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Research Paper

Impaired mechanics and matrix metalloproteinases/inhibitors expression in female ascending thoracic aortic aneurysms

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ABSTRACT

We hypothesized that female gender may have a specific negative impact on the mechanical characteristics, composition, and expression of matrix metalloproteinases/tissue inhibitors (MMPs/TIMPs) in the wall of ascending thoracic aortic aneurysms (ATAAs). Degenerative ATAAs were resected from 35 patients (age: 67±2 years, male: 20, ATAA diameter: 5.5±0.1 cm) undergoing elective surgery. Tissue specimens were grouped by gender, region, and direction and submitted to immunohistochemistry for semi-quantitative assessment of MMP-2, MMP-9, TIMP-1, and TIMP-2 expressions, i.e. of staining intensity in extracellular matrix and immunoreactivity in vascular cells, as well as to histology for quantitation of elastin/collagen contents. Biomechanical characterization by the Fung-type model and examination of failure properties was performed. Gender differences in patient age, ATAA diameter, and ATAA diameter/body-surface area were non-significant. Increased MMP-2 and MMP-9, and decreased TIMP-1 and TIMP-2 expressions were observed in females. Elastin/collagen contents were higher in males than females, as was failure stress in circumferential but not longitudinal specimens. In both directions, failure stretch was invariant, while the Fung-type model parameters and elastic moduli calculated at physiologic stress levels were higher in females, suggestive of increased wall stiffness compared to males. MMP and TIMP expressions did not differ with region, unlike failure stress longitudinally that was greater posteriorly than anteriorly. The female gender is associated with impaired ATAA strength and increased stiffness, relating to the more extensive extracellular matrix breakdown and significantly higher ratio of MMP/TIMP expression witnessed in females. The present data may aid to identify the underlying pathophysiology accountable for the higher rupture risk, documented by epidemiologic studies in females.

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1. Introduction

The ascending thoracic aortic aneurysm (ATAA) is a lethal pathology. Notwithstanding the continuous advances in diagnostic and surgical interventions, ATAA rupture and dissection carries a high degree of morbidity, mortality, and medical cost. Despite the predominance of male over female patients with ATAA, a higher incidence of ruptures has been documented by epidemiologic studies in females. Specifically, Coady et al. (1997) and Davies et al. (2002) have reported that the yearly and cumulative rates of aortic dissection or rupture before operative repair rise notably with aortic diameter above the 5-cm threshold; for women, multivariate analysis showed a higher probability of negative events. Juvonen et al. (1997) have disclosed a greater proportion of females among patients with ruptured TAA in univariate analysis that was not apparent in their multivariate model. The association of females with lethal outcomes may be reminiscent of body size differences, assuming that a particular aortic dimension represents a larger size and thus greater threat in females, whose body frame is smaller. Accounting, however, for body-surface area in gender differences of aortic complications, Davies et al. (2006) deduced that the extra risk accompanying women was not only an epiphenomenon of their smaller size.

Causative factors other than body size per se may underlie the higher risk in females, e.g. an imbalanced activity of inflammatory mediators and matrix metalloproteinases (MMPs) (Ailawadi et al., 2004) and a more pronounced age-related stiffening of proximal aorta in women (Waddell et al., 2001); both effects consistent with changes in female hormonal status. MMPs serve to degrade the extracellular matrix, assuming pivotal roles in ATAA pathogenesis. They are a family of zinc-proteolytic endopeptidases, sharing homologous protein sequences, with conserved domain structures and specific domains, associated to substrate specificity and recognition of other proteins (Nagase et al., 2006). Owing to their specificity for elastin and collagen, MMP-2 and MMP-9 (gelatinase A and B), and their endogenous tissue inhibitors TIMP-1 and TIMP-2 (Freestone et al., 1995) that modulate the activity of those matrix-degrading enzymes, are important regulators of arterial micro-architecture and, thereby, of ATAA pathogenesis.

Our hypothesis was that gender directly affects the biomechanical strength and stiffness of ATAAs. The protective role of male gender may be attributable to changes in these characteristics, as rupture is a biomechanical event arising when the hemodynamic loads acting on the aortic wall exceed its strength (Vorp, 2007). Our study sought to assess gender differences in the mechanical characteristics by tensile-testing. Elastin/collagen contents were also determined in relation to region via quantitative histology, together with semi-quantitation of MMP-2, MMP-9, TIMP-1, and TIMP-2 expressions through immunohistochemical staining, serving to substantiate our mechanical findings.

2. Material and methods

2.1. Aortic tissue collection

Whole degenerative ATAAs were resected (67 ± 2 years age; 20/15 male/female ratio [60% male]; 5.5 ± 0.1 cm ATAA diameter) during elective surgery at the Department of Cardiothoracic Surgery of the Athens Medical Center over a 32-month period. Informed consent was obtained from the patients and the study was approved by the institutional review board. Patients with bicuspid aortic valve, aortic dissection, or hereditary connective tissue disorders were excluded. Maximum ATAA diameter was measured by the surgeons or during magnetic resonance imaging and computed tomography. Within 24 h of resection, multiple specimens were cut from the anterior, posterior, right and left lateral quadrants with circumferential (CIRC) and longitudinal (LONG) direction for the biomechanical tests. An individual CIRC-directed specimen from each region was formalin-fixed over 24 h for histological processing.

2.2. Light microscopy

Paraffin-embedded 5-μm sections were stained with hematoxylin-eosin, Sirius red, and orcein, using standard techniques and reagents. Measurements were conducted via image-analysis software (Image-Pro Plus v4.5; Media Cybernetics Inc) on polychromatic images acquired by a digital camera (Altra20; Soft Imaging System) fitted to light microscopy, as detailed by our group (Iliopoulos et al., 2009a; Sokolis et al., 2012a). The extent of staining was quantified through color segmentation; specific colors (red for collagen in Sirius red and orange for elastin in orcein-stained sections) were isolated and quantified as percentage area of the total field staining positive. Values for each specimen were averages from ten random regions and three sections.

2.3. Immunohistochemistry

The tissue sections were deparaffinized with xylene, immersed in graded alcohol, rinsed, and subjected to heat-induced epitope retrieval. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in phosphate-buffered saline (PBS) and nonspecific binding was blocked using blocking reagent Sniper (Biocare Medical) for 5 min. The slides were incubated afterwards with primary antibodies against MMP-2 (MBL International Corp), MMP-9 (Santa Cruz Biotechnology Inc), TIMP-1 (Abcam Inc), and TIMP-2 (Chemicon International Inc) in a dilution of 1:100 for 1 h at room temperature. They were then rinsed with PBS and incubated with secondary biotinylated anti-mouse or anti-rabbit or anti-goat IgG (DakoCytomation) for 30 min, and with streptavidin peroxidase to form avidin-biotin complexes (Elite ABC Reagent; Vector Laboratories) for 30 min; all incubations performed at 20–25 °C with appropriate rinses between steps. Enzyme activity was detected by a diaminobenzidine

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