Temporal disparity in the induction of matrix metalloproteinases and tissue inhibitors of metalloproteinases after thoracic aortic aneurysm formation

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Background: An important component of matrix remodeling during thoracic aortic aneurysm progression is the balance between matrix metalloproteinases and their endogenous inhibitors (tissue inhibitors of metalloproteinases). However, whether and to what degree matrix metalloproteinase/tissue inhibitor of metalloproteinases profiles change over time with an evolving thoracic aortic aneurysm remains unclear.

Methods: Descending thoracic aortic aneurysms were induced in mice (FVB strain, 15 minutes of 0.5 mol/L $CaCl_2$ exposure) and followed for 24 hours, 72 hours, 1 week, 2 weeks, 4 weeks, or 8 weeks (each group, n = 13). Thoracic aortic aneurysm size was determined by means of video micrometry, and immunoblotting was used to measure aortic matrix metalloproteinase 2, 8, 9, and 12 and tissue inhibitor of metalloproteinases 1 and 4 levels (expressed as a percentage of control values, n = 13).

Results: Increased aortic diameter was detected by 72 hours and reached a maximal size at 4 weeks (135% ± 4% increase from baseline, $P < .05$), which is consistent with thoracic aortic aneurysm progression. Active matrix metalloproteinase 8 (collagenase) levels increased at 72 hours (178% ± 49%, $P < .05$ from control), and active matrix metalloproteinase 12 (elastase) levels increased by 24 hours (138% ± 11%, $P < .05$), whereas active matrix metalloproteinase 2 levels increased at 72 hours and 1 week after thoracic aortic aneurysm induction (72 hours: 158% ± 12%, 1 week: 162% ± 19%; $P < .05$). At 1 week after thoracic aortic aneurysm induction, active matrix metalloproteinase 9 and 12 levels decrease (matrix metalloproteinase 9: 55% ± 5%; matrix metalloproteinase 12: 63% ± 5%; $P < .05$); however, matrix metalloproteinase 9 and 12 levels were increased from these values at 4 and 8 weeks ($P < .05$). Tissue inhibitor of metalloproteinases 1 levels were decreased at 1 week (52% ± 15%, $P < .05$) and later returned to control values, whereas tissue inhibitor of metalloproteinases 4 levels increased at the late thoracic aortic aneurysm time points (4 weeks: 278% ± 46%; 8 weeks: 213% ± 40%; $P < .05$).

Conclusions: These findings show 2 phases of matrix metalloproteinase abundance during murine thoracic aortic aneurysm formation. The late tissue inhibitor of metalloproteinases 4 increase might explain prevention of further aortic dilation past 4 weeks. Unique matrix metalloproteinase/tissue inhibitor of metalloproteinases temporal relationships occurred during the natural history of thoracic aortic aneurysm progression that might hold both diagnostic and therapeutic relevance.

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Thoracic aortic aneurysms (TAAs) represent a serious and potentially lethal disease with high mortality and morbidity rates. TAA formation and progression is a multifactorial process that involves both cellular and extracellular processes.[1,2] Ascending and descending TAAs likely differ in cause and natural progression,[3] and anatomic and cellular differences between the ascending

Abbreviations and Acronyms
ECM = extracellular matrix
MMP = matrix metalloproteinase
TAA = thoracic aortic aneurysm
TIMP = tissue inhibitor of metalloproteinases

and descending aorta might explain why TAAs that develop in these areas differ. With respect to the extracellular matrix (ECM), degradation and remodeling universally occurs with descending TAAs, but the underlying mechanisms remain poorly understood.

ECM degradation in aneurysm tissue is caused in part by a family of endopeptidases termed matrix metalloproteinases (MMPs).[4,5] Several members of the MMP family have been implicated in the pathogenesis of aortic aneurysms, including gelatinase A (MMP-2), gelatinase B (MMP-9), and macrophage elastase (MMP-12).[6-9] The major structural component that lends strength to the aortic wall is elastin. Elastin fiber fragmentation is a frequent histologic finding in aneurysmal tissue.[10] MMP-12, released from macrophages, has the ability to directly degrade elastin within the aortic wall, facilitating aneurysmal dilatation with the loss of structural integrity. Neutrophil collagenase (MMP-8) and MMP-9 are released in their preformed states from neutrophils in the early stages of inflammation. In addition, it has been established that the endogenous tissue inhibitors of the MMPs (TIMPs) play a role in the modulation of MMP activity.[11,12] Radiolabeled MMP inhibitors, combined with in vivo scintigrapghy in experimental animal models, have opened the door for noninvasive clinical diagnostic methods in MMP-related diseases.[13] Although previous studies have demonstrated clear roles for these specific MMPs and TIMPs in aneurysmal disease, no study to date has determined the relative changes in abundance over the course of aneurysmal development. We hypothesized that in a model of descending TAA,[14] temporal changes in these critical MMP and TIMP types occur throughout TAA progression. Hence the present study examines the changes in these species after experimental descending TAA induction.

Methods

Experimental Design

Animals used in the study were adult wild-type FVB mice (n = 91). Equal numbers of male (n = 47, 52%) and female (n = 44, 48%; $P = .54$) mice were used, and all mice were between 8 and 12 weeks of age at the time of the initial operation. Mice were randomly assigned to one of 6 groups for terminal harvest and analysis 24 hours (n = 13), 72 hours (n = 13), 1 week (n = 13), 2 weeks (n = 13), 4 weeks (n = 13), or 8 weeks (n = 13) after the initial operation. From each group, the aortas from 3 mice were formaldehyde fixed for histologic studies, and the aortas from 10 mice were frozen for biochemical analysis. One mouse each from the 24-hour and 1-week groups were excluded because of inadequate tissue. For biochemical analysis, the above animals were compared with age-matched, control unoperated animals (n = 10). All animals were treated and cared for in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (National Research Council, Washington, DC, 1996), and the protocols were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee (protocol no. 2146).

Operative Procedure

Murine TAA production was induced as previously described.[12,15] Briefly, periadventitial calcium chloride ($CaCl_2$), which has been shown to lead to structural disruption of the medial layer of the aortic wall and the induction of a localized inflammatory response, was applied to the abluminal surface of the descending thoracic aorta to induce aneurysmal dilatation.[14] This experimental model of aortic aneurysm was selected for this study, as opposed to the elastase perfusion model, because the modest dilation it produces likely mimics the early stages of aneurysmal formation.

Anesthetized and orotracheally intubated animals were subjected to fifth intercostal space thoracotomy with exposure of the descending thoracic aorta. Aortic diameter measurements were obtained. A sponge soaked in 0.5 mol/L $CaCl_2$ was placed on the distal half of the descending thoracic aorta for 15 minutes. After 15 minutes, the sponge was removed, the chest was irrigated, and the lung was re-expanded. The operative mortality rate was 20%. The mice then survived for the time points indicated before terminal study and aortic harvest.

Aortic Diameter Measurements

Aortic diameter, as assessed by means of video micrometry, was measured for each mouse at both the baseline and predetermined time point terminal operation. Digital images of the descending thoracic aorta were obtained by using a color CCD camera (KP DZ0B, Hitachi Kokusai Electric Inc, Tokyo, Japan) linked to a laptop computer with digital imaging software (WinTV2000, Hauppauge Computer Works, Inc, Hauppage, NY). Aortic diameter measurements were made through a digital video caliper (DMZR, Techni-Quip, Polk City, Fla). Terminal aortic size was expressed as a percentage increase from respective baseline measurement.

Aortic Harvest

With the animal anesthetized, the left thoracotomy was reopened and extended beneath the xiphoid process. Terminal aortic diameter measurements were obtained in vivo before death to ensure that the aorta was sufficiently pressurized. The aorta was then carefully harvested from its root to the aortic bifurcation. The descending thoracic portion was then divided from the ascending aorta, as well as the abdominal aorta.

MMP and TIMP Abundance

For this study, the relative abundances of MMPs/TIMPs were examined by using quantitative immunoblotting techniques, which have been described in detail previously.[16] Briefly, 10 μg of aorta extract was loaded onto a 4% to 12% Bis-Tris gradient gel and subjected to fractionation by means of electrophoresis. The fractionated proteins