydrolysis, to disrupt the source tissue. As a result of increasing demand, and this anxiety, commercial CS is sourced increasingly from porcine,⁶ chicken¹⁸ or shark tissues⁶ rather than bovine, and other sources are being actively examined.^{19,44}

The long-term outcomes of prolonged CS consumption have not been examined and while there are no data sug-

gesting adverse outcomes, it will be important to remain vigilant in this regard. There are an increasing number of biological processes in which CS is implicated⁴⁵; impacting only some of those processes, and not others, remains a significant therapeutic opportunity and a major scientific challenge.

Chondroitin sulphate impacts on biological systems

Data showing a role for CS in fundamental biological processes, including cell division and development of the central nervous system is growing.^{11,16,46} The best described function of CS is a structural role within cartilage, the osmotic swelling it induces resists compression and supports weight bearing.⁴⁷ However, well established tissue-, pathology- and age-related sulphation changes point to more fundamental biochemical roles.^{7,20}

Joint health and osteoarthritis (OA)

Osteoarthritis is one of the most common musculoskeletal conditions globally⁴⁸ and at present there are no pharmaceutical therapies with a disease-modifying outcome. Indeed, a recent trial has shown that a candidate drug which inhibited degradative enzymes linked to OA progression, showed no benefit and had significant adverse effects.⁴⁹

The largest body of research data concerning CS as a therapeutic is concerned with the potential it has to impact joint health, with most studies examining the impact on knee⁵⁰ or hip joints,⁴⁵ although it has also been examined in hand OA.⁵¹ All of the work undertaken so far has been done with people who have OA and explores the impact upon the condition. There have been no studies of, and hence there is no data supporting, a role for CS in preventing OA development in healthy people.

A large body of data exists from *in vivo* studies of CS, however, meta-analyses are united in rejecting many studies because they are poorly designed or poorly performed.⁵² However, robust meta-analysis,^{52–57} well performed studies²¹ and a strong recommendation by EULAR as an intervention for hip OA⁴⁵ and for knee OA⁵⁰ have not presented an unequivocal judgement on the efficacy of CS and the topic remains controversial.

The mode of action of CS in this condition is not clear. *In vitro* studies suggest that CS can impact processes associated with cartilage degeneration; promoting synthesis of proteoglycans which are lost during cartilage degeneration,⁵⁸ inhibiting elastase^{59,60} and cathepsin G activity,⁶⁰ reducing gene expression for a range of proteolytic enzymes⁶¹ and, in combination with glucosamine, reducing subcondral bone resorption.⁶² Other data point to an anti-inflammatory effect. However, these and other studies of CS function, often use intact chains while the predominant form taken up following CS consumption are small oligosaccharides.³¹

Important questions remain to be answered in respect of the impact of CS on joint pathology, including the possibility that a sub-set of OA sufferers will benefit more from the impact of CS than others. Indeed, the need for further work to examine the impacts of CS on low grade OA has been

highlighted.⁵² Other outstanding issues include the possibility that CS from different sources, hence having differing structures, may bring about differing therapeutic outcomes. In addition without adequate quality control and characterisation it will be impossible to discount the possibility that poor quality CS has compromised experimental data. It is noteworthy that recent trials have used test materials which were subject to rigorous quality control.^{21,63}

Other potential therapeutic roles

Anti-inflammatory action. Many pathologies which CS has been suggested to impact are linked by the presence of a pathological inflammatory response. During a clinical trial of a mainly 4-sulphated CS in OA, patients suffering from Psoriasis underwent a significant improvement in their condition⁶⁴ and a mixture of shark and bovine derived CS were reported to have a dramatic positive impact on Colitis.⁶⁵

Inhibition of TNF- α activity reduces Inflammatory Bowel Disease (IBD)⁶⁶ and psoriasis severity.^{67,68} Other data show that CS binds to TNF- α ⁶⁹ and our unpublished data show that CS can inhibit TNF- α activity, although the extent varies with structure. Binding to TNF- α is strongest for CS with a high level of sulphation (CS-E – see Table 1) but small oligosaccharides were able to bind⁶⁹ so those features reported to be taken up following consumption could exert an influence. In addition, data by Cho et al.³⁰ show that a partially depolymerised tracheal CS leads to a reduction in serum TNF- α levels in mice. Taken together these data suggest that CS may impact these pathologies by inhibiting TNF- α activity.

CS may also impact inflammatory processes by inhibition of expression of pro-inflammatory molecules,⁶¹ reducing IL-1 β -induced NF- κ B nuclear translocation⁷⁰ and TNF- α -induced NF- κ B signalling,⁷¹ both important pro-inflammatory events. However other data suggest that it may enhance NF- κ B activation.⁷²

Malaria. CS has a central role in the sequestration of *Plasmodium falciparum* infected red blood cells to the human placenta; and so represents a therapeutic target. The 4-sulphated form of CS was found to be required for this interaction while the presence of 6-sulphates inhibited the interaction,¹⁰ highlighting the importance of establishing structural parameters in CS used in function studies. The size of CS oligosaccharides also has a significant impact on this interaction, an octasaccharide being the minimum required to inhibit the interaction.⁷³

Anti-oxidant. The potential of CS to impact oxidative processes central to the initiation of atherosclerotic plaque development has attracted significant interest. However, a better understanding of the structure/function relationships in this process are required. A mainly 4-sulphated tracheal CS does appear to have a free radical scavenging activity^{74,75} but no data are available to show the impact of oligosaccharide size upon this activity and so the capacity of consumed CS to have an effect after depolymerisation and uptake remains unclear.

Allergic response. A range of studies, using shark derived material, has highlighted the inhibition of antigen-induced IgE production in mice by CS,^{76–78} suggesting that shark CS may reduce allergic response and have an anti-allergic

effect. However, as described above other data suggest that tracheal CS may lead to the generation of anaphylactic mediators⁴³ and highly sulphated CS is now strongly associated with pathological responses.⁴⁰

Urinary pathology. Preliminary trials of CS taken orally, or instilled, show improvements in symptoms of interstitial cystitis (IC).^{79–81} Exogenous CS has been shown to localise to areas of bladder damage in a mouse model.⁸² It may coat areas of bladder damage in IC sufferers while in others it is likely to be excreted normally without significantly interacting with the bladder surface. Other similar molecules, such as pentosan polysulphate, hyaluronan and heparin, which like CS, are negatively charged, have similar impacts upon IC, and so variations in CS structure may not impact its therapeutic potential in this condition.

CS is also reported to inhibit urinary stone formation⁸³ and urinary CS composition differs between stone formers and non-formers.⁸⁴ There are a range of ways in which CS could impact urinary stone formation including inhibition of mineral nucleation, or, increasing mineral binding sites so preventing one dominant site forming and easing excretion of partially mineralised forms.

The future

The role of CS as a therapeutic remains the topic of debate, mainly focussed upon roles it may have in joint health. However, data now show a wide range of pathologies and processes in which CS may have an impact. The variation of CS structure with source is well established, and studies examining CS function need to reflect upon the CS structure employed.

In reflecting upon the therapeutic potential of CS it is important to consider the size of oligosaccharide required to have an effect, and relate that to data which suggest that the majority of CS taken up following consumption is not polymeric material but smaller oligosaccharides.³¹ In addition, the impact of highly sulphated CS in human systems differs dramatically from that in the rat or rabbit⁴³ and so studies using animal models will need careful interpretation.

The range of processes in which CS may be involved and the details of these remain, in most cases, to be fully elucidated and further studies in these areas are required. Although we are acquiring a better understanding of the structure/function relationships of CS, more data are required to clarify the roles CS has, and, to establish the features which bring these about. The prospect of using CS motifs with impacts upon a single pathology, or biological process, is a significant scientific and clinical challenge.

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