

# Osteoarthritis and Cartilage



## Modulation of inflammation by chondroitin sulfate

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### SUMMARY

**Objective and methods:** To evaluate the immune-modulator effect of chondroitin sulfate (CS) by means of the review of the literature.

**Results:** Inflammatory reactions are primarily originated by infectious agents, immune reactions and by sterile tissue lesions that activate membrane receptors by means of pathogen-associated molecular patterns, tissue breakdown products and cytokines. The activation of membrane receptors triggers the phosphorylation of mitogen activated protein kinases and of the nuclear factor  $\kappa$ B (NF- $\kappa$ B). The binding of NF- $\kappa$ B to the promoter of target genes enhances the expression of pro-inflammatory cytokines, inducible nitric oxide synthase, cyclooxygenase 2, phospholipase A2, and matrix metalloproteases, proteins that contribute to tissue damage and to the inflammatory reaction. The activation of NF- $\kappa$ B has a key role in the immune homeostasis and the inflammatory response and therefore, in the pathogenesis of numerous diseases. Chondroitin sulfate (CS) is able to diminish NF- $\kappa$ B activation and nuclear translocation in chondrocytes and synovial membrane, effects that may explain the benefits of CS in osteoarthritis. In addition, systemic CS reduces NF- $\kappa$ B nuclear translocation in macrophages and hepatocytes, raising the hypothesis that CS might be of benefit to treat other diseases with a strong inflammatory component. There is preliminary evidence in humans that CS improves moderate to severe psoriasis. Moreover, experimental and clinical data suggest that CS might be a useful therapeutic agent in diseases such as inflammatory bowel diseases, atherosclerosis, Parkinson's and Alzheimer's diseases, multiple sclerosis, amyotrophic lateral sclerosis, rheumatoid arthritis and systemic lupus erythematosus.

**Discussion:** These results urge for double blinded placebo-controlled trials to confirm the utility of CS in diseases with immune and inflammatory components.

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### Inflammation

The deployment of defensive responses of the organism caused by infectious pathogens or by sterile tissue damage characterizes any inflammatory reaction (Fig. 1). Infections caused by bacteria, protozoa, fungi, and viruses activate the pattern-recognition receptors (PRRs) in immune cells by means of cell-membrane molecules and/or of lipopolysaccharides (LPS). The most frequently activated membrane PRRs are toll-like receptors 1–13 (TLR1–13), scavenger receptors such as CD36, and complement receptors. Pathogen-associated molecular patterns (PAMPs) and LPS can also activate a cytoplasmic PRR, the nucleotide-oligomerization domain (NOD-like receptor – NLR)<sup>1–3</sup>.

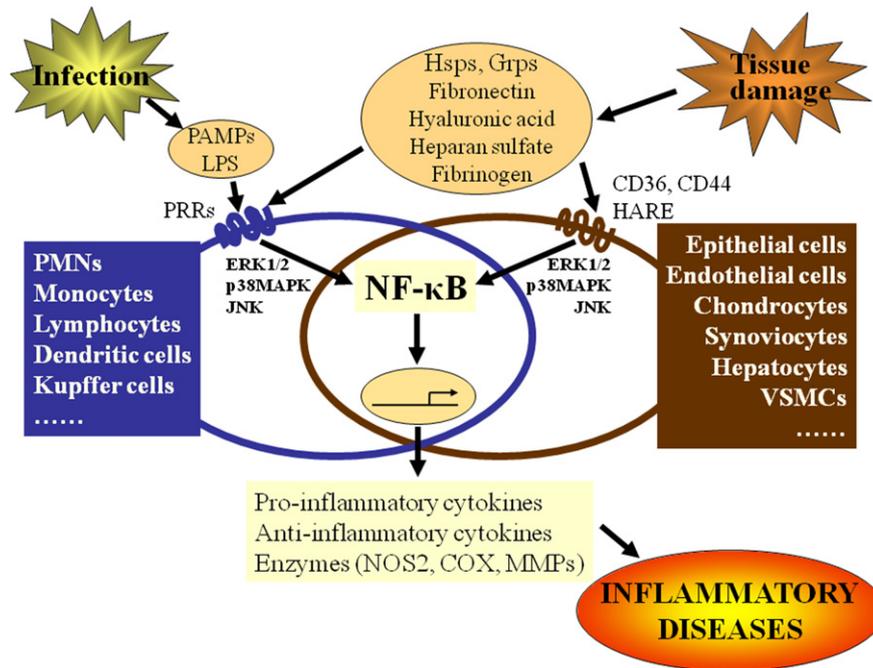
Following a sterile tissue lesion, heat shock proteins 60 and 70 (Hsp60, Hsp70), glucose regulated proteins (Grps), fibrinogen,

surfactant protein A, and breakdown products of tissue matrix (fibronectin, hyaluronic acid, and heparan sulfate) are released in the nearby interstitial space. These endogenous compounds will activate PRRs but may also activate other receptors in immune and other tissue cells.<sup>4</sup> For instance, fibronectin fragments bind to  $\alpha$ 5 $\beta$ 1-integrin and CD44, hyaluronic acid fragments stimulate CD44 and TLRs, and heparan sulfate proteoglycans and fibrinogen activate TLRs<sup>5,6</sup>. It is noteworthy that Hsps may penetrate into the bloodstream and through cell-surface receptors, such as TLRs and scavenger receptor CD36, produce an effect at distant sites in the body<sup>7</sup>.

In immune cells and cells in any type of tissue, the activation of PRRs,  $\alpha$ 5 $\beta$ 1-integrin, and CD44 by PAMPs, LPS, Hsps, and endogenous breakdown products will lead to the phosphorylation and activation of extracellular signal-regulated kinase 1/2 (ERK1/2), p38 mitogen activated protein kinase (p38MAPK), and c-Jun NH2-terminal kinase (JNK)<sup>8,9</sup>. These kinases phosphorylate the I $\kappa$ B kinase (IKK) which will phosphorylate and inactivate the inhibitor  $\kappa$ B $\alpha$  (I $\kappa$ B $\alpha$ ) that is forming a complex with the p65/p50 heterodimer or nuclear factor- $\kappa$ B (NF- $\kappa$ B) in the cytosol; as a consequence of I $\kappa$ B $\alpha$  inactivation, NF- $\kappa$ B is released and initiates the translocation to the

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**Fig. 1.** In immune cells, bacteria, protozoa, fungi and viruses activate the PRRs by means of PAMPs and/or LPS, and as a consequence, activate the ERK1/2, p38MAPK, and JNK. These kinases stimulate the nuclear translocation of the p65/p50 heterodimer or NF- $\kappa$ B and binding to target gene, followed by the increase of expression of pro- and anti-inflammatory cytokines, NOS2, COX-2, PLA2 and MMPs, factors that will perpetrate the inflammatory reaction and the inflammatory disease. On the other hand, a sterile tissue lesion will release Hsps, Grps, fibrinogen, surfactant protein A and breakdown products of tissue matrix (fibronectin, hyaluronic acid, and heparan sulfate), compounds that will activate PRRs in immune cells and scavenger receptors in cells of any tissue to further activate the nuclear translocation of NF- $\kappa$ B.

nucleus<sup>10,11</sup>. The NF- $\kappa$ B dimer binds to  $\kappa$ B sites within the promoters/enhancers of hundreds of target genes, in such way that NF- $\kappa$ B regulates the transcription of genes that are involved in inflammation, immunity, apoptosis, cell proliferation and differentiation. As a result of NF- $\kappa$ B binding to target genes, the expression of many proteins increases, such as pro- and anti-inflammatory cytokines, e.g., interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and enzymes, such as inducible nitric oxide synthase (NOS2), cyclooxygenase 2 (COX-2), phospholipase A2 (PLA2), matrix metalloproteases (MMPs), e.g., MMP-3, MMP-9, MMP-13, etc., all factors that will perpetrate the inflammatory reaction<sup>3,12</sup>.

There is evidence that the activation of NF- $\kappa$ B has a causative role in the pathogenesis of numerous diseases, such as inflammatory bowel disease<sup>13</sup>, gastritis<sup>14</sup>, rheumatoid arthritis<sup>15</sup>, psoriasis<sup>16</sup>, atherosclerosis<sup>17</sup>, Alzheimer's disease<sup>18</sup>, Parkinson's disease<sup>19</sup>, asthma, acute respiratory distress syndrome<sup>20</sup>, sepsis and systemic inflammatory response syndrome<sup>21</sup>, cancer<sup>22</sup>, systemic lupus erythematosus<sup>15</sup>, amyotrophic lateral sclerosis, multiple sclerosis<sup>23</sup>, and type I diabetes<sup>18,24</sup>. The key role of NF- $\kappa$ B in inflammatory responses and immune homeostasis explains the interest of compounds targeting any step leading to its nuclear translocation for the treatment of diseases with an inflammatory component.

It is noteworthy that the evolution and prognosis of the above listed diseases, where nuclear translocation of NF- $\kappa$ B is a fundamental event, is associated with blood biomarkers of inflammation, primarily IL-6 and C-reactive protein (CRP). For instance, plasma concentrations of IL-6 and CRP are closely associated to the evolution and severity of inflammatory bowel diseases<sup>25,26</sup>, psoriasis<sup>27</sup>, atherosclerosis and myocardial infarction<sup>28,29</sup>, Parkinson's and Alzheimer's diseases<sup>30,31</sup>, amyotrophic lateral sclerosis<sup>32</sup>, cancer<sup>33,34</sup>, and diabetes<sup>35</sup>. In some instances, such as cancer, its relationship with serum concentrations of biomarkers of inflammation is close enough to formulate the hypothesis that NF- $\kappa$ B system is part of the pathophysiology of cancer formation.

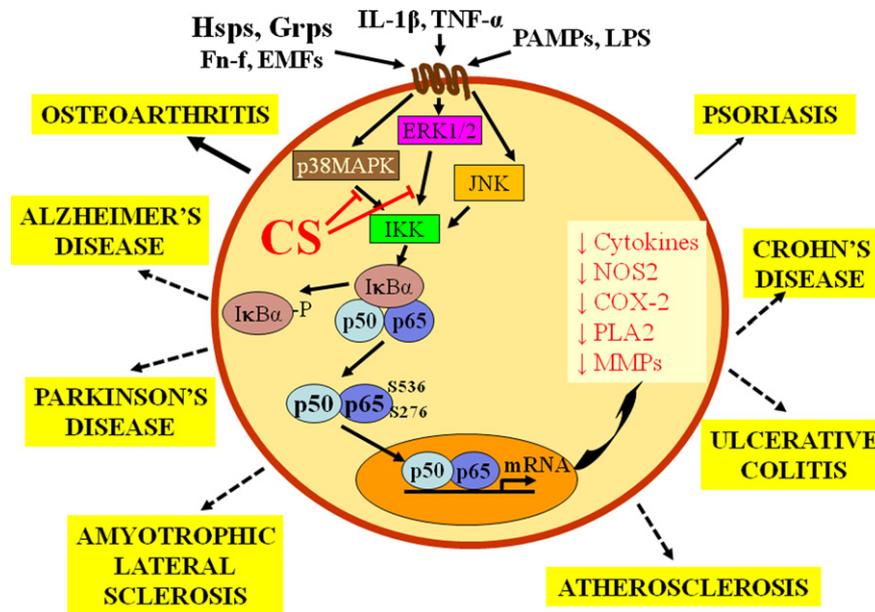
Therefore, it has been proposed that anti-inflammatory agents that suppress NF- $\kappa$ B or NF- $\kappa$ B-regulated products should be potentially helpful in both the prevention and treatment of cancer<sup>36</sup>.

### Modulation of inflammation by chondroitin sulfate

#### Osteoarthritis

Osteoarthritis is characterized by focal areas of loss of joint cartilage, with varying degrees of osteophyte formation, subchondral bone change and synovitis. The pathophysiology of osteoarthritis appears to be associated to multiple microtraumas to the joint cartilage and formation of extracellular matrix fragments (EMFs)<sup>37</sup>. These fragments bind to  $\alpha$ 5 $\beta$ 1 integrin receptor, CD44 and TLRs on the chondrocytes and on the macrophages of the synovial membrane where they activate ERK1/2, p38MAPK and JNK, and subsequently trigger the nuclear translocation of the activator protein-1 (AP-1) and NF- $\kappa$ B which enhance the expression of MMPs, IL-1 $\beta$ , TNF- $\alpha$ , PLA2, COX-2 and NOS2 (Fig. 2)<sup>38</sup>. This chronic inflammatory reaction will be at the origin of the synovitis and the damage and loss of cartilage<sup>39,40</sup>.

*In vitro*, in chondrocytes, IL-1 $\beta$  activates ERK1/2, p38MAPK, and JNK, and induces the nuclear translocation of NF- $\kappa$ B and AP-1<sup>41</sup>. Using the chondrocyte stimulated by IL-1 $\beta$  as experimental model, it has been shown that bovine chondroitin sulfate (CS) diminishes IL-1 $\beta$ -induced NF- $\kappa$ B nuclear translocation. In addition, CS inhibits IL-1 $\beta$ -induced p38MAPK and ERK1/2 phosphorylation.<sup>42</sup> Moreover, the disaccharides  $\Delta$ di-4S and  $\Delta$ di-6S of CS also reduce IL-1 $\beta$ -induced NF- $\kappa$ B nuclear translocation in the chondrocyte<sup>39</sup>. It is unclear how CS elicits its effects. It has been shown that CS is internalized in the cell *via* the hyaluronan receptor for endocytosis (HARE)<sup>43</sup>, and other scavenger receptors such as CD36<sup>44</sup> and CD44<sup>45</sup>. The mechanism by which CS reduces p38MAPK and ERK1/2 phosphorylation and NF- $\kappa$ B nuclear translocation remains unclear but it has been reported that inhibition of hyaluronan binding to CD44 by CS diminishes ERK1/2



**Fig. 2.** CS, by reducing the phosphorylation of ERK1/2 and p38MAPK, diminishes the nuclear translocation of NF-κB triggered by Hsps, Grps, fibronectin (Fn-f) and EMFs, pro-inflammatory cytokines, IL-1β and TNF-α, PAMPs and LPS. As a consequence, CS reduces the expression of pro-inflammatory cytokines, NOS2, COX-2, PLA2 and MMPs, and diminishes the inflammatory reaction. This mechanism of action may explain the effect of CS in diseases with a strong inflammatory component. Solid large arrow indicates solid clinical evidence, solid narrow arrow denotes preliminary clinical evidence, and broken arrow indicates experimental and/or rather preliminary clinical evidence.

phosphorylation<sup>46</sup>. Since the activation of these signaling pathways is redox-sensitive, once internalized by HARE, the antioxidant activity of CS may have contributed to these effects<sup>47</sup>.

There is strong evidence that nuclear translocation of NF-κB is pivotal to the presentation and maintenance of synovitis. Following the *in vitro* transfection of the inhibitory subunit IκBα in synovio-cytes, IL-1β-induced NF-κB nuclear translocation is inhibited, as is the production of IL-6, of chemokines monocyte chemoattractant protein-1 (MCP-1) and RANTES, of p75 soluble TNF-α receptor, of MMP-1, MMP-3, MMP-13, and of the expression of aggrecanase-1 or ADAMTS<sup>48</sup>. On the other hand, in the rat adjuvant arthritis model, specific inhibition of IKK-β-mediated NF-κB activation locally in the inflamed joint, using a NEMO-binding domain (NBD) peptide, reduces synovial inflammation<sup>49</sup>.

CS reduces the signs of synovitis in animal models as well as in humans. In DBA/1J mice with a type II collagen-induced arthritis and treated for 9 weeks with various dosages of CS, the infiltration of inflammatory cells, granulated tissue formation and proliferation of synovial lining cells, as well as swelling, were partially prevented by treatment with 1000 mg/kg/day of CS orally for 63 days<sup>50</sup>. In patients with knee OA, bovine CS diminished the number of patients with signs of synovitis from 90 of 307 at baseline to 38 at the end of 24 weeks of treatment<sup>51</sup>.

Because of the high sulfate and carboxyl group content, CS has strong negative charges, property that allows CS to interact with a wide range of proteins, including receptors, enzymes, cytokines, chemokines, lipoproteins, and adhesion molecules, and confers CS with its antioxidant properties. However, there is no information about the specific role of CS disaccharides, sulfated and non-sulfated, when CS is administered systemically. *In vitro*, CS disaccharides inhibit NF-κB nuclear translocation induced by IL-1β<sup>39</sup>, the Δdi-4,6S being more potent than the Δdi-6S and the Δdi-4S. In the experimental autoimmune encephalomyelitis, Δdi-6S promotes recovery and neuronal survival, effect associated with an inhibition of NF-κB nuclear translocation<sup>52</sup>. These reports suggest that CS disaccharides show pharmacological activity but unfortunately, there is no information about the efficacy and potency of the disaccharides as a function of their content in sulfate.

Based on these reports, we may speculate that the effect of CS on the symptomatology and evolution of osteoarthritis in patients<sup>53–55</sup> is associated to the inhibition of NF-κB nuclear translocation in chondrocytes, synovial macrophages and synovio-cytes. On the other hand, using the combined *in vivo* model of chronic arthritis and atherosclerosis, intraperitoneal bovine CS diminishes NF-κB nuclear translocation in peripheral blood mononuclear cells<sup>56</sup>. Unpublished results from our laboratory show that *in vivo* orally administered CS abrogates the hepatic nuclear translocation of NF-κB induced by an aseptic inflammatory reaction. Altogether, these reports suggest that *in vivo* CS may reduce NF-κB nuclear translocation in many tissues.

Is it noteworthy that as a consequence of the inhibition of NF-κB nuclear translocation by CS, biomarkers of inflammation are reduced *in vivo*. In rabbits with the combined model of chronic arthritis and atherosclerosis, intraperitoneal administration of CS reduces serum concentrations of IL-6 and CRP, and diminishes the production of the chemokine MCP-1 by blood mononuclear cells<sup>56</sup>. The effect of CS on NF-κB and biomarkers of inflammation, CRP and IL-6, further support the hypothesis that CS might be of therapeutic value for diseases associated with an inflammatory reaction.

#### Psoriasis

Nuclear expression of NF-κB is detected in 66% of psoriatic lesions and over-expressed in psoriasis compared with normal skin<sup>57</sup>. Compared with non-lesional psoriatic skin, in lesional psoriatic skin, NF-κB DNA binding to the p53κB site is decreased, whereas NF-κB binding to the proinflammatory IL-8 κB site is increased<sup>58</sup>. In a hospital based case–control study, including 519 patients with psoriasis vulgaris and 541 matched controls who were genotyped for NFκB1 (encodes for p50 protein) polymorphisms, it was observed that NFκB1 wild-type genotype was associated to an increased risk for psoriasis vulgaris; the association was stronger in the subgroups of onset age 40 years, Psoriasis Area and Severity Index (PASI) score around 20, and male patients<sup>59</sup>. Further supporting the role of NF-κB in the incidence/severity of psoriasis, effective treatment with etanercept, a recombinant

human TNF receptor fusion protein, produced a significant down-regulation of phosphorylated NF- $\kappa$ B, reduction that correlated with the improvement of skin histology and clinical outcomes<sup>60</sup>.

In order to assess the efficacy of CS for the treatment of psoriasis, in an open non-controlled trial, 11 patients with knee OA and long-standing, moderate-to-severe psoriasis resistant to conventional therapy, received 800 mg/day of bovine CS for 2 months. The results showed that swelling, redness, flaking and itching of the skin was diminished, and hydration and softening of the skin was enhanced with a reduction of scaling; in addition, epidermal thickness was decreased, the number of keratinocytes were reduced and the severity of psoriasis activity diminished<sup>61</sup>. These results suggest that bovine CS might be a helpful agent to treat selected patients with moderate-to-severe psoriasis.

#### Inflammatory bowel disease

An eventual benefit of CS for the treatment of inflammatory bowel diseases has been explored. Using the murine dextran sulfate sodium-induced colitis (DSS-IC) model, it was shown *in vivo* that oral CS reduces the bloody stools as well as erosions, and impedes the increase of white blood cells more effectively than 5-aminosalicylic acid<sup>62</sup>. On the other hand, with the same DSS-IC model, it was shown that oral glucosamine improved the clinical symptoms and suppressed colonic inflammation and tissue injury; moreover, glucosamine inhibited the activation of intestinal epithelial cells, as demonstrated by a diminished nuclear translocation of NF- $\kappa$ B in the intestinal mucosa<sup>63</sup>. The experimental evidence reviewed support the hypothesis that CS might reduce the incidence and severity of relapse of inflammatory bowel diseases in humans.

#### Atherosclerosis

The incidence and progression of atherosclerosis and subsequent cardiovascular complications, e.g., myocardial infarction and stroke, are closely associated to inflammation. There is strong evidence that NF- $\kappa$ B is a pivotal transcription factor in the heart and cardiovascular system<sup>64</sup>, and that pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  play a crucial role in the disruption of macrovascular and microvascular circulation both *in vivo* and *in vitro*<sup>65</sup>. Moreover, the biomarkers CRP and IL-6 are highly predictive of cardiovascular events<sup>66</sup>. The beneficial effect of acetylsalicylic acid on cardiovascular events has been in part explained by a reduction of NF- $\kappa$ B nuclear translocation<sup>67</sup>. There is experimental<sup>56,68</sup> and clinical data<sup>69,70</sup> suggesting that CS might be of utility to prevent or diminish the incidence of atherosclerosis and its complications. Additional placebo-controlled trials are required to demonstrate the usefulness of CS for the treatment of atherosclerotic diseases.

In conclusion, the activation of NF- $\kappa$ B is pivotal to immune homeostasis and the inflammatory response and therefore, in the pathogenesis of numerous diseases. The benefits of CS in osteoarthritis may be explained by reduction of NF- $\kappa$ B nuclear translocation in chondrocytes and synovial membrane. In addition, systemic CS reduces NF- $\kappa$ B nuclear translocation in macrophages and hepatocytes, raising the hypothesis that CS might be of benefit to treat other diseases with a strong inflammatory component. Supporting such statement, there is evidence in humans that CS improves moderate to severe psoriasis.

#### Conflict of interest

Patrick du Souich reports receiving research grants and lecture fees from Bioibérica S.A. No funds were provided for writing this manuscript.

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