ARD Online First, published on July 20, 2007 as 10.1136/ard.2006.068882

Biochemical basis of the effect of chondroitin sulfate on osteoarthritis articular tissues

Jordi Monfort, MD[1], Jean-Pierre Pelletier, MD[2], Natalia Garcia-Giralt, PhD[1], and Johanne Martel-Pelletier, PhD[2]

[1]Hospital del Mar, Passeig del Mar, Barcelona 08003, Spain; [2]Osteoarthritis Research Unit, University of Montreal Hospital Centre, Notre-Dame Hospital, 1560 rue Sherbrooke Est, Montreal, Quebec H2L 4M1, Canada.

Address correspondence and reprint requests to:

Johanne Martel-Pelletier, PhD, Osteoarthritis Research Unit, University of Montreal Hospital Centre, Notre-Dame Hospital, 1560 rue Sherbrooke Est, Montreal, Quebec, Canada H2L 4M1, Email: jm@martelpelletier.ca

Keywords*:* biochemical factors; chondroitin sulfate; osteoarthritis

ABSTRACT

Osteoarthritis is a chronic disease characterized by irreversible damage to joint structures, including loss of articular cartilage, osteophyte formation, alterations in the subchondral bone and synovial inflammation. Pain, functional disability and impairment of health-related quality of life are major complaints in patients with osteoarthritis. Several compounds have been investigated for their positive effects on the relief of clinical symptoms and improvement of structural changes in osteoarthritis. It has been shown that chondroitin sulfate interferes with the progression of structural changes in joint tissues and is used in the management of patients with osteoarthritis. This review summarizes data from relevant reports describing the mechanisms of action of chondroitin sulfate involved in the beneficial effects of the drug.

INTRODUCTION

Osteoarthritis (OA) is a progressive degenerative joint disease and the most common form of arthritis, especially in older persons. The disease is thought to result from biochemical changes and biomechanical stresses affecting articular tissue structure. The disorder is multifactorial, increases with aging, and has genetic and hormonal influences. Articular tissues, including the cartilage, synovial membrane and subchondral bone, are major sites of changes during the course of the disease.

Exposure of the articular cartilage to localized physical or chemical stresses, including mechanical, inflammatory, and oxidative phenomena may contribute to degradation of cartilage matrix macromolecules [1-6]. From a biochemical perspective, OA may be considered as a breakdown of the extracellular matrix, in which homeostasis depends on the balance between catabolic and anabolic events. At the early stage of the disease process, there is a 'hypermetabolic' response in the cartilage considered to be a reactive feedback response of chondrocytes to proteoglycan depletion in the matrix. This response is believed to be a protective attempt to counteract the effects of environmental stress and agents, and may retard the progression of cartilage degeneration.

Although destruction of the articular cartilage characterizes OA, inflammation of the synovial membrane plays an important role in the progression of joint tissue lesions. However, synovial inflammation is a secondary phenomenon related to multiple factors, such as cartilage matrix degradation, and excess amounts of nitric oxide (°NO) and reactive oxygen species (ROS), rather than the primary cause of the disease. In addition, it has been shown that the subchondral bone is the site of several dynamic morphological changes that are involved in the disease process [7]. These changes are associated with a number of locally abnormal biochemical pathways related to the altered metabolism of osteoblasts [8-10].

Some compounds have been shown to have a slow acting symptomatic effect in OA and were termed 'symptomatic slow-acting drugs for OA (SySADOA)'. Most of the compounds suggested as SySADOA are physiological molecules contained in the articular tissues and include glucosamine sulfate (GS) and chondroitin sulfate (CS). Chondroitin sulfate is a major component of the extracellular matrix of many connective tissues, including cartilage, bone, skin, ligaments, and tendons. It is a sulfated glycosaminoglycan composed of a long unbranched polysaccharide chain with a repeating disaccharide structure of N-acetylgalactosamine and glucuronic acid. Most of the N-acetylgalactosamine residues are sulfated, particularly in the 4- or 6-position (CS4 and CS6), making CS a strongly charged polyanion. In the articular cartilage, aggrecan binds collagen fibrils and confines water content by limiting the degree to which glycosaminoglycans can separate. The high content of CS in the aggrecan plays a major role in allowing cartilage to resist tensile stresses during various loading conditions by providing this tissue with resistance and elasticity. It has been shown that changes occur in the structure of CS in OA tissues [11] and a diminished ratio of CS6/CS4 has been found in OA synovial fluid [12]. This could then constitute a way by which cartilage degenerates during this disease. Therefore, knowing the mechanism of action of this molecule is of particular importance.

Bioavailability and pharmacokinetics of chondroitin sulfate

Chondroitin sulfate used in studies is mainly obtained from bovine, porcine and marine cartilage; however most studies use a CS purified from bovine trachea (95% purity), which is the same as that used in clinical trials [13]. The naturally occurring CS has a molecular weight of 50 to 100 kDa, and after the extraction process, its molecular weight is 10 to 40 kDa, depending on the raw material.

Pharmacokinetic studies performed on humans and experimental animals after oral administration of CS revealed that it can be absorbed orally [14-17]. More specifically, Volpi et al [18] showed, in a study on oral bioavailability of CS in healthy male volunteers, a significant increase in plasma levels of CS compared with predose levels over a 24 hour period. Chondroitin sulfate plasma levels increased (more than 200%) in all subjects, with a peak concentration after 2 hours, the increase reaching significance from 2 to 6 hours. Absorbed CS reaches the blood compartment as high, intermediate and low molecular mass derivatives. Moreover, from the above studies, CS shows first-order kinetics up to single doses of 3,000 mg. Multiple doses of 800 mg in patients with OA do not alter the kinetics of CS. The bioavailability of CS ranges from 15% to 24% of the orally administered dose. Of the absorbed fraction of CS, 10% is in the form of CS and 90% in the form of depolymerized derivatives with a lower molecular weight. More particularly, on the articular tissue, Ronca et al [19] reported that CS is rapidly absorbed in the gastro-intestinal tract and a high content of labeled CS is found in the synovial fluid and cartilage.

Of note is that in in vitro studies, a large range of CS concentrations has been used (e.g. 12.5 to 2000 µg/mL, but generally ≤ 200 µg/mL) and one could question the relevance of using the highest drug concentrations. Possible explanations could be as follows. Chondroitin sulfate is a slow-acting drug for the treatment of OA, characterized by a slow onset of action, with a maximal effect being attained after several months of treatment with a carry over effect that persists after cessation of therapy. Drugs of biologic origin, such as CS, are difficult to measure in the biologic fluids and to differentiate from endogenous molecules. Moreover, due to the rapid degradation of this drug to compounds with lower molecular weight, the relationship between response and plasma concentration is not obvious. Therefore, although in vivo concentrations of CS are low, the disaccharide concentrations are consequently high, so the in vivo effect of CS may be the sum of CS and disaccharide concentrations. As a consequence of the above, it is difficult to calculate the Cmax of this type of drug. However, du Souich et al [20] used an alternative approach to classical methods in which they suggested to predict the Emax of the drug. The authors estimated that in humans the half-life of CS and its derivatives in plasma is approximately 15 hours (ie, steady state is attained in 3-4 days); however, approximately 3 to 6 months may be needed to obtain the maximal effect. Moreover, it was predicted that in patients with knee OA of moderate severity, 50% of Emax is achieved in approximately 35 days. Hence, in order to reproduce in vitro an effect observed in vivo obtained after several weeks of treatment, it appears necessary to increase the drug concentrations used in vitro. This could then provide a rationale as to why, in in vitro experiments, CS is sometimes at a high concentration.

Effects of chondroitin sulfate on articular tissues

Anti-apoptotic effects of chondroitin sulfate

Chondrocytes synthesize components of the extracellular matrix and regulate cartilage metabolism; therefore, the number and function of chondrocytes preserves the morphological and biological characteristics of cartilage. Death of chondrocytes is considered an important factor contributing to the depletion of cartilage matrix in OA. Cell death includes necrosis and apoptosis. In OA cartilage, a higher number of chondrocytes showing signs of apoptosis compared with normal cartilage [21-23]. In a study performed on an experimental mouse model, which spontaneously developed OA [24], the effects of orally administered CS (0.3 mg/day for 12 consecutive days) on the apoptotic index of chondrocytes was evaluated after 30 days of treatment. The results showed that CS induced a significant reduction in apoptotic chondrocytes. In vitro, it was also reported that CS (200 µg/mL) decreased the chondrocyte susceptibility to SNP-induced apoptosis, which appears to be concurrent with CS diminishing the phosphorylation and activation of p38 mitogen-activated protein kinase (MAPK) [25].

Chondroitin sulfate increases the synthesis of proteoglycans

Studies have shown that, in vitro, CS significantly induces the production of proteoglycans by human OA chondrocytes as well as the interleukin-1ß (IL-1ß) depletion of proteoglycan production [26,27]. In vivo, CS was also shown to induce proteoglycans. Indeed, in a model of chymopapain-induced articular cartilage injury, oral treatment and intramuscular injection with CS (80 mg/day) showed a protective effect on the damaged cartilage, with a significant increase in the synthesis of articular cartilage proteoglycans [28]. Chondroitin sulfate is believed to provide building blocks for the synthesis of proteoglycans and increase the sulfate incorporation in OA proteoglycans [29]; therefore, increasing its concentration could favor proteoglycan production and account for its beneficial effects.

Chondroitin sulfate reduces the effects of proteases

Extracellular matrix components modulate cellular behavior by creating an influential cellular environment. Thus, the turnover of extracellular matrix components is an integral part of development, morphogenesis, and tissue remodelling. While various types of proteases participate in matrix turnover, one group of key enzymes has specifically been related to articular tissues, this being the metalloproteases (MMP). This enzyme family of calcium-dependent zinc-containing endopeptidases is known to play important roles in tissue remodelling during physiological as well as pathological processes. In cartilage, MMPs are the principal proteases capable of degrading a wide variety of the extracellular matrix components [30]. Metalloprotease activity is regulated by specific inhibitors named tissue inhibitors of MMPs (TIMPs) [31]. The balance between MMPs and TIMPs regulates tissue remodelling under normal conditions. A deregulation of this balance is found in pathological conditions, such as OA [32,33]. It has been shown that stromelysin-1, gelatinases and collagenases are of major importance in cartilage degradation. Thus, decreasing the

effects of the MMPs by reducing their synthesis and/or activity could then account for the beneficial effects of CS treatment.

Hence, data show that in chondrocytes from normal human knee femoral cartilage, the addition of chondroitin polysulfate (10 µg/mL) to the culture media stimulated the accumulation of molecules such as aggrecan, hyaluronan and type II collagen in the cell-associated matrix, due in part to the downregulation of MMPs [27].

Stromelysin-1 or MMP-3 is known to play an important role in proteoglycan cleavage and is critical for cartilage proteoglycan homeostasis. Furthermore, MMP-3 is involved in the activation of other pro-MMPs, including pro-collagenases [34,35]. In human OA chondrocytes, CS (150 µg/mL) was shown to inhibit by 28% the IL-1ß-induced MMP-3 synthesis [36].

Release and activation of the gelatinase B or MMP-9 triggers bone and cartilage degradation, which can exacerbate joint degeneration [37-39]. In an arthritis rat model using Freund's adjuvant, treatment with CS in a dietary bar formulation at a concentration of 18 mg CS/g alone and in combination with GS (22.5 mg/g) prevented the increased joint MMP-9 levels associated with arthritis [40].

Collagenase-3 or MMP-13 is a major enzyme involved in cartilage degradation in OA. It preferentially cleaves type II collagen and is five to ten times more active on this substrate than collageanse-1 or MMP-1 [41]. Recently, it was shown that CS (25 µg/mL) down-regulated lipopolysaccharide (LPS)-induced MMP-13 in chondrocytes, which was concurrent with a reduction of p38 MAPK and extracellular signal-regulated kinase 1/2 (ERK1/2) activation [42]. In another study, CS (20 µg/mL) alone or in combination with GS (5 µg/mL) also decreased the IL-1ß-induced MMP-13 expression by chondrocytes [43].

Chondroitin sulfate has anti-inflammatory properties

Joint lubrication is naturally provided, at least in part, by hyaluronic acid in the synovial fluid. Hyaluronic acid is present in abundance in normal young and healthy joints. In degenerative OA, a dilution of high molecular weight hyaluronate has been reported [44]. It is considered that an increase in hyaluronate contributes to reducing inflammation in the articular tissues. The addition of CS (10 - 400 µg/mL) to monolayer cultures of synovial lining cells during the log phase of growth stimulates the synthesis of hyaluronate by 11% [45]. Moreover, when added during the stationary phase of growth, hyaluronate synthesis was increased by about 88% [45].

Recent evidence has implicated a number of cytokines, and more particularly IL-1ß in the OA pathological process. It is believed that IL-1ß is the principal cytokine responsible for the degeneration of extracellular matrix components in the articular tissues of patients with OA [6]. This cytokine induces a cascade of catabolic events including the upregulation of expression of other pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-6, MMPs, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and

microsomal prostaglandin E synthase-1 (mPGES-1), and the release of NO and prostaglandin E_2 (PGE_2).

Among the different catabolic pathways that are activated by inflammatory factors, NO is of interest for at least two main reasons. First, NO and its byproducts are capable of inducing the inflammatory component of OA. Secondly, it may induce tissue damage and tissue destruction; therefore it could be responsible not only for the symptoms but also the disease process per se (Table 1).

It is well established that PGE_2 plays a crucial role in the pathogenesis of arthritis. Prostaglandin E_2 induces pain and increases the production of catabolic factors including pro-inflammatory cytokines, MMPs and ROS, which in turn contribute to alterations in cartilage, synovial membrane, and bone. Moreover, in addition to exerting inflammatory effects on its own, PGE_2 can also potentiate the effects of other mediators of inflammation. The critical role of PGE_2 in the pathology of arthritis has been demonstrated in animal models of arthritis and mice lacking COX-2 or PGE_2 receptors [46-49].

Until recently, COX activity had been considered the key step in prostaglandin synthesis. However, a terminal enzyme responsible for isomerization of the COX derived-PGH_2 (inactive prostaglandin precursor of PGE_2) into PGE_2 has been identified; this enzyme was designated prostaglandin E synthase (PGES). To date, three distinct PGES isoforms have been identified (Table 2). From the three isoforms, it has been shown that the mPGES-1 is expressed in human articular tissues, and its level is increased upon IL-1ß stimulation and is upregulated in OA tissues [50-52].

In vitro, bovine articular cartilage explant treatment with CS (20 µg/mL) was effective in suppressing IL-1ß induced iNOS, COX-2 and mPGES-1 gene expression [53,54]. Furthermore, the effect of CS (20 µg/mL) in combination with GS (5 µg/mL) was more effective in the reduction of all these genes, and their expression levels reverted to normal values. The same authors showed that CS also reduced the production of PGE_2, but only the combination of CS and GS reverted the IL-1ß-induced PGE_2 synthesis to control levels as well as the IL-1ß-induced NO production. These data showing that the combination of both CS and GS is more efficient at reducing the level of expression of genes involved in inflammatory conditions, could account for the increased therapeutic effectiveness observed when both compounds were used together in a recent clinical trial of patients with knee OA [55].

Similar data were reported for human articular chondrocytes. Indeed, in a model of chondrocyte cultivated in clusters [26], IL-1ß induced an increase in PGE_2 synthesis with a stronger effect during the first two weeks of the experiment than thereafter (from day 16 to day 32). With this model, addition of CS (500-1000 µg/mL) significantly reduced the IL-1ß-induced PGE_2 during the first 16 days of culture but not during the last two week. Recently it was reported that CS (200 µg/mL) reduces IL-1ß-induced ERK1/2 and p38 MAPK phosphorylation and the nuclear transactivation of nuclear factor-κB (NF-κB), which may contribute to the anti-catabolic effects of this compound [25].

However, these results could also be explained by the fact that CS has been found to inhibit several markers of inflammation in vitro and in vivo in animal models. In the murine collagen-induced arthritis (CIA) model, the effect of CS on joint histopathology was evaluated. Chondroitin sulfate at a dose of 1000 mg/kg significantly diminished parameters of synovitis including cell infiltration, fibrosis and proliferation of synovial lining cells [56]. In another study using a rat model with Freund's adjuvant arthritis [40], treatment with CS in a dietary bar formulation at a concentration of 18 mg CS/g significantly reduced IL-1ß levels in joint tissues but not in serum. However, the combination of CS and GS (22.5 mg/g bar) reduced IL-1ß levels both in serum and tissue.

In the same animal model, treatment with CS (1 mL/kg, intraperitoneally once a day starting at the onset of arthritis and for 10 days) caused a significant reduction in malonaldehyde (an indicator to estimate the extent of lipid peroxidation in the damaged cartilage) and blunted the depletion of endogenous antioxidant reduced glutathione (GSH) and superoxide dismutase (SOD), probably by competing in scavenging for free radicals and therefore contributing to preserve the integrity of cellular membranes in the injured cartilage [57]. The production of oxygen free radicals that occurs with the development of arthritis in articular cartilage leads to decreased GSH and SOD levels as a consequence of their consumption during oxidative stress and cellular lysis [58,59]. This contributes to increased cellular damage by favoring attack by free radicals. Therefore, CS could protect against hydrogen peroxide formation and superoxide anions.

Interaction of ROS with DNA can induce a multiplicity of products of varying structures and with differing biological impacts. The antioxidant cell defence system intercepts ROS and normally inhibits cellular and nuclear damage. When the amount of ROS produced overwhelms these endogenous defences, an increase in oxidative DNA injury occurs [60]. Various studies have shown that CS4 acts as an antioxidant, thereby protecting cells from ROS damage [57,61,62].

A recent study has demonstrated that the marked increases in TNF-α levels and myeloperoxidase activity in the plasma of CIA rats were significantly reduced by treatment with CS (25 mg/kg) [57]. The reduction in the myeloperoxidase strongly suggests that CS induces a decrease in polymorphonuclear cell infiltration that occurs in the joint synovial tissue. This decrease and the other biochemical parameters (influx of inflammatory cells, synovial hyperplasia and erosion of bone and cartilage) were evaluated by histological analysis of joints from hind limbs and confirmed the protective effects of the CS4 and hyaluronic acid polymers.

Finally Cho et al [63] showed, using the same animal model (CIA), that treatment with CS (1200 mg/kg) also significantly reduced serum IL-6 levels. However, the high doses of CS used in this study should be taken into account when considering the results.

Effect of chondroitin sulfate on subchondral bone alterations

Osteoarthritis is considered a complex illness in which tissues of the joint play significant roles in the initiation and/or progression of the pathophysiology. We still do not completely

understand what initiates the degradation and loss of cartilage. However, recent evidence suggests a key role for the subchondral bone in the progression and/or initiation of OA and that these changes are related to altered osteoblast metabolism [7-10]. Recently, three major factors that play a role in bone metabolism have been identified. These are osteoprotegerin (OPG), the receptor activator of NF-κB ligand (RANKL) and RANK. The first two factors are synthesized by osteoblasts and RANKL is essential for osteoclast differentiation and bone loss. OPG is a decoy receptor that blocks the binding of RANKL to its receptor RANK (on osteoclasts), thereby preventing osteoclastogenesis and, as a result, inhibiting bone resorption. Recently, the effect of CS (200 µg/mL) on the bone remodelling factors, RANKL and OPG, before and after stimulation with $1,25(OH)_2D_3$ (vitamin D_3; 50 nM) was evaluated on human OA subchondral bone osteoblast [64]. Data showed that CS upregulates OPG expression and production under basal conditions and in the presence of vitamin D_3. Chondroitin sulfate significantly inhibited RANKL expression under basal conditions, and although vitamin D_3 drastically upregulated its expression, the drug under vitamin D_3 downregulated RANKL levels. Consequently, under basal conditions, CS significantly upregulated the expression ratio of OPG/RANKL, vitamin D_3 decreased this ratio, but CS in the presence of vitamin D_3 reversed this decrease. These data are of great importance, as the expression of RANKL is increased in abnormal osteoblasts [64] and thereby affects the balance of OPG/RANKL resulting in bone destruction. Therefore, by increasing the OPG/RANKL ratio, CS could exert a positive effect on OA structural changes at the subchondral bone level.

CONCLUSION

This article reviewed several mechanisms of action of CS that could be responsible for symptomatic relief properties of this drug in patients with OA. Chondroitin sulfate has been shown to reduce pro-inflammatory factors, modify the cellular death process and improve the anabolism/catabolism balance of extracellular cartilage matrix. At the same time it has proven to have a positive effect on some of the pathological processes involving the synovial tissue and subchondral bone. These mechanisms could, therefore, account for the beneficial results observed in some clinical trials [65-71]. In a recent study [55], CS was shown to have a significant advantage over placebo by decreasing the incidence of joint swelling, and/or effusion. The same clinical trial [55] also showed that the combination of both CS and GS appears more effective than either alone, and this combination could explain the induced higher in vivo effect on symptoms in the subset of patients with moderate to severe knee pain. In addition, a recent meta-analysis [72] has suggested the possibility that CS alone has a clinically relevant effect in patients with low grade OA.

Table I. Effects of Nitric Oxide on Articular Tissues

Cartilage Damage	**Inflammation, Swelling and Pain**
Induction • Chondrocyte death (apoptosis) [3,73-76] Synthesis/activity of MMPs [77] **Reduction** • Collagen and aggrecan (proteoglycan) synthesis [78,79] • IL-1Ra synthesis [80]	**Induction** • COX-2 activity [75,81] • IL-1β converting enzyme (ICE), IL-18 [82]

Table 2 : Prostaglandin E Synthase (PGES)

• **Cytosolic PGES (cPGES)**	Ubiquitous, function remains to be determined
• **Microsomal PGES (mPGES)**	• mPGES-1: Inducible, functional coupling with COX-2 [83-86] • mPGES-2: Ubiquitous, functional coupling with COX-1 [87]

REFERENCES

1. Henrotin Y, Deby-Dupont G, Deby C, De Bruyn M, Lamy M, Franchimont P. Production of active oxygen species by isolated human chondrocytes. Br J Rheumatol. 1993;**32**:562-7.

2. Guilak F, Meyer BC, Ratcliffe A, Mow VC. The effects of matrix compression on proteoglycan metabolism in articular cartilage explants. Osteoarthritis Cartilage. 1994;**2**:91-101.

3. Pelletier JP, Jovanovic DV, Lascau-Coman V, Fernandes JC, Manning PT, Connor JR, et al. Selective inhibition of inducible nitric oxide synthase reduces progression of experimental osteoarthritis in vivo: possible link with the reduction in chondrocyte apoptosis and caspase 3 level. Arthritis Rheum. 2000;**43**:1290-9.

4. Tiku ML, Shah R, Allison GT. Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation. Possible role in cartilage aging and the pathogenesis of osteoarthritis. J Biol Chem. 2000;**275**:20069-76.

5. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilage. 2003;**11**:747-55.

6. Martel-Pelletier J, Lajeunesse D, Pelletier JP. Etiopathogenesis of osteoarthritis, In Koopman WJ, ed. *Arthritis & Allied Conditions. A Textbook of Rheumatology*. Baltimore: Lippincott, Williams & Wilkins, 2005: 2199-226.

7. Lajeunesse D, Massicotte F, Pelletier JP, Martel-Pelletier J. Subchondral bone sclerosis in osteoarthritis: Not just an innocent bystander. Modern Rheumatology. 2003;**13**:7-14.

8. Hilal G, Martel-Pelletier J, Pelletier JP, Ranger P, Lajeunesse D. Osteoblast-like cells from human subchondral osteoarthritic bone demonstrate an altered phenotype *in vitro*: Possible role in subchondral bone sclerosis. Arthritis Rheum. 1998;**41**:891-9.

9. Massicotte F, Lajeunesse D, Benderdour M, Pelletier J-P, Hilal G, Duval N, et al. Can altered production of interleukin 1ß, interleukin-6, transforming growth factor-ß and prostaglandin E2 by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. Osteoarthritis Cartilage. 2002;**10**: 491-500.

10. Sanchez C, Deberg MA, Piccardi N, Msika P, Reginster JY, Henrotin YE. Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic chondrocytes. Osteoarthritis Cartilage. 2005;**13**:988-97.

11. Caterson B, Mahmoodian F, Sorrell JM, Hardingham TE, Bayliss MT, Carney SL , et al. Modulation of native chondroitin sulphate structure in tissue development and in disease. J Cell Sci. 1990;**97**:411-7.

12. Shinmei M, Miyauchi S, Machida A, Miyazaki K. Quantitation of chondroitin 4-sulfate and chondroitin 6-sulfate in pathologic joint fluid. Arthritis Rheum. 1992;**35**:1304-8.

13. Barnhill JG, Fye CL, Williams DW, Reda DJ, Harris CL, Clegg DO. Chondroitin product selection for the glucosamine/chondroitin arthritis intervention trial. J Am Pharm Assoc (Wash DC). 2006;**46**:14-24.

14. Conte A, Palmieri L, Segnini D, Ronca G. Metabolic fate of partially depolymerized chondroitin sulfate administered to the rat. Drugs Exp Clin Res. 1991;**17**:27-33.

15. Conte A, de Bernardi M, Palmieri L, Lualdi P, Mautone G, Ronca G. Metabolic fate of exogenous

chondroitin sulfate in man. Arzneimittelforschung. 1991;**41**:768-72.

16. Conte A, Volpi N, Palmieri L, Bahous I, Ronca G. Biochemical and pharmacokinetic aspects of oral treatment with chondroitin sulfate. Arzneimittelforschung. 1995;**45**:918-25.

17. Palmieri L, Conte A, Giovannini L, Lualdi P, Ronca G. Metabolic fate of exogenous chondroitin sulfate in the experimental animal. Arzneimittelforschung. 1990;**40**:319-23.

18. Volpi N. Oral bioavailability of chondroitin sulfate (Condrosulf) and its constituents in healthy male volunteers. Osteoarthritis Cartilage. 2002;**10**:768-77.

19. Ronca F, Palmieri L, Panicucci P, Ronca G. Anti-inflammatory activity of chondroitin sulfate. Osteoarthritis Cartilage. 1998;**6 Suppl A**:14-21.

20. du Souich P, Vergés J. Simple approach to predict the maximal effect elicited by a drug when plasma concentrations are not available or are dissociated from the effect, as illustrated with chondroitin sulfate data. Clin Pharmacol Ther. 2001;**70**:5-9.

21. Blanco FJ, Guitian R, Vazquez-Martul E, de Toro FJ, Galdo F. Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. Arthritis Rheum. 1998;**41**:284-9.

22. Kim HA, Lee YJ, Seong SC, Choe KW, Song YW. Apoptotic chondrocyte death in human osteoarthritis. J Rheumatol. 2000;**27**:455-62.

23. Kouri JB, Aguilera JM, Reyes J, Lozoya KA, Gonzalez S. Apoptotic chondrocytes from osteoarthrotic human articular cartilage and abnormal calcification of subchondral bone. J Rheumatol. 2000;**27**:1005-19.

24. Caraglia M, Beninati S, Giuberti G, D'Alessandro AM, Lentini A, Abbruzzese A, et al. Alternative therapy of earth elements increases the chondroprotective effects of chondroitin sulfate in mice. Exp Mol Med. 2005;**37**:476-81.

25. Vergés J, Montell E, Martinez G, Heroux L, du Souich P. Chondroitin sulfate reduces nuclear translocation factor NF-kappaB and does not modify the activation state of activating protein-1 (AP-1). Osteoarthritis Cartilage. 2004;**12**:194 (Abstract)

26. Bassleer CT, Combal JP, Bougaret S, Malaise M. Effects of chondroitin sulfate and interleukin-1 beta on human articular chondrocytes cultivated in clusters. Osteoarthritis Cartilage. 1998;**6**:196-204.

27. Wang L, Wang J, Almqvist KF, Veys EM, Verbruggen G. Influence of polysulphated polysaccharides and hydrocortisone on the extracellular matrix metabolism of human articular chondrocytes in vitro. Clin Exp Rheumatol. 2002;**20**:669-76.

28. Uebelhart D, Thonar EJ, Zhang J, Williams JM. Protective effect of exogenous chondroitin 4,6-sulfate in the acute degradation of articular cartilage in the rabbit. Osteoarthritis Cartilage. 1998;**6 Suppl A**:6-13.

29. Schwartz NB, Dorfman A. Stimulation of chondroitin sulfate proteoglycan production by chondrocytes in monolayer. Connect Tissue Res. 1975;**3**:115-22.

30. Nagase H, Woessner JF Jr. Matrix metalloproteinases. J Biol Chem. 1999;**274**:21491-4.

31. Bode W, Fernandez-Catalan C, Tschesche H, Grams F, Nagase H, Maskos K. Structural properties of matrix metalloproteinases. Cell Mol Life Sci. 1999;**55**:639-52.

32. Dean DD, Martel-Pelletier J, Pelletier JP, Howell DS, Woessner JF Jr. Evidence for metalloproteinase and

metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. J Clin Invest. 1989;**84**:678-85.

33. Su S, Grover J, Roughley PJ, Di Battista JA, Martel-Pelletier J, Pelletier JP, et al. Expression of the tissue inhibitor of metalloproteinases (TIMPs) gene family in normal and osteoarthritic joints. Rheumatol Int. 1999;**18**:183-91.

34. Suzuki K, Enghild JJ, Morodomi T, Salvesen G, Nagase H. Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). Biochemistry. 1990;**29**:10261-70.

35. Nagase H, Suzuki K, Morodomi T, Enghild JJ, Salvesen G. Activation mechanisms of the precursors of matrix metalloproteinases 1, 2 and 3. Matrix Suppl. 1992;**1**:237-44.

36. Monfort J, Nacher M, Montell E, Vila J, Verges J, Benito P. Chondroitin sulfate and hyaluronic acid (500-730 kda) inhibit stromelysin-1 synthesis in human osteoarthritic chondrocytes. Drugs Exp Clin Res. 2005;**31**:71-6.

37. Hirose T, Reife RA, Smith GN Jr, Stevens RM, Mainardi CL, Hasty KA. Characterization of type V collagenase (gelatinase) in synovial fluid of patients with inflammatory arthritis. J Rheumatol. 1992;**19**:593-9.

38. Sopata I, Wize J, Filipowicz-Sosnowska A, Stanislawska-Biernat E, Brzezinska B, Maslinski S. Neutrophil gelatinase levels in plasma and synovial fluid of patients with rheumatic diseases. Rheumatol Int. 1995;**15**:9-14.

39. Tetlow LC, Adlam DJ, Woolley DE: Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes. Arthritis Rheum 2001;**44**:585-94.

40. Chou MM, Vergnolle N, McDougall JJ, Wallace JL, Marty S, Teskey V, et al. Effects of chondroitin and glucosamine sulfate in a dietary bar formulation on inflammation, interleukin-1beta, matrix metalloprotease-9, and cartilage damage in arthritis. Exp Biol Med. 2005;**230**:255-62.

41. Reboul P, Pelletier JP, Tardif G, Cloutier JM, Martel-Pelletier J. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes: A role in osteoarthritis. J Clin Invest. 1996;**97**:2011-9.

42. Holzmann J, Brandl N, Zemann A, Schabus R, Marlovits S, Cowburn R, et al. Assorted effects of TGFbeta and chondroitinsulfate on p38 and ERK1/2 activation levels in human articular chondrocytes stimulated with LPS. Osteoarthritis Cartilage. 2006;**14**:519-25.

43. Chan PS, Caron JP, Orth MW. Effect of glucosamine and chondroitin sulfate on regulation of gene expression of proteolytic enzymes and their inhibitors in interleukin-1-challenged bovine articular cartilage explants. Am J Vet Res. 2005;**66**:1870-6.

44. Dahl LB, Dahl IM, Engstrom-Laurent A, Granath K. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. Ann Rheum Dis. 1985;**44**:817-22.

45. Verbruggen G, Veys EM. Influence of sulphated glycosaminoglycans upon proteoglycan metabolism of the synovial lining cells. Acta Rhumatol Belg. 1977;**1**:75-92.

46. Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, et al. The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. Arthritis Rheum. 2000;**43**:2687-93.

47. McCoy JM, Wicks JR, Audoly LP. The role of prostaglandin E2 receptors in the pathogenesis of rheumatoid arthritis. J Clin Invest. 2002;**110**:651-8.

48. Averbeck B, Rudolphi K, Michaelis M. Osteoarthritic mice exhibit enhanced prostaglandin E2 and unchanged calcitonin gene-related peptide release in a novel isolated knee joint model. J Rheumatol. 2004;**31**:2013-20.

49. Honda T, Segi-Nishida E, Miyachi Y, Narumiya S. Prostacyclin-IP signaling and prostaglandin E2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. J Exp Med. 2006;**203**:325-35.

50. Kojima F, Naraba H, Miyamoto S, Beppu M, Aoki H, Kawai S. Membrane-associated prostaglandin E synthase-1 is upregulated by proinflammatory cytokines in chondrocytes from patients with osteoarthritis. Arthritis Res Ther. 2004;**6**:R355-65.

51. Masuko-Hongo K, Berenbaum F, Humbert L, Salvat C, Goldring MB, Thirion S. Up-regulation of microsomal prostaglandin E synthase 1 in osteoarthritic human cartilage: critical roles of the ERK-1/2 and p38 signaling pathways. Arthritis Rheum. 2004;**50**:2829-38.

52. Li X, Afif H, Cheng S, Martel-Pelletier J, Pelletier JP, Ranger P, et al. Expression and regulation of microsomal prostaglandin E synthase-1 in human osteoarthritic cartilage and chondrocytes. J Rheumatol. 2005;**32**:887-95.

53. Chan PS, Caron JP, Rosa GJ, Orth MW. Glucosamine and chondroitin sulfate regulate gene expression and synthesis of nitric oxide and prostaglandin E(2) in articular cartilage explants. Osteoarthritis Cartilage. 2005;**13**:387-94.

54. Chan PS, Caron JP, Orth MW. Short-term gene expression changes in cartilage explants stimulated with interleukin beta plus glucosamine and chondroitin sulfate. J Rheumatol. 2006;**33**:1329-40.

55. 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.

56. Omata T, Sagawa Y, Itosaku Y, Inoue N, Tanaka Y. Effects of chondroitin sulphate-C on bradykinin-induced proteoglycan depletion in rats. Arzneimittelforschung. 2000;**49**:577-81.

57. Campo GM, Avenoso A, Campo S, Ferlazzo AM, Altavilla D, Calatroni A. Efficacy of treatment with glycosaminoglycans on experimental collagen-induced arthritis in rats. Arthritis Res Ther. 2003;**5**:R122-31.

58. Kiziltunc A, Cogalgil S, Cerrahoglu L. Carnitine and antioxidants levels in patients with rheumatoid arthritis. Scand J Rheumatol. 1998;**27**:441-5.

59. Hassan MQ, Hadi RA, Al-Rawi ZS, Padron VA, Stohs SJ. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J Appl Toxicol. 2001;**21**:69-73.

60. Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. Toxicology. 2002;**181-182**:219-22.

61. Albertini R, Passi A, Abuja PM, De Luca G. The effect of glycosaminoglycans and proteoglycans on lipid peroxidation. Int J Mol Med. 2000;**6**:129-36.

62. Campo GM, Avenoso A, Campo S, D'Ascola A, Ferlazzo AM, Calatroni A. Reduction of DNA fragmentation and hydroxyl radical production by hyaluronic acid and chondroitin-4-sulphate in iron plus ascorbate-induced oxidative stress in fibroblast cultures. Free Radic Res. 2004;**38**:601-11.

63. Cho SY, Sim JS, Jeong CS, Chang SY, Choi DW, Toida T, et al. Effects of low molecular weight chondroitin sulfate on type II collagen-induced arthritis in DBA/1J mice. Biol Pharm Bull. 2004;**27**:47-51.

64. Kwan Tat S, Pelletier JP, Vergés J, Montell E, Lajeunesse D, Fahmi H, et al. Chondroitin and glucosamine sulfate in combination decrease the pro-resorptive properties of human osteoarthritis subchondral bone osteoblasts. (Submitted)

65. Conrozier T. Anti-arthrosis treatments: efficacy and tolerance of chondroitin sulfates (CS 4&6). Presse Med. 1998;**27**:1862-5.

66. 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.

67. Verbruggen G, Goemaere S, Veys EM. Chondroitin sulfate: S/DMOAD (structure/disease modifying anti-osteoarthritis drug) in the treatment of finger joint OA. Osteoarthritis Cartilage. 1998;**6 Suppl A**:37-8.

68. Malaise M, Marcolongo R, Uebelhart D, Vignon E. Efficacy and tolerability of 800 mg oral chondroitin 4&6 sulfate in the treatment of knee osteoarthritis: a randomised, double-blind, multicentre study versus placebo. Litera Rheumatologica. 1999;**24**:31-42.

69. Uebelhart D, Malaise M, Marcolongo R, DeVathaire 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.

70. Michel BA, Stucki G, Frey D, De Vathaire F, Vignon E, Bruehlmann P, et al. Chondroitins 4 and 6 sulfate in osteoarthritis of the knee: a randomized, controlled trial. Arthritis Rheum. 2005;**52**:779-86.

71. Kahan, A. STOPP (STudy on Osteoarthritis Progression Prevention): a new two-year trial with chondroitin 4&6 sulfate (CS). 2006; www.ibsa.ch/eular_2006_amsterdam_vignon-2.pdf

72. Reichenbach S, Sterchi R, Scherer M, Trelle S, Bürgi E, Bürgi U et al: Meta-analysis: chondroitin for osteoarthritis of the knee or hip. Ann Intern Med 2007;**146**:580-90.

73. Blanco FJ, Ochs RL, Schwarz H, Lotz M. Chondrocyte apoptosis induced by nitric oxide. Am J Pathol. 1995;**146**:75-85.

74. Hashimoto S, Takahashi K, Amiel D, Coutts RD, Lotz M. Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis. Arthritis Rheum. 1998;**41**:1266-74.

75. Notoya K, Jovanovic DV, Reboul P, Martel-Pelletier J, Mineau F, Pelletier JP. The induction of cell death in human osteoarthritis chondrocytes by nitric oxide is related to the production of prostaglandin E2 via the induction of cyclooxygenase-2. J Immunol. 2000;**165**:3402-10.

76. Jovanovic DV, Mineau F, Notoya K, Reboul P, Martel-Pelletier J, Pelletier J-P. Nitric oxide-induced cell death in human osteoarthritc synoviocytes is mediated by tyrosine kinase activation and hydrogen peroxide and/or superoxide formation. J Rheumatol. 2002;**29**:2165-75.

77. Murrell GAC, Jang D, Williams RJ. Nitric oxide activates metalloprotease enzymes in articular cartilage. Biochem Biophys Res Commun. 1995;**206**:15-21.

78. Hauselmann HJ, Oppliger L, Michel BA, Stefanovic-Racic M, Evans CH. Nitric oxide and proteoglycan biosynthesis by human articular chondrocytes in alginate culture. FEBS Lett. 1994;**352**:361-4.

79. Taskiran D, Stefanovic-Racic M, Georgescu HI, Evans CH. Nitric oxide mediates suppression of cartilage

proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun. 1994;**200**:142-8.

80. Pelletier JP, Mineau F, Ranger P, Tardif G, Martel-Pelletier J. The increased synthesis of inducible nitric oxide inhibits IL-1Ra synthesis by human articular chondrocytes: possible role in osteoarthritic cartilage degradation. Osteoarthritis Cartilage. 1996;**4**:77-84.

81. Amin AR, Attur MG, Patel RN, Thakker GD, Marshall PJ, Rediske J, et al. Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. J Clin Invest. 1997;**99**:1231-7.

82. Boileau C, Martel-Pelletier J, Moldovan F, Jouzeau JY, Netter P, Manning PT, et al. The in situ up-regulation of chondrocyte interleukin-1-converting enzyme and interleukin-18 levels in experimental osteoarthritis is mediated by nitric oxide. Arthritis Rheum. 2002;**46**:2637-47.

83. Naraba H, Murakami M, Matsumoto H, Shimbara S, Ueno A, Kudo I, et al. Segregated coupling of phospholipases A2, cyclooxygenases, and terminal prostanoid synthases in different phases of prostanoid biosynthesis in rat peritoneal macrophages. J Immunol. 1998;**160**:2974-82.

84. Brock TG, McNish RW, Peters-Golden M. Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E2. J Biol Chem. 1999;**274**:11660-6.

85. Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A. 1999;**96**:7220-5.

86. Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, et al. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. J Biol Chem. 2000;**275**:32783-92.

87. Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I. Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. J Biol Chem. 2000;**275**:32775-82.

Biochemical basis of the effect of chondroitin sulfate on osteoarthritis articular tissues

Jordi Monfort, Jean-Pierre Pelletier, Natalia Garcia-Giralt and Johanne Martel-Pelletier

Ann Rheum Dis published online July 20, 2007

Notes