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Association of Matrix Metalloproteinase Levels with Collagen Degradation in the Context of Abdominal Aortic Aneurysm

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WHAT THIS PAPER ADDS

This study attempts to clarify the role of matrix metalloproteinases (MMPs) in collagen degradation in abdominal aortic aneurysm (AAA) wall and compares the expression of MMPs at the mRNA and protein levels. Interestingly, MMPs showed no correlation between mRNA and protein, indicating that MMPs are differently regulated and their activity is apparently independent of their expression at mRNA level. Furthermore, the data show that collagen III seems to be the most important collagen in advanced AAA to maintain the aortic wall integrity. At the protein level, MMP-8, MMP-9, and MMP-12, in particular, seem to be associated with collagen I and collagen III and their degradation.

Objective/Background: Matrix metalloproteinases (MMPs) have already been identified as key players in the pathogenesis of abdominal aortic aneurysm (AAA). However, the current data remain inconclusive. In this study, the expression of MMPs at mRNA and protein levels were investigated in relation to the degradation of collagen I and collagen III.

Methods: Tissue samples were obtained from 40 patients with AAA undergoing open aortic repair, and from five healthy controls during kidney transplantation. Expression of MMPs 1, 2, 3, 7, 8, 9, and 12, and tissue inhibitor of metalloproteinase (TIMP)1, and TIMP2 were measured at the mRNA level using quantitative reverse transcription polymerase chain reaction. At the protein level, MMPs, collagen I, and collagen III, and their degradation products carboxy-terminal collagen cross-links (CTX)-I and CTX-III, were quantified via enzyme linked immunosorbent assay. In addition, immunohistochemistry and gelatine zymography were performed.

Results: In AAA, significantly enhanced mRNA expression was observed for MMPs 3, 9, and 12 compared with controls ($p \leq .001$). MMPs 3, 9, and 12 correlated significantly with macrophages ($p = .007$, $p = .018$, and $p = .015$, respectively), and synthetic smooth muscle cells with MMPs 1, 2, and 9 ($p = .020$, $p = .018$, and $p = .027$, respectively). At the protein level, MMPs 8, 9, and 12 were significantly elevated in AAA ($p = .006$, $p = .0004$, and $p < .001$, respectively). No significant correlation between mRNA and protein was observed for any MMP. AAA contained significantly reduced intact collagen I (twofold; $p = .002$), whereas collagen III was increased (4.6 fold; $p < .001$). Regarding degraded collagen I and III relative to intact collagens, observations were inverse (1.4 fold increase for CTX-1 [$p < .001$]; fivefold decrease for CTX-III [$p = .004$]). MMPs 8, 9, and 12 correlated with collagen I ($p = .019$, $p < .001$, and $p = 0.003$, respectively), collagen III ($p = .015$, $p < .001$, and $p < .001$, respectively), and degraded collagen I ($p = .012$, $p = .049$, and $p = .001$, respectively).

Conclusion: No significant relationship was found between mRNA and protein and MMP levels. MMPs 9 and 12 were overexpressed in AAA at the mRNA and protein level, and MMP-8 at the protein level. MMP-2 was detected in synthetic SMCs. Collagen I and III showed inverse behaviour in AAA. In particular, MMPs 8, 9, and 12 appear to be associated with collagen I, collagen III, and their degradation products.

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INTRODUCTION

Recent data state the prevalence of abdominal aortic aneurysm (AAA) to be between 1.7% and 12.7%.[1–4] The main risk of AAA is that it remains asymptomatic over years. However, in the case of rupture the estimated total mortality rate is 78–83%.[5–7] In addition, approximately one third of patients with ruptured AAA die before reaching

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hospital. Even in hospital, the mortality of ruptured AAA ranges from 28.4% to 29.9% for endovascular repair to 40.8% to 43.9% for open surgical repair.[8,9] Thus, timely recognition of patients at such risk is essential. Currently, only the diameter of the aneurysm is an established diagnostic tool with which to evaluate the risk of rupture in clinical practice.[10] For more individualised treatment of patients with AAA, other parameters for risk stratification are necessary. Of course, this requires a comprehensive understanding of AAA pathogenesis and pathophysiology. In general, the aneurysmal aortic wall shows several decisive changes compared with healthy tissue. Accumulation of inflammatory cells, neovascularisation, degradation of collagen, loss of elastic fibres, and apoptosis of smooth muscle cells result in severe remodelling of the extracellular matrix (ECM) of the aortic wall, especially within the tunica media and adventitia,[10] in turn increasing wall stiffness and thinning. Continuous augmentation of mechanical stress and strain finally exceeds the stability of the AAA wall, which ultimately leads to rupture and consequent clinical events.[10,11]

The vessel wall remodelling is especially mediated by various matrix metalloproteinases (MMPs).[11–13] These proteolytic enzymes play an important role in the homeostasis of ECM in healthy tissues, as well as in the formation of aneurysms.[12] Chase and Newby indicated that three main characteristics of MMPs lead to their decisive pathogenic role in vascular diseases: the ability to cleave fibrillar collagen, the ability to degrade the whole spectrum of ECM proteins, and the ability to be activated in a cascade.[13]

Many studies have already focused on the expression of MMPs in AAA. Comparative data regarding expression of these proteases at mRNA and protein level, as well the comparison of their expression and occurrence with the amount of collagen and their degradation products within AAA wall, are still lacking.[14,15] Abdul-Hussien et al. analysed MMPs 1, 8, 9, 13, and 14, and measured degradation of collagen I.[14] The results showed enhanced collagen I turnover in correlation with MMP-8. Besides collagen I, collagen type III is the second fibrillar collagen present in the aortic wall.[16] Bode et al. found increased collagen type III in AAA comparing type III collagen in aortic occlusive disease, AAA, and healthy controls.[15]

So far, most studies have focused on the expression of MMPs at the mRNA level, or have analysed blood of affected patients for surrogate markers. Few studies have measured MMPs at protein level. Furthermore, there is still a lack of information about the relationship between mRNA and protein, particularly with regard to MMPs.

Therefore, in the present study, the main focus was on the main fibrillar collagens of the vessel wall (collagen I and collagen III),[16] their degradation products, and relevant MMPs (at mRNA and protein levels), known to play an important role in AAA: collagenases (MMPs 1 and 8) cleaving intact collagen; gelatinases (MMPs 2 and 9) cleaving denatured collagen; stromelysins (MMPs 3 and 7) with a broad substrate specificity; and macrophage elastase MMP-12. The aim was to identify relevant MMPs in the pathogenesis of AAA and their individual roles in the degradation of collagen I and collagen III.

MATERIALS AND METHODS

Patients and tissue specimens

Samples of human aortic tissue were collected from the Department of Vascular and Endovascular Surgery at Klinikum rechts der Isar (Munich, Germany) from patients scheduled for open aortic repair from the anterior sac of the infrarenal abdominal aorta between September 2012 and October 2014. In total, 40 patients with AAAs and nine control samples from kidney donors during kidney transplantations at the Department of General Surgery, Klinikum rechts der Isar, were included. The tissue samples were embedded in paraffin for histological analysis, or frozen in liquid nitrogen and stored at −80 °C for polymerase chain reaction (PCR) and protein analysis within 2 h of surgical excision. Patient demographic data, risk factors, comorbidities, and medication are shown in Table 1. The study was performed according to the Guidelines of the World Medical Association Declaration of Helsinki. The local ethics committee approved the study, and written informed consent was given by all patients.

RNA extraction and quantitative reverse transcription PCR

After mechanical homogenisation of the frozen tissue, RNA was isolated with RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Next, cDNA was synthesised with RevertAid First Strand

Table 1. Patient characteristics.

	AAA (n = 40)	Control (n = 5)
Sex		
Female	6 (15)	1 (20)
Male	34 (85)	4 (80)[a]
Median (range) age (y) median (range)	67 (40–86)	57 (47–66)[a]
Mean ± SD diameter (mm)		
Female	51 ± 7	
Male	70 ± 19	
Ruptured aneurysms	9 (23)	
Hypertension	30 (75)	
Smokers	12 (30)	
Hyperlipidaemia	15 (38)	
Hypercholesterolaemia	2 (5)	
Coronary heart disease	6 (15)	
Peripheral arterial disease	4 (10)	
Chronic kidney insufficiency	9 (23)	
Diabetes mellitus	2 (5)	
ASA or clopidogrel	30 (75)	
Beta-blockers	26 (65)	
ACE inhibitors	21 (53)	
Diuretics	18 (45)	
Statins	22 (55)	

Note. Data are n (%) unless otherwise indicated. ASA = acetylsalicylic acid; ACE = angiotensin converting enzyme. [a] No significant differences.

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