Atherosclerosis 209 (2010) 96–103

Contents lists available at ScienceDirect

Atherosclerosis

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

Cathepsin K gene disruption does not affect murine aneurysm formation☆

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ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form 27 August 2009
Accepted 1 September 2009
Available online 6 September 2009

Keywords:
Cathepsin K
Aneurysm
Elastolytic capacity
Collagenolytic activity
Angiotensin II

ABSTRACT

Cathepsin K (catK), a lysosomal cysteine protease, exerts strong elastinolytic and collagenolytic activity and is implicated in a range of pathological disorders including cardiovascular disease. CatK expression was found to be elevated in human aortic aneurysm pointing to a role in this vasculopathy. In the angiotensin II (Ang II)-induced mouse model for aneurysm formation, catK, S and C expression was strongly upregulated. Therefore, we investigated the effect of catK deficiency on Ang II-induced aneurysm formation in the abdominal aorta of apoE−/− mice.

Contrary to our expectations, catK deficiency did not protect against aneurysm formation, nor did it affect medial elastin breaks. Proteolytic activity in abdominal aortic lysates were comparable between apoE−/− and catK−//−apoE−/− mice. Adventitial presence of catS- and catC-expressing cells was significantly increased in catK−/−//apoE−/− versus apoE−/− mice, which might have compensated for the deficiency of catK-derived proteolysis in the aneurysm tissue of catK deficient apoE−/− mice. Circulating granulocytes and activated T cell numbers were significantly increased in Ang II-infused catK−/−//apoE−/− mice, which is consistent with the borderline significant increase in adventitial leukocyte content in catK−/−//apoE−/− compared to apoE−/− mice. Strikingly, despite unchanged proteolytic activity in AAA lesions, collagen content in the aneurysm was significantly increased in catK−//−apoE−/− mice. In conclusion, while catK deficiency has major impact on various vasculopathies, it did not affect murine aneurysm formation.

1. Introduction

Abdominal aortic aneurysms (AAAs) are permanent dilations of the arterial wall in the abdominal aorta [1], which after rupture can cause life-threatening bleeding. The prevalence of AAA in western society, as diagnosed by means of autopsy, ultrasound screening, and hospital discharge data, is substantial, ranging from 19–34% in women to 66–81% in men [2]. Women are usually older when they undergo AAA repair [2]. Although a history of atherosclerotic disease predisposes to aneurysm formation, several other factors contribute to its ontogenesis, including genetic predisposition, inflammation, hypertension and hyperlipidemia [3,4].

Excessive degradation of extra cellular matrix (ECM) components such as elastin and collagen [5,6], a critical process in AAA, was shown to be at least in part dependent on a local disbalance between matrix metalloproteinases (MMPs) and cognate inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Indeed doxycycline, a broad spectrum MMP inhibitor, was shown to prevent angiotensin II (Ang II)-induced aneurysm formation in hyperlipidemic LDL receptor deficient mice [7]. In addition to MMP, other cysteine proteases like cathepsins are involved in ECM degradation [8].

Cathepsin K (catK) is a papain-like cysteine protease that has a distinct ECM degrading potential. It is one of the most potent elastases in mammals, and in addition harbors unique collagenolytic activity [9–12]. In AAA wall, protein levels and activities of catK, L and S were elevated compared to that in healthy arteries, whereas levels of the endogenous cysteine protease inhibitor cystatin C were decreased [13–15]. In a previous study, we already showed that catK deficiency in apoE−/− mice reduced plaque progression with a concomitant increase in plaque collagen content and macrophage size [16]. In addition, the number of elastin breaks in the media underlying the atherosclerotic plaque was sharply decreased in

☆ The authors (MJAPD, KC, EALB, SH) participate in the European Vascular Genomics Network (http://www.evgn.org), a Network of Excellence supported by the European Community's Sixth Framework Program for Research Priority 1 (Life Sciences, Genomics, and Biotechnology for Health; contract LSHM-CT-2003-503254). This study was supported by grant 2003T201 from the Netherlands Heart Foundation (EALB).

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doi:10.1016/j.atherosclerosis.2009.09.001

advanced lesions of catK−/−//apoE−/− compared to apoE−/− mice. Taken together, the profound effect of catK on atherosclerosis, an established risk factor for AAA, and its enhanced expression in human aneurysms point to a potentially important, adverse role for catK in aneurysm formation.

This hypothesis was tested in an established mouse model of AAA in which aneurysm formation is induced by sustained subcutaneous infusion of Ang II in apoE−/− and catK−/−//apoE−/− mice. Surprisingly, our data showed that catK deficiency neither reduced aneurysm formation nor reduced proteolytic activity in abdominal aortic lysates, although we did notice increased collagen content in the aneurysm itself. Remarkably, catS and C staining revealed a more abundant presence of catS- and catC-positive cells in the adventitia of catK deficient animals. Apparently, in the absence of catK, other cysteine proteinases may compensate for the loss in catK-derived proteolytic activity in the arterial wall. Moreover catK deficiency dramatically increased circulating granulocyte and activated T cell numbers in Ang II-infused but not in untreated mice, which might counteract the potentially protective pro-fibrotic effect of catK deficiency in AAA.

2. Methods

2.1. Animals

Male apoE−/− mice on a C57Bl6 background were obtained from IffaCredo (Lyon, France). The catK−/− mice, kindly provided by Dr P Saftig, were generated on an outbred 129SVJ-C57BL/6J genetic background [17]. After import, we subsequently intercrossed the catK−/− mice at least 9 times with apoE−/− mice to generate catK−/−//apoE−/− mice and syngenic catK+/+//apoE−/− control mice. Animals were maintained in accordance with the Dutch government guidelines and animal experiments were approved by the regulatory authority of the University of Maastricht. Mice were fed a normal chow diet throughout the experiment.

2.2. Induction of aneurysm formation by chronic Ang II infusion

Aneurysm formation was induced by subcutaneous Ang II infusion [18]. Alzet mini-osmotic pumps (model 2004; ALZA Scientific products, Mountain View, California, USA) were implanted into apoE−/− (n = 19 mice) and catK−/−//apoE−/− mice (n = 20 mice) at 10 weeks of age. Pumps were filled with Ang II (Sigma Chemical Co., St. Louis, MO, USA) dissolved in phosphate buffered saline (10 mM sodium phosphate, 150 mM sodium chloride; pH 7.4) to mediate the subcutaneous delivery of 1 ng/g/min of Ang II for 28 days. Pumps were placed subcutaneously in the neck of anesthetized mice through a small incision that was closed using a silk suture (5-0).

2.3. Tissue harvesting and histological analysis

After 4 weeks of Ang II infusion, mice were sacrificed and the arterial system was perfused with PBS containing 0.1 mg/ml nitroprusside (Sigma, St. Louis, MO) through a catheter inserted into the left cardiac ventricle and subsequently with 1% paraformaldehyde. The aortic piece comprising the ascending aorta to the aortic bifurcation was fixed overnight in 1% paraformaldehyde and embedded in paraffine. We also investigated aortic tissue from mice which died before sacrificing. Ten cross-sections (4 μm thick) were cut at 200 μm intervals throughout the abdominal aorta starting at the middle of the aneurysm. From each level, a cross-section was stained with Elastica-von Gieson (EvG) for total vessel area and number of elastic lamina breaks. Transmural breaks (complete medial disruption) and more focal destructions of single elastic lamina were both scored as elastin break but at a different extent. More specifically, a transverse section containing a focal destruction of a single elastic lamina was scored as one break; while a transmural break encompassing four to five elastic lamina breaks were counted as four or five breaks, respectively.

The relative collagen area in the media and the aneurysm area, i.e. the percentage of total area that stained positive for Sirius red, was determined under a microscope coupled to a computerized morphometry system (Quantimet 570, Leica). Morphometric analysis was performed by one blinded investigator (LBai; intra-observer variability was <10%).

2.4. Quantification of aneurysm formation

Severity of aneurysms in the suprarenal region of the aorta was analyzed according to Daugherty et al. [19]. In this scoring system, a healthy artery not obviously different from untreated apoE-KO mice was classified type 0, a dilated aorta/lumen without thrombus as type I; remodeled tissue often containing thrombus as type II; a pronounced bulbous form of type II containing thrombus as type III; while lesions with multiple aneurysms containing thrombus as type IV. A numerical score was allocated to each mouse. For statistical comparison of disease progression stages, a semi-quantitative pathology score was calculated by averaging the numerical aneurysm score of apoE−/− and catK−/−//apoE−/− mice [20].

2.5. Immunohistochemistry

Ruptured aneurysm lesions (containing fatal and non-fatal rupture) were used to study lesion morphology. Sections were stained with the following antibodies: MAC3 rat monoclonal antibody (1:30, BD-Biosciences Pharmingen) to detect macrophages, CD45 (1:5000, BD-Biosciences Pharmingen) to detect leukocytes, α-smooth muscle actin (α-SMA, 1:500, Dako) to detect smooth muscle cells and Ly-6G (1:200, BD-Biosciences Pharmingen) to detect granulocytes. Immunostaining of catS and catC were performed using rabbit anti-mouse catS (1:500, Calbiochem) and goat anti-mouse catC (1:30, Santa Cruz, California) antibodies. Mouse lymph node was used as positive control for CD45 staining, mouse spleen as positive control for Ly-6G staining, and mouse atherosclerotic lesions as positive control of both Mac3 and catS/C staining. Deletion of the primary antibody served as negative control and did not show any staining for any of the antibodies.

The section containing the largest aneurysm area was used to quantify the number of CD45+, MAC3+ and Cats/C+ cells. The relative macrophage, leukocyte and granulocyte content in aneurysm and adventitial area was calculated by correcting the MAC3+ and CD45+ cell number for the total cell content of aneurysm and adventitia, respectively. As Ly-6G+ cells were not present in every slide, the number of Ly6G+ cells counted in 6 slides was divided by the sum of 6 corresponding aneurysm lesion area. CatC+ and catS+ cells were primarily detected in the adventitia of the aneurysm lesion. To quantify the relative catC+ and catS+ cell density, the number of catC+ and catS+ cells in either the aneurysm region or in the adventitia was divided by the total number of cells in the aneurysm and adventitia, respectively. All measurements were conducted by one investigator (LBai; intra-observer variability was <10%).

2.6. Bone marrow-derived macrophage isolation and culturing

Bone marrow–derived macrophages were isolated from the femur and tibia of apoE−/− mice. Cells were cultured in standard RPMI containing L-glutamine, HEPES, 10% fetal calf serum, 100 IU/ml penicillin/streptomycin, and 15% L929 cell conditioned medium. After 7 days of culturing, bone marrow-derived