

Osteoarthritis and Cartilage



Antioxidant, antiinflammatory and neuroprotective actions of chondroitin sulfate and proteoglycans

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SUMMARY

The antiinflammatory and antiapoptotic effects of chondroitin sulfate (CS) are being used to treat osteoarthritis. Recent evidence has revealed that those peripheral effects of CS may also have therapeutic interest in diseases of the central nervous system (CNS). We review here such evidence. Perineuronal nets (PNNs) formed by chondroitin sulfate proteoglycans (CSPGs) may have a neuroprotective action against oxidative stress potentially involved in neurodegeneration. On the other hand, in human neuroblastoma SH-SY5Y cells CS has antioxidant and neuroprotective effects by activating the signaling pathway PKC/PI3K/Akt and inducing the antioxidant enzyme hemoxygenase-1. Consistent with this is the observation that protein kinase C (PKC) blockade overcomes inhibition of neurite outgrowth elicited by CSPGs. In addition, CS protects cortical neurons against excitotoxic death by phosphorylation of intracellular signals and the suppression of caspase-3 activation. Of interest is the finding that a disaccharide derived from CSPG degradation (CSGP-DS) protects neurons against toxicity both *in vitro* and *in vivo*. Furthermore, CSGP-DS efficiently protects against neuronal loss in experimental autoimmune encephalomyelitis and uveitis, decreases secretion of tumor necrosis factor- α (TNF- α) and block necrosis factor kappa B (NF- κ B) translocation. In conclusion, CS may have neuroprotective properties linked to its antioxidant and antiinflammatory effects.

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Introduction

Chondroitin sulfate (CS) is a natural glycosaminoglycan (GAG) present in the extracellular matrix (ECM) surrounding cells, especially in the cartilage, skin, blood vessels, ligaments, tendons and brain, where it constitutes an essential component of proteoglycans (PGs)¹. Multiple controlled clinical trials in patients with osteoarthritis (OA) have reported clinical benefits of CS (800–1200 mg) to reduce pain, joint swelling and effusion and improve joint function with an excellent safety profile^{2–4}. The majority of the clinical efficacy literature describes the results of studies in which the objective was to determine the efficacy of CS in the symptomatic treatment of knee OA through placebo-controlled studies, and also through comparator studies in which CS treatment has been directly compared with diclofenac sodium⁵ or celecoxib⁶. Several studies have also been reported that

assessed efficacy in finger/hand OA⁷ and long-term studies provided evidence for a disease-modifying effect of CS^{8,9}. Accordingly, CS has been classified as a symptomatic slow acting drug in osteoarthritis (SYSADOA) and a structure/disease-modifying anti-osteoarthritis drug (S/DMAOD)¹⁰. Likewise, the OsteoArthritis Research Society International (OARSI) and the European League Against Rheumatism (EULAR) support the usefulness of CS as a SYSADOA for the management of knee OA in their last recommendation guidelines^{11,12}.

However, the symptomatic effectiveness of CS in the osteoarthritic joint is still controversial and CS studies have been sometimes criticized for small sample sizes or short length of therapy. It is noteworthy that OA is a chronic disease which often develops slowly, so that it can take from years to decades to develop from early OA, where only metabolic events can be detected, to clinical OA, where symptomatic events are manifest. Even though good quality randomized clinical trials have been performed with CS for periods ranging from a couple of months to 2 years, this time interval is still short to study a chronic disease. The combination of this factor and the fact that CS presents a slow but gradual decrease of the clinical symptoms of OA patients could explain some of the weaknesses observed in the clinical trials carried out with this drug.

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On the other hand, in a meta-analysis recently published¹³ it was concluded that the symptomatic benefit of CS is minimal. However, the authors acknowledge that the 20 trials included showed a high degree of heterogeneity, which obviously imposes serious uncertainties on the adequacy of the conclusions drawn in the study¹⁴. However, the rest of meta-analyses performed with CS have concluded that it is effective in treating the symptoms of OA with possible disease-modifying effects. Combined with a strong safety profile and a carry-over effect, such conclusions have created support for the use of CS in the treatment of OA^{15–17}. The beneficial effects of CS described above are likely due to its chondroprotective effects linked to reduction of chondrocyte apoptosis, decrease of synthesis and/or activity of ECM metalloproteases and augmentation of the synthesis of articular cartilage PGs as seen in several *in vitro* and *in vivo* studies with CS. Furthermore, CS exhibits well documented immunomodulatory effects such as reduction of NF- κ B nuclear translocation, decrease in the production of proinflammatory cytokines Interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), and diminution of the expression and activity of nitric oxide synthase-2 (NOS-2) and cyclooxygenase-2 (COX-2)¹⁴. More recent studies show that these properties of CS may also have therapeutic applications in diseases of the central nervous system (CNS).

Neurodegenerative diseases and stroke have pathogenic mechanisms related to oxidative stress, inflammation, apoptosis and neuronal loss. Thus, therapeutic strategies based in immunomodulatory and antiinflammatory drugs are being studied¹⁸. One of such drugs is CS that exhibits antioxidant, immunomodulatory and neuroprotective effects in neuronal tissues. Furthermore, there is increasing interest in clarifying the role of chondroitin sulfate proteoglycans (CSPGs) in regeneration and plasticity of the CNS¹⁹. Hence, we will review next the evidence supporting a role for CS and CSPGs in neurological repair and neuroprotective mechanisms that could inspire development of novel medicines with potential therapeutic impact in neurodegenerative diseases, stroke and CNS trauma.

CSPGs in the CNS: role on brain plasticity and repair

CSPGs are the most abundant types of PGs in the mammalian CNS. They mainly act as barrier molecules that affect cell migration, axon regeneration and brain plasticity, particularly through their GAG chains. Actions of GAG chains have been explored with (1) chondroitinase, that digests GAGs to form disaccharides^{20,21} and (2) GAG synthase inhibitors such as sodium chlorate and β -D-xylosides²². On the basis of these studies, it seems that removing CS-GAG chains, possibly combined with treatments that enhance the intrinsic regenerative or plastic capabilities of adult CNS neurons, may be of considerable promise as a therapeutic strategy to augment CNS repair after injury²³.

Antioxidant and neuroprotective actions of CS

Specialized forms of the ECM are perineuronal nets (PNNs), first described by Ramón y Cajal and Golgi as reticular structures covering cell bodies and proximal dendrites in subpopulations of neurons. They are formed by aggregating CSPGs whose GAGs form highly negative charged structures that can contribute to reduce local oxidative stress by scavenging and binding redox-active iron, thus providing neuroprotection to net-associated neurons. These neurons have been found to be less frequently affected by lipofuscin accumulation than neurons without a net both in normal-aged and Alzheimer's disease (AD) human brains. As lipofuscin is an intralysosomal pigment composed of cross-linked proteins and lipids generated by iron-catalysed oxidative processes, the above results suggest a neuroprotective function of PNNs against oxidative stress, potentially involved in neurodegeneration²⁴.

We have recently reported evidence proving that CS has antioxidant and neuroprotective actions in human neuroblastoma SH-SY5Y cells²⁵. In these studies, CS used was highly purified chondroitins 4 and 6 sulfate of bovine origin in a concentration not less than 98% with an average molecular weight of ≈ 15 –16 kD, an intrinsic viscosity of ≈ 0.02 – 0.06 m³/kg and a ratio between the sulfated groups located in positions 4 and 6 on *N*-acetyl-D-galactosamine of 2. To produce neuroprotective and antiapoptotic effects, CS was incubated for 24 h before adding the free radical producing agents (H₂O₂ or combined oligomycin plus rotenone). CS drastically reduced the generation of free radicals produced by H₂O₂ or combined oligomycin plus rotenone. Furthermore, CS augmented the phosphorylation of Akt and heme oxygenase-1 (HO-1), suggesting that this signaling pathway was involved in its neuroprotective effects. In fact, CS augmented Akt phosphorylation, an effect that was prevented by chelerythrine, a protein kinase C (PKC) inhibitor. Consistent with this idea is the observation that chelerythrine and LY294002, a PI3K/Akt inhibitor, prevented the neuroprotective effect of CS. On the basis of these results, we suggested that CS could protect SH-SY5Y cells under oxidative stress conditions by activating PKC, which phosphorylates Akt that *via* the PI3K/Akt pathway, induces the synthesis of the antioxidant protein HO-1 (Fig. 1). Consistent with this signaling pathway is the observation that PKC blockade overcomes the effects of CSPGs, i.e., the inhibition of neurite outgrowth²⁶.

Still more recently, it was shown that CS elicits neuroprotective effects in an *in vitro* model of calcium-dependent excitotoxicity. Thus preincubation of rat cortical neurons with a highly sulfated CS (CS-E) reduced death induced by *N*-methyl-D-aspartate (NMDA), (*S*)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or kainate. Other less sulfated CS preparations or highly sulfated polysaccharides such as heparin and dextran sulfate had no neuroprotective effects. The neuroprotective effects of CS-E seems to be related with phosphorylation of intracellular signals and the suppression of caspase-3 activation²⁷. It is noteworthy that in this study, CS had to be preincubated during 24 h before adding the neurotoxic agent in order to exert its neuroprotective action. This

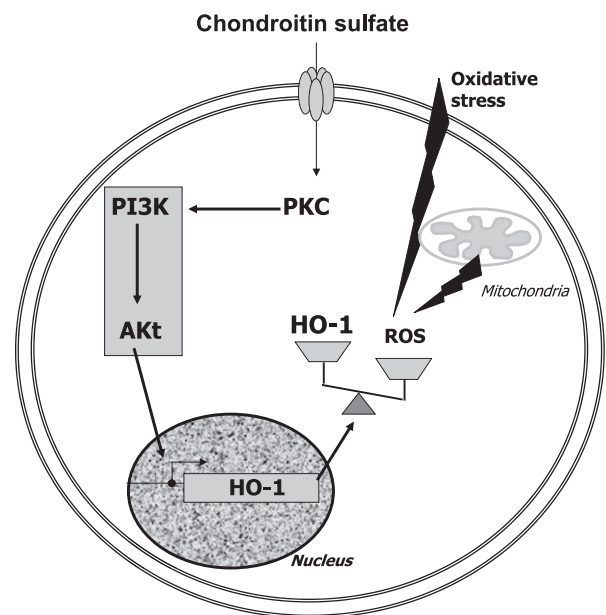


Fig. 1. Proposed mechanism for the antioxidant and neuroprotecting action of CS. CS activates PKC that will increase the phosphorylation of phosphoinositide 3-kinase (PI3K) and activates the Akt signaling pathway which will induce the synthesis of the antioxidant HO-1. Stress will increase the production of reactive oxygen species (ROS) that may be neutralized by HO-1.

agrees with the report of Canas *et al.*²⁵, who showed that CS also requires 24 h preincubation to exert its neuroprotective effects against oxidative stress. We might speculate that this time is required to induce antioxidant enzymes that will protect neurons against oxidative stress. It could be interesting to explore whether a common signaling pathway mediates the neuroprotective actions in rat cortical neurons stressed with calcium-dependent excitotoxicity²⁷ and human neuroblastoma cells subjected to oxidative stress²⁵. In this latter study, it was reported that CS did not exert neuroprotection in a calcium-overload model of cell death.

CS effects in neuroinflammation

Inflammation has been actively related with the onset of several neurodegenerative disorders, including AD. A current hypothesis considers that an extracellular insult to neurons could trigger the production of cytokines such as TNF- α , IL-1 β and IL-6 that could affect normal neuronal activity. For instance, TNF- α is produced by activated microglia²⁸ mainly in response to amyloid beta (A β ₁₋₄₀ and A β ₁₋₄₂), oxidative stress²⁹, glutamate³⁰, and lipopolysaccharide (LPS)³¹. These effects have been linked to AD pathogenesis.

On the other hand, IL-6 production occurs in activated glia such as astrocytes and microglial cells³². This cytokine has been implicated in the pathogenesis of AD with acute or chronic inflammatory components³³, Parkinson's disease, multiple sclerosis and HIV encephalopathy³⁴. An interesting recent finding reveals that peripheral LPS injection in IL-6 knockout mice is refractory to develop involvement of IL-6 in impairment of working memory providing an additional support to the AD³⁵. The possible role of other inflammatory mediators in AD and the controversy surrounding them have been recently reviewed³⁶.

A disaccharide degradation product of CSGP (CSGP-DS) modulates inflammatory responses. CSGP-DS has been generated *ex vivo* by degrading CSPG by using chondroitinase ABC; this degradation is likely to produce large amounts of Di-6S on the galactosamine unit. In addition, other DS molecules, including Di-0S are expected to be produced as a result of degradation^{37,38}. This is particularly interesting because of the small size of this disaccharide consisting of 4-deoxy-L-threo-hex-4-enopyronasyluronic acid and N-acetyl-D-galactosamine-6-sulfate. In experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune uveitis (EAU), the dramatic increase in T cells infiltrating the CNS is far in excess of the number needed for regular maintenance. The disaccharide CSGP-DS markedly alleviated the clinical symptoms of EAE and protected against neuronal loss in EAU. The disaccharide decreases the secretion of TNF- α and blocks NF- κ B translocation³⁸. This, together with the previous report showing that the disaccharide protects neurons against toxicity both *in vitro* and *in vivo*³⁷, suggests that disaccharides derived from CS may have therapeutic potential for the modulation of the local immune response in general, and to overcome inflammation associated with neurodegenerative diseases in particular.

Perspective and therapeutic potential

Because CSPGs act as a barrier that impairs axonal growth and brain repair after brain injury, stroke or neurodegenerative diseases, it seems clear that removal promotes plasticity, providing potential treatments for those CNS disorders. However, although short term degradation of PNNs with for instance chondroitinase ABC may promote plasticity³⁹, it is uncertain whether PNNs absence for long periods may damage neurons. An interesting point is the idea that a therapeutic window for CSPG degradation may exist i.e., during the acute phase of spinal cord injury, CSPG degradation may promote repair and plasticity; at later stages this intervention will have opposite effects⁴⁰. This hypothesis should be

more widely tested. In the light that chondroitinase ABC is being expected to become a useful therapy for CNS injury, the time window for its application has to be considered carefully.

The pharmacological modulation of the PKC/PI3K/Akt pathway may also be a good strategy for neuroprotection with for instance CS²⁵. Induction of the HO-1 by CS will reinforce the antioxidant capability of vulnerable neurons. The large molecular weight of CS does not limit its access to the brain when systemically administered; indeed, following oral and intravenous administration of ¹³¹I labeled CS to rats, it was demonstrated that CS and disaccharides are found in the brain but at concentrations lower than in blood. Supporting these reports, it has been found that systemic administration of the Δ di-6S disaccharide of CS to mice does elicit directly or indirectly an effect in the CNS³⁸. The observation that a small disaccharide derived from CSPG degradation has potent anti-neuroinflammatory and neuroprotective actions in EAE and EAU^{37,38} should stimulate the use of such small molecules to explore their potential neuroprotective effects both *in vitro* and *in vivo* models of neuronal death.

Conflict of interest

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