Atherosclerosis 245 (2016) 82–87

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Treatment with chondroitin sulfate to modulate inflammation and atherogenesis in obesity

Pedro Melgar-Lesmes [a, *], Fernando Garcia-Polite [a, b], Paula Del-Rey-Puech [a, b], Elisabet Rosas [a, b], Juliana L. Dreyfuss [c], Eulàlia Montell [d], Josep Vergés [d], Elazer R. Edelman [a, e], Mercedes Balcells [a, b]

[a] Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, USA
[b] Bioengineering Department, Institut Químic de Sarrià, Ramon Llull Univ, Barcelona, Spain
[c] Federal University of São Paulo, Escola Paulista de Medicina, Department of Health Informatics, São Paulo, Brazil
[d] Pre-Clinical R&D Area, Pharmascience Division, Bioibérica SA, Barcelona, Spain
[e] Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Article history:
Received 6 September 2015
Received in revised form
3 December 2015
Accepted 10 December 2015
Available online 13 December 2015

Keywords:
Inflammation
Extracellular matrix
Immunology
Vascular biology
Atherosclerosis

ABSTRACT

Background and aims: Osteoarthritic patients treated with high doses of chondroitin sulfate (CS) have a lower incidence of coronary heart disease – but the mechanistic aspects of these beneficial effects of CS remain undefined. We examined how CS treatment affects the formation of atheroma via interaction with endothelial cells and monocytes.

Methods: We characterized arterial atheromatous plaques by multiphoton microscopy and serum pro-inflammatory cytokines by immunoenzymatic techniques in obese mice receiving CS (1 g/kg/day, i.p.) or vehicle for 6 days. Effects of CS on signaling pathways, cytokine secretion and macrophage migration were evaluated in cultures of human coronary endothelial cells and in a monocyte cell line stimulated with TNF-α by Western blot, immunoenzymatic techniques and transwell migration assays.

Results: Treatment of obese mice with CS reduced the extension of foam cell coverage in atheromatous plaques of arterial bifurcations by 62.5%, the serum concentration of IL1β by 70%, TNF-α by 82% and selected chemokines by 25–35%. Cultures of coronary endothelial cells and monocytes stimulated with TNF-α secreted less pro-inflammatory cytokines in the presence of CS ($P < 0.01$). CS reduced the activation of the TNF-α signaling pathway in endothelial cells (pErk 36% of reduction, and NFκB 33% of reduction), and the migration of activated monocytes to inflamed endothelial cells in transwells (81 ± 6 vs. 13 ± 2, $P < 0.001$).

Conclusions: CS interferes with the pro-inflammatory activation of monocytes and endothelial cells driven by TNF-α thus reducing the propagation of inflammation and preventing the formation of atherosclerotic plaques.

1. Introduction

Obesity and atherosclerosis are chronic inflammatory processes very closely integrated, characterized by activation of immune system and endothelium where TNF-α plays a pivotal role [1–4]. Circulating monocytes adhere to inflamed endothelium, infiltrate atherosclerotic lesions, differentiate into macrophages [5,6], and deliver pro-inflammatory mediators such as TNF-α which participate decisively in the development and exacerbation of atherosclerosis [7,8].

Glycosaminoglycans are large linear polysaccharides constructed of repeating disaccharide units. Among them, heparan sulfate has been suggested as critical to regulation of vascular repair after injury and promoting atherogenesis [9,10]. The glycosaminoglycan chondroitin sulfate (CS) has traditionally being associated to the prevention of cardiac events, but its role in atherosclerosis has not been completely elucidated as yet. In the early 70s, 60 patients with coronary heart disease treated with commercial CS showed a

Abbreviations: CS, chondroitin sulfate; HCAEC, human coronary artery endothelial cells; EC, endothelial cell; MCP-1, monocyte chemotactic protein 1; PMA, phorbol-12-myristate-13-acetate; TNF-α, tumor necrosis factor α.

* Corresponding author.
E-mail address: pedroml@mit.edu (P. Melgar-Lesmes).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.12.016
0021-9150/

seven-fold lower incidence of coronary events compared to the control group [11]. After 6 years of follow-up, only 10% of those CS-treated patients presented an acute cardiac event, of which four died, compared with 70% of which 14 died in the control group [12]. Few years later, a different clinical trial showed similar beneficial effects of CS therapy in mortality on atherosclerotic subjects [13]. However, since then, the therapeutic use of CS has been focused mainly to the treatment of osteoarthritis as CS is present in the extracellular matrix (ECM) especially in the cartilage, skin, blood vessels, ligaments and tendons [14]. Recently, new evidences raised in patients with osteoarthritis and CS therapy. Interestingly, osteoarthritic patients treated with CS showed a reduction of seven-fold in the incidence of coronary events [15]. Animal studies have demonstrated the anti-inflammatory potential of CS inhibiting hind paw edema, synovitis and destruction of the articular cartilage in a dose-dependent manner [16]. In advanced atherosclerosis, there is now a notion that there is a decrease in CS 4S (mainly composed of glucuronic acid and galactosamine residues sulfated in position 4; the disaccharides are Δdi-4S) and/or CS 6S (mainly composed of Δdi-6S), with a concomitant increase in arterial walls of dermatan sulfate (CS with the glucuronic acid moiety is replaced by its C-5 epimer, the iduronic acid) [14]. Administration of CS 6S has been described to interfere with the proinflammatory response of activated murine macrophages [17], but no studies have described the potential immunomodulatory effects of CS in atherogenesis. We therefore aimed to investigate whether CS could modulate inflammation in activated monocytes and endothelial cells to prevent atherogenesis in obese mice.

2. Materials and methods

2.1. Animal studies

Male diet induced obesity (DIO) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animals were maintained in a temperature-controlled room (22 °C) on a 12-h light–dark cycle under institutional and NIH guidelines. After arrival, mice were continuously fed *ad libitum* with high fat diet (60% of kcal from fat) until euthanasia. CS (1 g/kg/day from bovine origin with a disaccharide sulfation profile of 63% of 4-sulfated, 31% of 6-sulfated and 6% of O-sulfated; Bioibérica, Barcelona, Spain) or saline solution (Vehicle, VH) was intraperitoneally injected for 6 days. Afterward animals were sacrificed and blood samples obtained and processed by standard procedure to obtain serum for Multiplex ELISA arrays (Quansys Bioscience Inc., Logan, UT) of the cytokines IL-1β, IL-6, IL-10, TNF-α, MIP-1α, KC, MCP-1, RANTES, TARC. Animal experiments were approved by the Animal Ethics Committee at Massachusetts Institute of Technology, MA, USA.

2.2. Whole-mount multiphoton imaging of macrophage presence and angiography in carotid bifurcations

Obese mice (14 weeks old) treated or non-treated with CS were anesthetized with isoflurane, injected with 100 μL of 20 mg/mL 70-kDa Texas red-dextran in PBS into the tail vein and, after 6 h, euthanized by overexposure to CO_2, and intracardially perfused with fluorescein isothiocyanate-labeled dextran (FITC-dextran, MW 2 × 106 Da, Sigma, St. Louis, MO). Carotid bifurcations and macrophage fluorescence were visualized using a multiphoton intravital microscope (Leica Microsystems, Heerbrugg, Switzerland). The description of this method is expanded in the Online Data Supplement.

2.3. Cell culture and live-dead assay

Human coronary artery endothelial cells (HCAEC) were grown on Endothelial Growth Medium-2 (EGM-2, Lonza, Walkersville, MD, USA) and the human monocyte cell line THP-1 on DMEM supplemented with 10% fetal bovine serum. HCAEC or THP-1 was seeded on 6-well plates at a density of 2×10^5 cells/well. Then, cells were pre-treated with either CS (200 μg/mL) or prednisolone (10 μmol/L) for 24 h and continuously treated when stimulated with TNF-α (3 ng/mL) for 16 h. Conditioned media was obtained after additional 24 h with TNF-α free media and used for multiplex ELISA arrays of cytokines and chemokines (Quansys Biosciences, Logan, UT, USA). Cytotoxicity was tested using a Live/Dead assay (Life Technologies, Grand Island, NY, USA). The description of this method is expanded in the Online Data Supplement.

2.4. In vitro migration assay

Migration of the human monocyte cell line (THP-1 cells) to HCAEC, was quantified in 24-well plate Transwell inserts with a 5 μm porous membrane (Corning) with or without PMA (100 ng/mL) in the presence or absence of CS or prednisolone (200 μg/mL) or in the presence or the absence of TNF-α (3 ng/mL) for 16 h. Cell migration was allowed for 16 h. Nuclei of migratory cells on the lower side of the membrane were stained with 4′,6-diamidino-2-phenylindole (DAPI, Vectashield, Vector laboratories, Burlingame, CA) and quantified in 6 different fields per sextuplicate using the ImageJ software. The description of this method is expanded in the Online Data Supplement.

2.5. Western blotting

Cell samples were lysed in RIPA buffer solution (Sigma, St Louis, MO) containing a cocktail of protease inhibitors (Sigma P8340). Whole lysates were used afterward for the analysis of protein abundance of phosphoErk/Erk, phosphoJnk/Jnk and NFkB. The description of this method is expanded in the Online Data Supplement.

2.6. Gene expression analysis by real-time PCR

Briefly, total RNA from HCAEC and THP-1 was extracted using RNeasy kit (Gibco-Invitrogen, Paisley, UK). A 1 μg of total RNA was reverse transcribed using a complementary DNA synthesis kit (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, California, USA). Taqman primers and probes for gene expression assays were selected from Applied Biosystems for human IL-6, IL-8, TNF-α and IL-1β. The description of this method is expanded in the Online Data Supplement.

2.7. Statistical analysis

Data are expressed as mean ± standard error. Statistical analysis of the results was performed by one-way analysis of variance (ANOVA), the Newman–Keuls test, and the unpaired Student's t test when appropriate. Differences were considered to be significant at a p value of 0.05 or less. Data sampled from Gaussian populations were used for the calculation of Pearson correlation coefficient. A Pearson correlation coefficient (r) value of >0.75 was considered to exhibit strong positive correlation between two variables.