Influence of Cardiovascular Risk Factors on Levels of Matrix Metalloproteinases 2 and 9 in Human Abdominal Aortic Aneurysms

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WHAT THIS PAPER ADDS

The results in this paper indicate that active smoking influences vascular cells to increase MMP-2 expression, which contributes to expansion and the rupture risk of AAA. This finding prompts more aggressive control of cardiovascular risk factors in the very early phase of the disease, and particularly AAA screening in smokers. This could even modify monitoring guidelines for AAA in the non-surgical range, and the approach should be more aggressive in patients who continue to smoke. It allows for the possibility that active smokers have a higher risk of rupture than those with the same arterial diameter who do not smoke or who have stopped smoking.

Objective: To evaluate the influence of cardiovascular risk factors on levels of matrix metalloproteinases (MMP) 2 and 9 in human abdominal aortic aneurysms (AAA).

Methods: Aortic samples were collected from patients who underwent AAA repair ($n = 89$). Patients were stratified according to the maximum transverse aorta diameter: small diameter (<55 mm), moderate diameter (55—69.9 mm) and large diameter (≥70 mm). Aortic walls were studied using real-time PCR and immunohistochemistry. MMP-2, MMP-9, α-actin, CD45, and CD68 transcript levels were determined relative to β-actin. Quantitative data were expressed as median (IQ-range).

Results: No differences were found in MMP-2 expression between the patient groups, which was mainly associated with vascular smooth muscle cells (VSMC); however, MMP-9 displayed the maximum level in the moderate-diameter group, associated with infiltrating macrophages. Current smoking (CS) and renal insufficiency (RI) significantly increased local levels of MMP-2 (CS 349.5 [219.5—414.1] vs. no-CS 184.4 [100.0—320.5]; $p < .008$; RI 286.8 [189.6—410.8] vs. no-RI 177.3 [99.3—326.9]; $p = .047$). Nevertheless, after stepwise linear regression analysis only CS remained as an independent variable predicting local levels of MMP-2 ($p = .002$). No risk factors influenced local levels of MMP-9.

Conclusions: The results show that local levels of MMP-2, an important factor for AAA development, were increased in current smoking AAA patients. MMP-2 was mainly associated with VSMC. It is suggested that MMP-2 could contribute significantly to the increased AAA growth rate observed in current smoking patients. These findings support inclusion of smokers in screening for aneurysmal disease, and emphasize the need for more aggressive monitoring of aneurysmal disease outside the surgical range in patients who smoke at the time of diagnosis and in those who continue to smoke during follow-up.

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INTRODUCTION

Abdominal aortic aneurysm (AAA) is a common, late age onset disorder in industrialized countries. Rupture of AAA has very high mortality rates. Screening studies have suggested that the prevalence of AAA in older men is about 5%.[1] Despite the aging of the population, the prevalence, incidence, and global mortality rate of AAA appear to have declined as a result of increased elective AAA repair, lower mortality from emergency repair, and a greater proportion of emergency admissions being offered emergency repair.[2,3]

The etiology of AAA is complex, but environmental and genetic factors are known to contribute to the risk.[4,5] The relationship between AAA and atherosclerosis is intriguing. Both disorders share some common features and AAA usually occurs in atherosclerotic individuals. Not only are there evident histopathological differences between AAA and atherosclerotic lesions, but differences have also been observed in cardiovascular risk factors. Diabetes, for example, is a well-established risk factor for atherosclerosis,

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but it has been reported to be protective for AAA,[6,7] whereas smoking seems to be a substantially greater risk factor for AAA than for atherosclerosis,[7,8] and chronic occlusive pulmonary disease (COPD) has been associated with AAA.[9]

Current evidence on the pathogenesis of AAA indicates that aortic wall inflammation and proteolytic degradation of the aortic wall play a fundamental role in the evolution and progression of the disease. A number of reports have documented increased expression of matrix metalloproteinases (MMP) in AAA, and genetic variants have been proposed to be associated with AAA.[10] The MMPs most prominently associated with AAA are MMP-2 (Gelatinase A) and MMP-9 (Gelatinase B),[11–14] both of which display elastolytic activity and similar proteolytic activity.[15] One fundamental characteristic of AAA is the hypervascularization of aortic tissue. It has been proposed that this vascularization might contribute to the development of aneurysms[16] and could play a causative role in the genesis of AAA.[11,17] As several studies have pointed to the essential role of MMP-2 and MMP-9 in the onset of angiogenesis,[18,19] local levels of MMP-2 and MMP-9 could be relevant in AAA progression.

Information is lacking on the influence of cardiovascular risk factors on local levels of MMPs in AAA. The aim of the present study was to evaluate the influence of the major cardiovascular risk factors on MMP-2 and MMP-9 levels in human AAA.

METHODS

Patients

The inclusion criteria for this study were patients undergoing elective open repair for atherosclerotic AAA. An infrarenal aorta biopsy was taken during the intervention. The exclusion criteria were absent or inadequate aortic biopsy and patients with pseudoaneurysms, or infectious or inflammatory aneurysms. Patients were stratified according to the maximum transverse aorta diameter determined by the use of a measurement transverse to the true lumen center line at infrarenal level, based on angio-CT with endovenous contrast, using Workstation AGFA IMPAX 6.4.0.4010 and OsiriX MD, FDA Cleared/CE IIa version, as a primary diagnostic. Three groups were defined: small diameter (SD, $<$55 mm), moderate diameter (MD, 55–69.9 mm) and large diameter (LD, $\geq$70 mm). Surgical repair is not usually indicated when the maximum transverse aortic diameter is $<$55 mm. The SD samples in this study were obtained from patients with maximum transverse aorta diameter $<$55 mm, who underwent surgery for a symptomatic or rapidly growing AAA ($>$5 mm/6 month or $>$10 mm/year) or for repair of a concomitant iliac artery aneurysm.

All patients underwent surgery at Hospital de la Santa Creu i Sant Pau (HSCSP). The study was approved by the local ethics committee, and patients gave written informed consent prior to surgery. All procedures were reviewed by the institutional review board at HSCSP. The study conformed to the principles of the Declaration of Helsinki. Clinical outcomes were taken from the clinical database.

Tissue samples

Biopsies were systematically obtained from the anterolateral wall of the remaining mid-infrarenal aortic wall after exclusion and prosthetic replacement of the AAA, at the level of the inferior mesenteric artery. Luminal thrombi, if present, were separated before the aortic biopsy was taken. Biopsies were processed immediately. A portion of each sample was placed in RNAlater solution (Qiagen GmbH, Hilden, Germany) and stored at 4 °C for 24 hours before long-term storage at −80 °C until further processing for RNA isolation. When possible, another portion was fixed in 10% formalin solution (Sigma-Aldrich, Inc St Louis, MO,USA) for 24 hours and embedded in paraffin for microscopic studies to locate the MMPs.

Endothelial cells and VSMC culture

Aortic human VSMC cultures were established from multiorgan donor aortas using an explant procedure as previously described.[20,21] VSMC were characterized by α-actin positive staining and used at passage 4-6. Endothelial cells were isolated by collagenase digestion from human umbilical cord veins (HUVEC) and cultured as previously described.[22] Cells were characterized by vWF positive staining and used at the first passage.

Risk factors

The risk factor definitions used in this study were: diabetes mellitus: glycated hemoglobin $>$5.8% or use of oral antidiabetic drugs or insulin; arterial hypertension (HTN): systolic blood pressure $\geq$140 mm Hg, diastolic blood pressure $\geq$80 mm Hg or use of antihypertensive medication; hyperlipidemia: a total cholesterol $>$6.2 mmol/L, LDL cholesterol $>$1.70 mmol/L or triglycerides $>$1.65 mmol/L; smoking was categorized into two groups: current smoking (CS): smokers and ex-smokers stopped smoking $<$1 year, and non-current smoking (N-CS): never-smoked and ex-smokers stopped smoking $>$1year; COPD: FEV1/FVC $<$0.7; and renal insufficiency (RI): estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation considering values of eGFR $\leq$60 mL/min/1.73 m^2 as RI.[23]

Analysis of mRNA levels in tissues and culture cells

Tissues were homogenized in the FastPrep-24 homogenizer and Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA). RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. For culture cells, total RNA was extracted using Ultraspec (Biotecx Laboratories, Inc., Houston, TX, USA) according to the manufacturer's instructions. cDNA was prepared by reverse transcribing 1 μg RNA with a High-Capacity cDNA Archive Kit with random hexamers (Applied Biosystems, Foster City, CA, USA). mRNA expression of the selected