Osteoarthritis and Cartilage 18 (2010) S24–S27

Osteoarthritis and Cartilage

Antioxidant, antiinflammatory and neuroprotective actions of chondroitin sulfate and proteoglycans

J. Egea †‡, A.G. García †‡§*, J. Verges ‖, E. Montell ‖, M.G. López †‡

† Instituto Teófilo Hernando, Facultad de Medicina, Universidad Autónoma de Madrid, Spain
‡ Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Spain
§ Servicio de Farmacología Clínica, Hospital Universitario la Princesa, Universidad Autónoma de Madrid, Spain
‖ Clinical Research Unit, Scientific Medical Department, Bioibérica SA, Barcelona, Spain

ARTICLE INFO

Article history:
Received 28 October 2009
Accepted 8 January 2010

Keywords:
Antiinflammatory
Antiapoptotic effects
Chondroitin sulfate

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 excytotoxic 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-κB) translocation. In conclusion, CS may have neuroprotective properties linked to its antioxidant and antiinflammatory effects.

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 sodium[5] or celecoxib[6]. Several studies have also been reported that assessed efficacy in finger/hand OA[7] 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/DMOAD)[10]. Likewise, the OsteoArthritis Research Society International (OARSI) and the European League Against Rheumatism (EULAR) support the usefulness of CS as an 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.

* Address correspondence and reprint requests to: Antonio G. García, Instituto Teófilo Hernando, Facultad de Medicina, Universidad Autónoma de Madrid, C/ Arzobispo Morcillo, 4, 28029 Madrid, Spain. Tel/Fax: 34-914973120.
E-mail address: agg@uam.es (A.G. García).

1063-4584/$ – see front matter © 2010 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.joca.2010.01.016

On the other hand, in a meta-analysis recently published[13] 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 study[14]. 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 condroprotective 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-κB nuclear translocation, decrease in the production of proinflammatory cytokines Interlekin-1 β (IL-1β) 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 studied[18]. 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 CNS[19]. 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-xylosides[22]. 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 injury[23].

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 neurodegeneration[24].

We have recently reported evidence proving that CS has antioxidant and neuroprotective actions in human neuroblastoma SH-SY5Y cells[25]. 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 m^3/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 (H_2O_2 or combined oligomycin plus rotenone). CS drastically reduced the generation of free radicals produced by H_2O_2 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 outgrowth[26].

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 activation[27]. 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

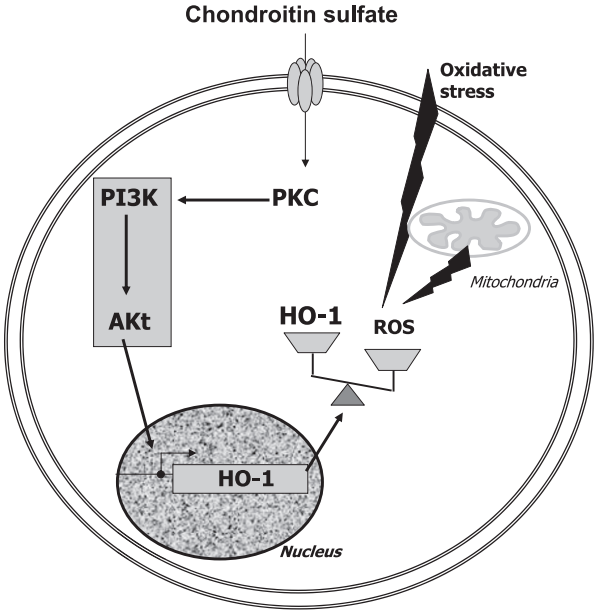

Fig. 1. Proposed mechanism for the antioxidant and neuroprotecting action of CS. CS activates PKC that will increase the phosphorylation of phosphoinositide 3-kinase (PI3K) and activates the Akt signaling pathway which will induce the synthesis of the antioxidant HO-1. Stress will increase the production 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 excitotoxicity[27] and human neuroblastoma cells subjected to oxidative stress[25]. 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-1β and IL-6 that could affect normal neuronal activity. For instance, TNF-α is produced by activated microglia[28] mainly in response to amyloid beta ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$), oxidative stress[29], glutamate[30], 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 cells[32]. This cytokine has been implicated in the pathogenesis of AD with acute or chronic inflammatory components[33], Parkinson's disease, multiple sclerosis and HIV encephalopathy[34]. 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 AD[35]. The possible role of other inflammatory mediators in AD and the controversy surrounding them have been recently reviewed[36].

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 EUA. The disaccharide decreases the secretion of TNF-α and blocks NF-κB translocation[38]. This, together with the previous report showing that the disaccharide protects neurons against toxicity both *in vitro* an *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 plasticity[39], 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 effects[40]. 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 CS[25]. 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 ^{131}I 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 Δdi-6S disaccharide of CS to mice does elicit directly or indirectly an effect in the CNS[38]. 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 *in vitro* and *in vivo* models of neuronal death.

Conflict of interest

(1) Funding for any research that has been done resulting in work described in manuscript, in particular funding from BioIberica: **Yes**.

(2) Honorarium, lecture fee, travel support or any other form of compensation received from BioIberica, for writing this manuscript, participating in symposium, or otherwise: **Yes**.

No funds were provided for writing this manuscript.

Acknowledgments

The work of the authors is being supported by "Cátedra Bioibérica/UAM de Inflamación Crónica y Citoprotección" (CABICYC) Spain, the Spanish Ministry of Science and Innovation Ref. SAF2009-12150 to MGL, Ref. SAF2006-03589 to AGG, the Spanish Ministry of Health (Instituto de Salud Carlos III) RETICS-RD06/0026, Comunidad Autónoma de Madrid, Spain Ref. SAL2006/0275, Fundación CIEN, IS Carlos III No. 85016/09, MICINN, No. P1016/09 and Agencia Lain Entralgo, CN no. NDE07/09, Comunidad de Madrid, Spain to AGG. We also thank the continued support of "Fundación Teófilo Hernando", Spain.

References

1. Hardingham TE, Muir H. Biosynthesis of proteoglycans in cartilage slices. Fractionation by gel chromatography and equilibrium density-gradient centrifugation. Biochem J 1972;126:791–803.
2. Bucsi L, Poor G. Efficacy and tolerability of oral chondroitin sulfate as a symptomatic slow-acting drug for osteoarthritis (SYSADOA) in the treatment of knee osteoarthritis. Osteoarthritis Cartilage 1998;6(Suppl A):31–6.
3. Uebelhart D, Thonar EJ, Delmas PD, Chantraine A, Vignon E. Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study. Osteoarthritis Cartilage 1998;6 (Suppl A):39–46.
4. Jackson CG, Plaas AH, Sandy JD, Hua C, Kim-Rolands S, Barnhill JG, *et al*. The human pharmacokinetics of oral ingestion of glucosamine and chondroitin sulfate taken separately or in combination. Osteoarthritis Cartilage 2010;18:297–302.
5. Morreale P, Manopulo R, Galati M, Boccanera L, Saponati G, Bocchi L. Comparison of the antiinflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. J Rheumatol 1996;23:1385–91.
6. Clegg DO, Reda DJ, Harris CL, Klein MA, O'Dell JR, Hooper MM, *et al*. Glucosamine, chondroitin sulfate, and the two in

combination for painful knee osteoarthritis. N Engl J Med 2006;354:795–808.
7. Verbruggen G, Goemaere S, Veys EM. Systems to assess the progression of finger joint osteoarthritis and the effects of disease modifying osteoarthritis drugs. Clin Rheumatol 2002;21:231–43.
8. Uebelhart D, Malaise M, Marcolongo R, de Vathaire F, Piperno M, Mailleux E, *et al*. Intermittent treatment of knee osteoarthritis with oral chondroitin sulfate: a one-year, randomized, double-blind, multicenter study versus placebo. Osteoarthritis Cartilage 2004;12:269–76.
9. Kahan A, Uebelhart D, De Vathaire F, Delmas PD, Reginster JY. Long-term effects of chondroitins 4 and 6 sulfate on knee osteoarthritis: the study on osteoarthritis progression prevention, a two-year, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2009;60:524–33.
10. Lequesne M. Symptomatic slow-action anti-arthritic agents: a new therapeutic concept? Rev Rhum Ed Fr 1994;61:75–9.
11. Jordan KM, Arden NK, Doherty M, Bannwarth B, Bijlsma JW, Dieppe P, *et al*. EULAR Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). Ann Rheum Dis 2003;62:1145–55.
12. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, *et al*. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008;16:137–62.
13. Reichenbach S, Sterchi R, Scherer M, Trelle S, Burgi E, Burgi U, *et al*. Meta-analysis: chondroitin for osteoarthritis of the knee or hip. Ann Intern Med 2007;146:580–90.
14. Monfort J, Martel-Pelletier J, Pelletier JP. Chondroitin sulphate for symptomatic osteoarthritis: critical appraisal of meta-analyses. Curr Med Res Opin 2008;24:1303–8.
15. McAlindon TE, LaValley MP, Felson DT. Efficacy of glucosamine and chondroitin for treatment of osteoarthritis. JAMA 2000;284:1241.
16. Richy F, Bruyere O, Ethgen O, Cucherat M, Henrotin Y, Reginster JY. Structural and symptomatic efficacy of glucosamine and chondroitin in knee osteoarthritis: a comprehensive meta-analysis. Arch Intern Med 2003;163:1514–22.
17. Hochberg MC, Clegg DO. Potential effects of chondroitin sulfate on joint swelling: a GAIT report. Osteoarthritis Cartilage 2008;16(Suppl 3):S22–4.
18. Townsend KP, Pratico D. Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs. FASEB J 2005;19:1592–601.
19. Galtrey CM, Fawcett JW. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res Rev 2007;54:1–18.
20. Moon LD, Asher RA, Rhodes KE, Fawcett JW. Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat Neurosci 2001;4:465–6.
21. Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, *et al*. Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 2002;416:636–40.
22. Grimpe B, Silver J. A novel DNA enzyme reduces glycosaminoglycan chains in the glial scar and allows microtransplanted dorsal root ganglia axons to regenerate beyond lesions in the spinal cord. J Neurosci 2004;24:1393–7.
23. Carulli D, Laabs T, Geller HM, Fawcett JW. Chondroitin sulfate proteoglycans in neural development and regeneration. Curr Opin Neurobiol 2005;15:116–20.
24. Morawski M, Bruckner MK, Riederer P, Bruckner G, Arendt T. Perineuronal nets potentially protect against oxidative stress. Exp Neurol 2004;188:309–15.
25. Canas N, Valero T, Villarroya M, Montell E, Verges J, Garcia AG, *et al*. Chondroitin sulfate protects SH-SY5Y cells from oxidative stress by inducing heme oxygenase-1 via phosphatidylinositol 3-kinase/Akt. J Pharmacol Exp Ther 2007;323:946–53.
26. Sivasankaran R, Pei J, Wang KC, Zhang YP, Shields CB, Xu XM, *et al*. PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. Nat Neurosci 2004;7:261–8.
27. Sato Y, Nakanishi K, Tokita Y, Kakizawa H, Ida M, Maeda H, *et al*. A highly sulfated chondroitin sulfate preparation, CS-E, prevents excitatory amino acid-induced neuronal cell death. J Neurochem 2008;104:1565–76.
28. Huang H, Lung HL, Leung KN, Tsang D. Selective induction of tumor necrosis factor receptor type II gene expression by tumor necrosis factor-alpha in C6 glioma cells. Life Sci 1998;62:889–96.
29. Nelson TJ, Alkon DL. Oxidation of cholesterol by amyloid precursor protein and beta-amyloid peptide. J Biol Chem 2005;280:7377–87.
30. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, *et al*. Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. J Biol Chem 2006;281:21362–8.
31. Culbert AA, Skaper SD, Howlett DR, Evans NA, Facci L, Soden PE, *et al*. MAPK-activated protein kinase 2 deficiency in microglia inhibits pro-inflammatory mediator release and resultant neurotoxicity. Relevance to neuroinflammation in a transgenic mouse model of Alzheimer disease. J Biol Chem 2006;281:23658–67.
32. Akama KT, Van Eldik LJ. Beta-amyloid stimulation of inducible nitric-oxide synthase in astrocytes is interleukin-1beta- and tumor necrosis factor-alpha (TNFalpha)-dependent, and involves a TNFalpha receptor-associated factor- and NFkappaB-inducing kinase-dependent signaling mechanism. J Biol Chem 2000;275:7918–24.
33. Bauer J, Strauss S, Volk B, Berger M. IL-6-mediated events in Alzheimer's disease pathology. Immunol Today 1991;12:422.
34. Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. Faseb J 2001;15:43–58.
35. Sparkman NL, Buchanan JB, Heyen JR, Chen J, Beverly JL, Johnson RW. Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers. J Neurosci 2006;26:10709–16.
36. Rojo LE, Fernandez JA, Maccioni AA, Jimenez JM, Maccioni RB. Neuroinflammation: implications for the pathogenesis and molecular diagnosis of Alzheimer's disease. Arch Med Res 2008;39:1–16.
37. Rolls A, Avidan H, Cahalon L, Schori H, Bakalash S, Litvak V, *et al*. A disaccharide derived from chondroitin sulphate proteoglycan promotes central nervous system repair in rats and mice. Eur J Neurosci 2004;20:1973–83.
38. Rolls A, Cahalon L, Bakalash S, Avidan H, Lider O, Schwartz M. A sulfated disaccharide derived from chondroitin sulfate proteoglycan protects against inflammation-associated neurodegeneration. Faseb J 2006;20:547–9.
39. Crespo D, Asher RA, Lin R, Rhodes KE, Fawcett JW. How does chondroitinase promote functional recovery in the damaged CNS? Exp Neurol 2007;206:159–71.
40. Rolls A, Shechter R, London A, Segev Y, Jacob-Hirsch J, Amariglio N, *et al*. Two faces of chondroitin sulfate proteoglycan in spinal cord repair: a role in microglia/macrophage activation. PLoS Med 2008;5:e171.