Osteoarthritis and Cartilage 18 (2010) S12–S16

Osteoarthritis and Cartilage

Improvement of experimental accelerated atherosclerosis by chondroitin sulphate

M.J. Martínez-Calatrava, R. Largo*, G. Herrero-Beaumont*

Joint and Bone Research Unit, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Spain

ARTICLE INFO

Article history:
Received 8 October 2009
Accepted 21 January 2010

Keywords:
Chondroitin sulphate
Atherosclerosis
Chronic inflammation
Arthritis
Rabbit model

SUMMARY

The rheumatic diseases have been associated with accelerated atherosclerosis. Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by persistent synovial inflammation which leads to disability and structural changes in joints. Epidemiological studies have demonstrated an increased cardiovascular mortality in patients with RA. In these patients, atherosclerotic plaque occurs earlier, and it has a faster evolution than in general population. Atherosclerosis (AT) is also an inflammatory disease partly mediated by cytokines, many of them involved on chronic synovitis. Our group has developed a rabbit experimental model of AT aggravated by chronic arthritis to study inflammatory mechanisms involved on the progression of vascular lesions and their response to drugs. A preliminary study using this model suggests a beneficial effect of chondroitin sulphate (CS), a drug recommended for the treatment of osteoarthritis, in controlling AT lesions. Yet clinical trials should be conducted with this compound to address the same hypothesis in human studies.

Atherosclerosis and rheumatic diseases

The vascular endothelium is involved in the pathogenesis of inflammatory rheumatic diseases. Rheumatoid arthritis (RA), systemic lupus erythematosus, systemic sclerosis and the anti-phospholipid syndrome have been associated with accelerated atherosclerosis. In this section, we will take RA as a prototype of these rheumatic diseases. RA is a chronic systemic inflammatory disease that primarily affects the joints, and it is marked by inflammatory changes in the synovial membranes and articular structures. RA is the most common chronic inflammatory condition, affecting approximately 1% of the adult population in developed populations[1]. In addition to their negative impact on quality of life, epidemiological studies have also consistently demonstrated an increased mortality in patients with RA, regardless of steroid use and the presence of rheumatoid factor, when compared with general population[2,3]. The major underlying cause for this excess mortality in RA seems to be the cardiovascular events subsequent to premature atherosclerosis. Cardiovascular mortality in RA is more frequent, regardless of RA manifestations, than would be expected from the profile of traditional cardiovascular risk factors (age, gender, diabetes mellitus, hypercholesterolaemia, hypertension, smoking status, sedentary lifestyle and family history of early coronary artery disease), especially in the youngest population where little or no atherosclerosis would be expected[4]. Several studies have demonstrated an increased prevalence of atherosclerotic plaque in patients with RA relative to controls[5,6] and that the atherogenic process is accelerated after the onset of RA[7]. Moreover, RA activity has been recently associated with carotid plaque instability and RA has been proposed as a novel cardiovascular risk factor with severity comparable to that of diabetes mellitus[8].

Atherosclerosis was formerly considered a passive disease of lipid deposition in arteries with formation of plaques that results in tissue ischaemia and thrombus, however, during the last decade it has become increasingly acknowledged that atherosclerosis is also a chronic inflammatory process. Atherosclerosis is influenced by rheumatoid factor[9], and mediators of synovitis (TNF-α, IL-6, IL-1) through their effect promoting macrophages, B and T lymphocytes, smooth muscle cells and endothelial cells activation, expression of surface adhesion molecules as the monocyte chemoattractant protein 1, and migration of leukocyte to the subendothelial layers. Here, these activated cells, in conjunction with oxidized low-density lipoproteins, integrate into the fatty streaks precursors of atherosclerotic plaques[10], insulin resistance[11], dyslipidemia[12] fibrinolysis[13] and circulating C-reactive protein (CRP) levels[14]. Thus, cardiovascular disease has been considered an extra-articular manifestation of RA[15].

Atherosclerosis and osteoarthritis (OA)

OA is the most prevalent joint disorder in ageing population of the developed world. OA is a disease affecting all joint tissues,

* Address correspondence and reprint requests to: Raquel Largo and Gabriel Herrero-Beaumont, Joint and Bone Research Laboratory, Fundación Jimenez Diaz, Reyes Católicos 2, 28040 Madrid, Spain. Tel: 34-915504978; Fax: 34-915442636.
E-mail address: gherrero@fjd.es (R. Largo).

1063-4584/$ – see front matter
doi:10.1016/j.joca.2010.01.014

although the articular cartilage and the subjacent bone often show the most marked changes. Established risk factors for OA are age, female gender, and obesity[16]. The association between obesity and OA is still not fully understood. Indeed, the obesity-related mechanical stress may explain this association for hip or knee OA, but it does not explain the reported association between obesity and hand OA. This leads one to think that other alterations related to obesity might be linked with OA. Obesity and OA are independent factors associated with atherosclerosis[17,18] and it has been postulated that a reduced blood supply to subchondral bone might underlie the development of OA[19]. The articular cartilage is an avascular tissue that receives more than 50% of nutrients and oxygen from the subchondral vessels[20]. In this context, the suggested consequences of bone ischaemia and endothelial dysfunction are: (1) reduction of the nutrients and oxygen supply to overlying cartilage causing catabolic and reparative processes in the cartilage; (2) osteocytes death and increased turnover of the subchondral bone[19]; (3) induction of bone marrow oedema[21], a potent risk factor for structural deterioration in knee OA[22,23]; (4) infiltration of leukocytes into the surrounding structures and initiation of inflammatory responses from these tissues[24]. All these alterations may compromise the shock absorption capacity of the subchondral bone leading to cartilage damage. Epidemiological studies reveal that the risk factors for progression are not the same as those for initiation[25]. Whether atherosclerosis participates in OA initiation or progression needs to be elucidated.

Animal models of atherosclerosis

Atherosclerosis is a polygenic and environmental-dependent disease. The inability to study atherosclerosis in a controlled manner in humans forces us to use animal models for studying atherosclerosis in depth. Experimental genetic murine models of atherosclerosis were developed in the late 1990s[26], but currently we know that the suitable specie in which to study atherosclerosis is the rabbit because it has a lipid metabolism similar to the human, and it is an animal susceptible to diet-induced atherosclerosis. In the literature we can find experimental murine models of accelerated atherosclerosis by systemic inflammation in which the inflammation is generated inducing hyperlipidemia[27], lupus[28], diet-induced obesity[29] or diabetes[30]. Recently, our group has developed an experimental model in rabbits to reproduce the setting of systemic inflammation associated with RA in order to study inflammation-related mechanisms of vascular lesions[31]. Studies were conducted on male New Zealand rabbits and the induction of chronic antigen-induced arthritis and atherosclerosis was synchronized as outlined in Fig. 1. To initiate atherosclerosis, animals were fed with a hyperlipidaemic diet enriched with 2% cholesterol and 6% peanut oil along all the study. Two weeks after the administration of this diet, an endothelial lesion was induced in both femoral arteries by infusion of gaseous nitrogen inside the femoral arteries. To induce antigen-arthritis, animals were given two intradermal injections of 1 ml ovalbumin in Freud's complete adjuvant. Five days after the last injection, 1 ml of ovalbumin was also injected into the knee joints on a weekly basis over the following 4 weeks. The control groups were induced to have either AIA alone or AT alone. Briefly, with that study we demonstrated that rabbits with combined atherosclerosis and chronic antigen-induced arthritis showed more pronounced systemic (higher serum CRP and IL-6 levels) and synovial inflammation, a higher activation of circulating mononuclear cells, and more secondary atherosclerotic lesions in the aorta than those animals with the single phenotype. Moreover, our results indicated that the presence of chronic arthritis in hyperlipemic rabbit results in a more vulnerable vessel wall at the site of the endothelial characterized for an increased expression of the pro-inflammatory molecules COX-2 and CCL-2. In summary, we characterized an experimental model that represents a new approach to the study of secondary atherosclerosis, and showed that chronic arthritis is a risk factor for the development of atherosclerotic lesions.

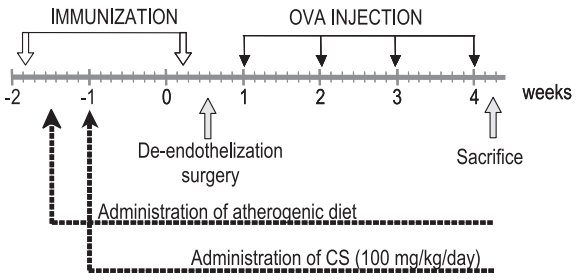

Fig. 1. Schematic representation of the experimental model.

Treatment with chondroitin sulphate (CS)

CS is a natural glycosaminoglycan predominantly found in the extra-cellular matrix surrounding cartilage, ligaments, tendons, blood vessels and skin[32,33]. CS belongs to the oral symptomatic slow-acting drugs and was included in the EULAR recommendations for the treatment of knee OA in 2003. It has been reported that CS reduces pain and swelling, improves articular function and prevents joint space narrowing[34]. Its therapeutic effect is probably the result of its anti-inflammatory activity in chondrocytes and synovial cells. In this sense, it has been demonstrated that CS decreases NF-κB nuclear translocation and consequently, the synthesis of several pro-inflammatory mediators (COX-2, NOSe, IL-1β, TNF-α, etc.)[35]. There is preliminary evidence suggesting that CS may also improve other inflammatory disorders such as atherosclerosis[36]. Accordingly, our group developed a study to examine the anti-inflammatory properties of CS on atherosclerotic lesions in the rabbit model of induced atherosclerosis above described[37]. In that study, 1 week after the first ovalbumin injection, rabbits were randomly allocated into two groups; one group received daily 100 mg/kg of CS and the other was given no treatment and was used as a control group. The results derived from that study demonstrated that, in rabbits, treatment with CS produces a clear reduction of systemic inflammation (serum CRP and IL-6 levels; Table I) and of pro-inflammatory activation of the circulating mononuclear cells, and an improvement in the inflammatory response of atherosclerotic lesions in femoral re-stenosis, as well as a decrease in the number of aortic atherosclerotic lesions. Regarding the molecular mechanisms underlying these anti-inflammatory effects, results from different studies[31,35,37–39], showed that CS and other glycosaminoglycans with similar structures such as glucosamine sulphate reduce NF-κB activation and consequently COX-2 and nitric oxide synthesis and PGE2 release in circulating mononuclear cells, chondrocytes, fibroblasts and macrophages. On the other hand, in our experimental model, CS reduced the intima/media thickness ratio in the arteries of injured rabbits (Fig. 2) and the neointimal macrophage infiltration,

Table I
Effect of chondroitin sulphate (CS) administration to rabbits with atherosclerosis plus chronic arthritis on IL-6 and CRP concentration in serum

	Healthy ($n = 15$)	NT ($n = 15$)	CS ($n = 9$)
IL-6 (pg/ml)	162.9 ± 22.2	$403.9 \pm 28.0^{*}$	$173.41 \pm 58.4\#$
CRP (μg/ml)	391.5 ± 40	$145{,}586.6 \pm 21{,}852.6^{*}$	$70{,}600.4 \pm 17{,}521.2^{*,\#}$

Data are shown as mean ± S.E.M.; $^{*}P < 0.01$ vs healthy controls; $\#P < 0.05$ vs NT rabbits.

suggesting that CS could interfere with the neointimal growth process linked to vascular injury and it could also interfere with inflammatory cell recruitment to the neointima of the femoral artery through the inhibition of CCL-2.

The use of CS or glucosamine could be limited by the results of a recent study showing that the treatment of OA with glucosamine worsens the insulin resistance, a pro-atherogenic alteration, in humans[40]. We have no data of insulin resistance in our rabbits, but

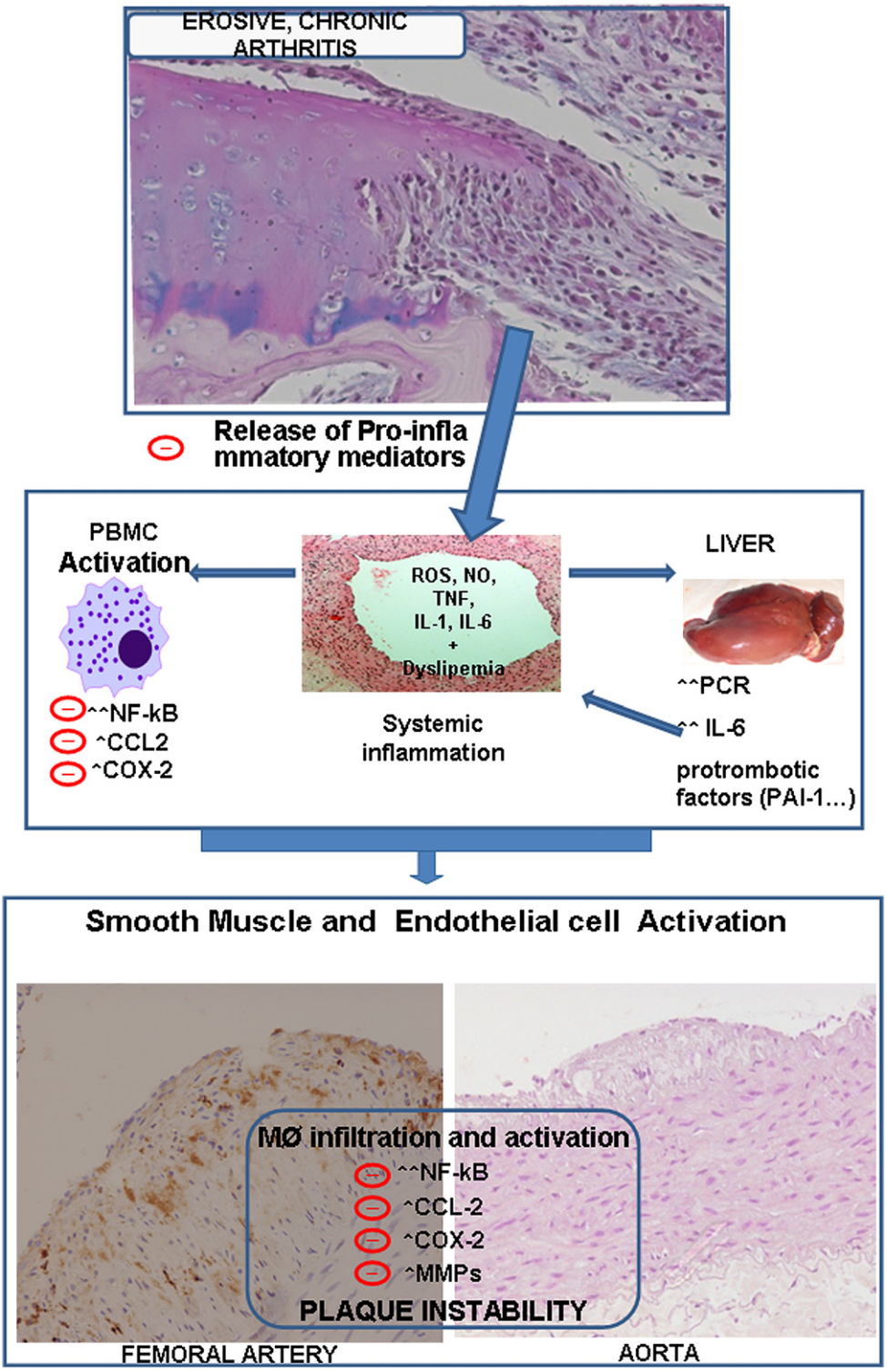

Fig. 2. Etiopathogenic mechanisms linking erosive, chronic arthritis and atherosclerosis. Rheumatoid joints secrete pro-inflammatory soluble factors in the blood stream that accelerate atherosclerosis by several mechanisms. Both, IL-6 and TNF-α stimulate the CRP, and PAI-1 synthesis and secretion by the liver, enhancing the systemic inflammation and inducing impaired fibrinolysis. CRP induces nuclear translocation of NF-κB in circulating mononuclear cells. NF-κB increases the gene expression of both COX-2 and CCL-2 in the vessel, stimulating the release and activation of matrix-degrading enzymes (MMP-2, MMP-9), and monocytes infiltration and activations. All these NF-κB-related mechanisms induce plaque instability and rupture. CS would favours the plaque stability by decreasing NF-κB nuclear translocation and consequently the synthesis of pro-inflammatory mediators (NO, IL-1, TNF-α, COX-2, etc.) and chemoattractant cytokines. The CS sites of actuation are marked in red.

a study developed in rats shows that CS does not induce insulin resistance[41].

In summary, this preliminary study suggests a beneficial effect of CS in controlling atherosclerotic lesions in rabbits. Clinical trials should be conducted with this compound to address the same hypothesis in human studies.

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**.

Gabriel Herrero-Beaumont has received speaking fees from BioIberica. Other authors declared that they have no conflict of interest. No funds were provided for writing this manuscript.

References

1. Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. Arthritis Res Ther 2009;11:229.
2. Young A, Koduri G, Batley M, Kulinskaya E, Gough A, Norton S, , *et al*Early Rheumatoid Arthritis Study (ERAS) Group. Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis. Rheumatology (Oxford) 2007;46:350–7.
3. Gabriel SE, Crowson CS, Kremers HM, Doran MF, Turesson C, O'Fallon WM, *et al*. Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years. Arthritis Rheum 2003;48:54–8.
4. del Rincón I, Freeman GL, Haas RW, O'Leary DH, Escalante A. Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis. Arthritis Rheum 2005;52:3413–23.
5. Roman MJ, Devereux RB, Schwartz JE, Lockshin MD, Paget SA, Davis A, *et al*. Arterial stiffness in chronic inflammatory diseases. Hypertension 2005;46:194–9.
6. del Rincón ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum 2001;44:2737–45.
7. del Rincón I, O'Leary DH, Freeman GL, Escalante A. Acceleration of atherosclerosis during the course of rheumatoid arthritis. Atherosclerosis 2007;195:354–60.
8. Stamatelopoulos KS, Kitas GD, Papamichael CM, Chryssohoou E, Kyrkou K, Georgiopoulos G, *et al*. Atherosclerosis in rheumatoid arthritis versus diabetes. A comparative study. Arterioscler Thromb Vasc Biol 2009.
9. Edwards CJ, Syddall H, Goswami R, Goswami P, Dennison EM, Arden NK, *et al*. Hertfordshire Cohort Study Group. The autoantibody rheumatoid factor may be an independent risk factor for ischaemic heart disease in men. Heart 2007;93:1263–7.
10. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685–95.
11. Hällgren R, Berne C. Glucose intolerance in patients with chronic inflammatory diseases is normalized by glucocorticoids. Acta Med Scand 1983;213:351–5.
12. Vallvé JC, Paredes S, Girona J, Uliaque K, Ribalta J, Hurt-Camejo E, *et al*. Tumor necrosis factor-alpha − 1031 T/C polymorphism is associated with smaller and more proatherogenic low density lipoprotein particles in patients with rheumatoid arthritis. J Rheumatol 2008;35:1697–703.
13. Lijnen HR, Alessi MC, Van Hoef B, Collen D, Juhan-Vague I. On the role of plasminogen activator inhibitor-1 in adipose tissue development and insulin resistance in mice. J Thromb Haemost 2005;3:1174–9.
14. Gonzalez-Gay MA, Gonzalez-Juanatey C, Piñeiro A, Garcia-Porrua C, Testa A, Llorca J. High-grade C-reactive protein elevation correlates with accelerated atherogenesis in patients with rheumatoid arthritis. J Rheumatol 2005;32:1219–23.
15. Ku IA, Imboden JB, Hsue PY, Ganz P. Rheumatoid arthritis. Circ J 2009;73:977–85.
16. Cicuttini FM, Baker JR, Spector TD. The association of obesity with osteoarthritis of the hand and knee in women: a twin study. J Rheumatol 1996;23:1221–6.
17. Koskinen J, Kähönen M, Viikari JS, Taittonen L, Laitinen T, Rönnemaa T, *et al*. Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults. The cardiovascular risk in young Finns study. Circulation 2009.
18. Jonsson H, Helgadottir GP, Aspelund T, Eiriksdottir G, Sigurdsson S, Ingvarsson T, *et al*. Hand osteoarthritis in older women is associated with carotid and coronary atherosclerosis: the AGES − Reykjavik study. Ann Rheum Dis 2008.
19. Findlay DM. Vascular pathology and osteoarthritis. Rheumatology (Oxford) 2007;46:1763–8.
20. Imhof H, Sulzbacher I, Grampp S, Czerny C, Youssefzadeh S, Kainberger F. Subchondral bone and cartilage disease: a rediscovered functional unit. Invest Radiol 2000;35:581–8.
21. Dodd JS, Raleigh JA, Gross TS. Osteocyte hypoxia: a novel mechanotransduction pathway. Am J Physiol 1999;277: C598–602.
22. Felson DTMS, Goggins J, LaValley MP, Gale ME, Totterman S, Li W, *et al*. Bone marrow edema and its relation to progression of knee osteoarthritis. Ann Intern Med 2003;139:330–6.
23. Hunter DJ, Zhang Y, Niu J, Goggins J, Amin S, LaValley MP, *et al*. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. Arthritis Rheum 2006;54:1529–35.
24. Miller D, Forrester K, Hart DA, Leonard C, Salo P, Bray RC. Endothelial dysfunction and decreased vascular responsiveness in the anterior cruciate ligament-deficient model of osteoarthritis. J Appl Physiol 2007;102:1161–9.
25. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, *et al*. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000;133:635–46.
26. Breslow JL. Mouse models of atherosclerosis. Science 1996;272:685–8.
27. Iacobini CMS, Ricci C, Scipioni A, Sansoni V, Cordone S, Taurino M, *et al*. Accelerated lipid-induced atherogenesis in galectin-3-deficient mice: role of lipoxidation *via* receptor-mediated mechanisms. Arterioscler Thromb Vasc Biol 2009;29:831–6.
28. Ma Z, Choudhury A, Kang SA, Monestier M, Cohen PL, Eisenberg RA. Accelerated atherosclerosis in ApoE deficient lupus mouse models. Clin Immunol 2008;127:168–75.
29. King VL, Hatch NW, Chan HW, de Beer MC, de Beer FC, Tannock LR. A murine model of obesity with accelerated atherosclerosis. Obesity (Silver Spring) 2009.
30. Renard CB, Kramer F, Johansson F, Lamharzi N, Tannock LR, von Herrath MG, *et al*. Diabetes and diabetes-associated lipid abnormalities have distinct effects on initiation and progression of atherosclerotic lesions. J Clin Invest 2004;114: 659–68.
31. Largo R, Sánchez-Pernaute O, Marcos ME, Moreno-Rubio J, Aparicio C, Granado R, *et al*. Chronic arthritis aggravates vascular lesions in rabbits with atherosclerosis: a novel model of atherosclerosis associated with chronic inflammation. Arthritis Rheum 2008;58:2723–34.

32. Wight TN, Merrilees MJ. Proteoglycans in atherosclerosis and restenosis. Key roles for versican. Circ Res 2004;94: 1158–67.
33. Teocharis AD, Tsolakis I, Tzanakakis GN, Karamanos NK. Chondroitin sulfate as a key molecule in the development of atherosclerosis and cancer progression. Adv Pharmacol 2006;53:281–95.
34. Iovu M, Dumais G, du Souich P. Anti-inflammatory activity of chondroitin sulfate. Osteoarthritis Cartilage 2008;16: S14–18.
35. Álvarez-soria MA, Largo R, Santillana J, Calvo E, Egido J, Herrero-Beaumont G. Differential anticatabolic profile of glucosamine sulfate versus other anti-osteoarthritic drugs on human osteoarthritic drugs on human osteoarthritic drugs on human osteoarthritic chondrocytes and synovial fibroblast in culture. Osteoarthritis Cartilage 2005;13:S153.
36. du Souich P, Garcia AG, Verges J, Montell E. Immunomodulatory and anti-inflammatory effects of chondroitin sulphate. J Cell Mol Med 2009.
37. Herrero-Beaumont G, Marcos ME, Sánchez-Pernaute O, Granados R, Ortega L, Montell E, *et al*. Effect of chondroitin sulphate in a rabbit model of atherosclerosis aggravated by chronic arthritis. Br J Pharmacol 2008;154:843–51.
38. Largo R, Alvarez-Soria MA, Díez-Ortego I, Calvo E, Sánchez-Pernaute O, Egido J, *et al*. Glucosamine inhibits IL-1beta-induced NFkappaB activation in human osteoarthritic chondrocytes. Osteoarthritis Cartilage 2003;11:290–8.
39. Rafi MM, Yadav PN, Rossi AO. Glucosamine inhibits LPS-induced COX-2 and iNOS expression in mouse macrophage cells (RAW 264.7) by inhibition of p38-MAP kinase and transcription factor NF-kappaB. Mol Nutr Food Res 2007;51:587–93.
40. Pham T, Cornea A, Blick KE, Jenkins A, Scofield RH. Oral glucosamine in doses used to treat osteoarthritis worsens insulin resistance. Am J Med Sci 2007;333:333–9.
41. Echard BW, Talpur NA, Funk KA, Bagchi D, Preuss HG. Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats. Mol Cell Biochem 2001;225:85–91.