Atherosclerosis 244 (2016) 195–203

Contents lists available at ScienceDirect

Atherosclerosis

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

Mechanisms involved in extracellular matrix remodeling and arterial stiffness induced by hyaluronan accumulation

Karen Axelgaard Lorentzen [a,*], Song Chai [a,1], Hui Chen [a,2], Carl Christian Danielsen [b], Ulf Simonsen [c], Lise Wogensen [a]

[a] The Research Laboratory for Biochemical Pathology, The Institute of Clinical Medicine, Aarhus University, Aarhus University Hospital, Noerrebrogade 44, Bld. 3B -1, DK-8000 Aarhus C, Denmark

[b] Institute of Biomedicine, Aarhus University, Wilhelm Meyers Allé 3, Bld. 1233, Room 132, 8000 Aarhus C, Denmark

[c] Pharmacology, Institute of Biomedicine, Research and Education, East. Aarhus University Bartholins Allé 6, Bld. 1242, Room 341, 8000 Aarhus C, Denmark

ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form
19 October 2015
Accepted 16 November 2015
Available online 1 December 2015

Keywords:
Diabetic angiopathy
Atherosclerosis
Hyaluronan
Extracellular matrix
Arterial stiffness
Inter-α-Inhibitor
Vascular smooth muscle cells

ABSTRACT

Background and aims: Hyperglycemia induces hyaluronan (HA) accumulation in the vasculature. Excessive accumulation of HA around the vascular smooth muscle cells (VSMC) results in increased aortic stiffness and strength and accelerated atherosclerosis in $ApoE^{-}/^{-}$ mice. We hypothesized that HA accumulation primes the vasculature for atherosclerosis by crosslinking and reorganizing the extracellular matrix (ECM) and by pushing VSMC differentiation towards a less mature phenotype.

Methods: Aortas from HAS-2 transgenic (Tg) mice and wild type mice were used for all experiments. Biomechanics and cross-sectional area measurements were performed before and after HA digestion. The vessel and ECM composition was examined by immunoblotting and electron microscopy. Primary VSMC cultures were examined by qPCR and thymidine incorporation.

Results: Tg mice aorta cross-sectional area was increased before (14%, $p = 0.0148$), but not after HA digestion ($p = 0.3437$). The increase in vessel stiffness (32%, $p = 0.0217$) and strength (31%, $p = 0.0043$) in the Tg aorta persisted after HA digestion. Crosslinking of HA by heavy chains from Inter-α-Inhibitor was increased (175%, $p = 0.0006$). The Tg VSMCs have the appearance of a synthetic phenotype supported by a 40% decrease in α-smooth muscle actin isoform X1 ($p = 0.0296$) and an increase in proliferation (63%, $p = 0.0048$) and osteoprotegerin production (133%, $p = 0.0010$) in cultured Tg VSMCs.

Conclusions: Our results show that induced HA accumulation is followed by increased HA crosslinking and create a shift in VSMC phenotype and proliferation. These findings may provide a mechanism for how hyperglycemia through HA accumulation prime the vascular wall for cholesterol and leucocyte accumulation and development of atherosclerosis.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Both diabetic angiopathy and atherosclerosis are characterized by increased arterial stiffness and hyaluronan (HA) deposition in the large elastic arteries. Atherosclerosis presents with local accumulations of HA around the affected areas, whereas diabetic angiopathy displays disseminated HA accumulation in the tunica media [1].

HA is a large non-sulfated glycosaminoglycan present in the extracellular matrix (ECM). Via interactions with various proteins it has viscous, space filling and water binding properties. HA presents as one of two physical shapes, which determines its function; long cable-like structures or as a pericellular coat. The covalent attachment of heavy chains (HCs) from the serum protein inter-α-

Abbreviations: $ApoE^{-/-}$, apolipoprotein E knock-out; αSMA, α-smooth muscle actin; ECM, extracellular matrix; HA, hyaluronan; HAS-2, hyaluronan synthase 2; HC, heavy chain; HC-HA, the covalent complex of a heavy chain bound to hyaluronan; IαI, inter-alpha-inhibitor; LDL, low density lipoprotein; MMP, matrix metalloprotease; OPG, osteoprotegerin; Tg, transgenic; TSG-6, tumor necrosis factor alpha stimulated gene 6; VSMC, vascular smooth muscle cell; WT, wild type/non-transgenic.

* Corresponding author.

E-mail addresses: Karen.Lorentzen@clin.au.dk (K.A. Lorentzen), Chai_song@hotmail.com (S. Chai), Chenhuilu67@aliyun.com (H. Chen), Ccd@biomed.au.dk (C.C. Danielsen), us@biomed.au.dk (U. Simonsen), lwb@ki.au.dk (L. Wogensen).

[1] Current address: Dept. of Ophthalmology, 2nd Hospital of Hebei Medical University, 215 Heping west rd., Shijiazhuang 050000, Hebei, China.

[2] Current address: Dept. of Endocrinology, The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning 530021, Guangxi, China.

http://dx.doi.org/10.1016/j.atherosclerosis.2015.11.016
0021-9150/© 2015 Elsevier Ireland Ltd. All rights reserved.

inhibitor (IαI) enhance both the HA cable structures and HAs avidity for leukocytes [2–5].

Tumor necrosis factor α stimulated gene 6 (TSG-6) catalyzes the transfer and covalent attachment of HCs from IαI onto HA creating HC-HA complexes, which stabilize HA in the ECM. TSG-6 is associated with inflammation and is expressed during inflammation and inflammation-like processes. IαI is a Kunitz-type serine protease constitutively expressed in the liver and available in vast amounts in the blood stream [6,7].

Another factor able to accommodate atherosclerosis is elastin fragments within the ECM as they form stable complexes with LDL due to their hydrophobicity and enhance vascular smooth muscle cell (VSMC) proliferation. Fragments of elastin within the ECM decreases elastin expression, which correlates with a change in VSMCs towards a less mature phenotype [8].

Osteoprotegerin (OPG) is also involved in the remodeling of vascular structures e.g. by promoting VSMC proliferation and migration, despite it was originally discovered as a protein required in bone remodeling. There is no clear causal link between OPG and atherosclerosis since ApoE$^{-/-}$ mice lacking OPG have increased atherosclerosis [9]. On the other hand, OPG levels correlate positively with both intima media thickness and atherosclerosis [10–12]. OPG is upregulated during vascular disease and has been suggested as a marker of vascular inflammation and disease [13].

In the present study we hypothesized that HA accumulation induces VSMC differentiation towards a less mature phenotype and prepares the vascular bed for calcification by crosslinking and reorganizing the ECM to accommodate the changes. To address the hypothesis we used a mouse model with overexpression of HA synthase (HAS)-2 by the α-smooth muscle actin (αSMA) promoter, thereby targeting the change to the VSMCs. These mice are characterized by accumulation of HA throughout the tunica media, increased vessel stiffness and strength, thinning of the elastic membranes and elastin fragments dispersed in the extracellular matrix (ECM). Additionally we found that HAS-2 upregulation in the VSMCs accelerates atherosclerosis in ApoE$^{-/-}$ mice [14]. In the present study we investigated the pre-atherosclerotic changes induced by HA accumulation by studying markers of VSMC differentiation and crosslinking of HA by *in vitro* and *in vivo* methods.

We found a change in VSMC phenotype and a change in the surrounding ECM more accommodating for leukocytes and low density lipoprotein (LDL), and hence atherosclerosis.

2. Materials and methods

2.1. Animals

Unless otherwise stated we used 4–5 months old age- and sex-matched HAS-2 transgenic (Tg) and non-transgenic (WT) mice on the genetic background of C57BL/6J mice as previously reported [14]. An αSMA promoter was used to obtain expression of HAS-2 in the VSMCs. The mice were housed at the animal facility at the University of Aarhus, and handled according to the guidelines and procedures recommended by The Animal Experiments Inspectorate, Denmark. They were kept at 21 °C with a 12 h day/night cycle and were given free access to standard chow and water. The project was approved by the Animal Experiments Inspectorate, Denmark (#2003/561-682, #2012-15-2935-00003) and the Danish Working Environment Service (#20090005631/2, #200300194040/3).

2.2. Biomechanical properties and mechanical testing with and without hyaluronidase

Freshly excised aortas from 11 to 12-months-old HAS-2 Tg (n = 5) and WT (n = 6) mice were cleaned of adhering fat and loose connective tissue, coded, and then cut into one mm high ring specimens (in average 10–13 specimens per aorta). They were soaked in 50 mM Tris–HCl, pH 7.4 (Sigma-Aldrich, St. Louis, MO, USA), and stored at −20 °C until analysis. For each triplet of rings in cranial-caudal direction (three triplets from each aorta), rings were randomized into three groups: A, B and C. The ring specimens were subjected to height (width) and wall thickness determination, as measured on a tapered capillary tube using a Nikon microscope (magnification: ×40, Nikon, Tokyo, Japan) (circular polarizer) with an ocular ruler (1 unit = 20.8 μm), placed in buffer reservoir between two parallel slides. Afterward the specimens were transferred to fresh buffer and mechanically tested in plate A: without treatment, B: after incubation in 0.1 M Tris–HCl, pH 7.4 (Sigma-Aldrich, St. Louis, MO, USA), 1 mM 1,10-phenanthroline (Sigma-Aldrich, St. Louis, MO, USA), and 10 μg ovomucoid/ml (T9253, Sigma-Aldrich, St. Louis, MO, USA), or C: after incubation in 0.1 M Tris–HCl, pH 7.4 (Sigma-Aldrich, St. Louis, MO, USA), 1 mM 1,10-phenanthroline (Sigma-Aldrich, St. Louis, MO, USA), 10 μg ovomucoid/ml (Sigma-Aldrich, St. Louis, MO, USA) and Streptomyces hyalurolyticus (0.2 U/ml) (Sigma-Aldrich, St. Louis, MO, USA). B and C were incubated at 30 °C on a tilting surface (2/min) for 24 h. Fresh 50 mM Tris–HCl, pH 7.4 (Sigma-Aldrich, St. Louis, MO, USA) was added for wash of the specimens. Measurements of height and wall thickness were repeated before mechanical testing. Plates were kept at room temperature during this procedure. The biomechanical analysis was performed as previously described [14]. Briefly, the specimens were stretched (10 mm/min) until failure by wires through vessel lumen and the maximal load and maximal stiffness were calculated from the recorded load-deformation values and load-strain curves. In calculation of strain, the original circumference of the ring specimen was defined as the circumference at which the specimen attained a minimal load (1% of maximum). Mean values of the triplicates were obtained for each mouse before group means were calculated.

2.3. Western blot

The heart and the entire aorta were excised and the aorta cleaned of adventitia. Two aortas of the same gender and genotype were pooled and the tissue was homogenized in 300 μl sample buffer (0.1% SDS (VWR International, Radnor, PA, USA), 1% NP-40 (Calbiochem, San Diego, CA, USA), 0.5% sodium deoxycholate (VWR International, Radnor, PA, USA), 100 mM NaCl (VWR International, Radnor, PA, USA), 0.1 mM sodium orthovanadate (Sigma-Aldrich, St. Louis, MO, USA), 1 mM sodium fluoride (Sigma-Aldrich, St. Louis, MO, USA), 50 mM Tris–HCl, pH 7.4 (Sigma-Aldrich, St. Louis, MO, USA)) containing a mixture of protease inhibitors (10 μg/ml aprotinin (Sigma-Aldrich, St. Louis, MO, USA), 10 μg/ml leupeptin (Sigma-Aldrich, St. Louis, MO, USA), 5 μg/ml pepstatin A (Sigma-Aldrich, St. Louis, MO, USA), and 10 μg/ml PMSF (Sigma-Aldrich, St. Louis, MO, USA)). Samples were centrifuged to pellet cell debris. The protein concentration was measured by the MicroBCA™ Protein Assay Reagent Kit (Pierce, Rockford, IL, USA).

For testing the presence of HC2-cross-linking equal amounts of total protein (20 μg) were non-treated or treated with either 5 units hyaluronidase (Streptomyces hyalurolyticus H1136, Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 37 °C, or 1 M NaOH (VWR International, Radnor, PA, USA) for 20 min at room temperature and the reaction was stopped with 4× volume 2 M Tris pH 7.6 (Sigma-Aldrich, St. Louis, MO, USA). The proteins were resolved by electrophoresis on a 4–12% SDS-polyacrylamide gel (4–12% acrylamide (40%, VWR International, Radnor, PA, USA), 0.375 M Tris-base pH 8.8 (Sigma-Aldrich, St. Louis, MO, USA), 0.5% SDS (VWR International, Radnor, PA, USA), 0–25% glycerol (Sigma-Aldrich, St. Louis, MO, USA), 0.5–1.25% ammonium persulfate (Sigma-Aldrich, St.

Download English Version:

https://daneshyari.com/en/article/5943684

Download Persian Version:

https://daneshyari.com/article/5943684

Daneshyari.com