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The matrix reloaded – Addressing structural integrity of the aortic wall in aneurysmal disease

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A B S T R A C T

Thoracic aortic aneurysms and dissections (TAADs) involve dilation of the aortic wall that can lead to tearing or rupture. Progressive extracellular matrix (ECM) degradation is common in TAAD, regardless of the underlying cause. TAAD treatments typically target cellular signaling pathways, rather than the ECM itself, due to the complex assembly process and long half-life of ECM proteins. Compounds that stabilize the ECM are proposed as an alternative TAAD therapy that addresses the underlying cause of aortic wall failure, namely compromised structural integrity. Compounds are discussed that revisit historical approaches to maintain and preserve structural integrity of biological tissues.

Thoracic aortic aneurysms and dissections (TAADs) are characterized by progressive dilation of the aortic wall that can lead to tearing or rupture and ultimately failure. About 20% of TAADs are associated with genetic mutations in pathways affecting TGFβ signaling, smooth muscle cell contractility, and extracellular matrix (ECM) proteins [2]. Regardless of the etiology, a common histopathologic finding is progressive degradation of the ECM, which may be caused by genetic mutations in ECM or ECM-associated proteins or downstream signaling pathways stimulated by other causes that eventually lead to pathologic ECM degradation and/or turnover. TAAD can be created in young animals by feeding them β-aminoproprionitrile (BAPN), which is an irreversible inhibitor of lysyl oxidase [3]. Lysyl oxidase is an enzyme necessary to crosslink elastin and collagen, the two main structural ECM proteins in the aortic wall.

Unlike cellular proteins that are continuously produced and have half-lives of 4–14 h, ECM proteins are produced mainly during developmental "construction" of the aortic wall and then additionally as necessary for maintenance and repair. Vascular collagen has a half-life of 60 – 70 days, while vascular elastin has a half-life of 60–70 years. The aortic wall contains a complex mix of ECM proteins that requires tight temporal and spatial coordination to produce the optimal mechanical behavior for cardiovascular function. Due to the long half-life, complexity, and lack of knowledge on how to exactly reproduce the aortic wall ECM, it has not typically been the target for treating TAADs, despite genetic evidence that ECM defects are often a primary cause of the disease.

TAAD treatments have instead focused on druggable cellular proteins that are usually related to TGFβ signaling or other downstream targets of altered mechanosensing in the aortic wall. For example, losartan, an angiotensin receptor blocker reduces TGFβ signaling and prevents aortic root dilation in mouse models of Marfan Syndrome [4]. Marfan Syndrome is a congenital disease characterized by TAAD and primarily caused by mutations in fibrillin-1, an ECM protein associated with elastin assembly and TGFβ sequestration in the aortic wall. However, a meta-analysis of several large clinical trials for Marfan Syndrome demonstrates that losartan has similar effectiveness in reducing aortic dilation to β-blockers, which work simply by lowering blood pressure and the resulting wall stresses [5]. One reason for the lack of impressive results in clinical trials for Marfan Syndrome may be that losartan is addressing an effect of, but not the "root" cause of aortic dilation and failure, namely the deterioration of aortic wall structural integrity over time due to progressive ECM degradation.

I propose that progressive ECM degradation in TAAD may be caused by initial defects in ECM assembly, due to genetic mutations in ECM components or related signaling pathways that interfere with the assembly and crosslinking process, that make the resultant ECM fibers more susceptible to failure from physiologic stresses due to blood pressure, blood flow, and axial tethering, and to enzymatic and immune cell attack during physiologic turnover (Fig. 1). I argue that treatments to stabilize the ECM from progressive degradation would address the fundamental problem of aortic wall structural integrity that leads to failure in TAAD. Preventing progressive ECM degradation would:

1. Maintain appropriate material behavior of the aortic wall, including strength, toughness, modulus, and elasticity, to prevent failure due to tearing or rupture.
2. Provide the proper mechanical environment for aortic wall cells and mitigate stimulation of mechanosensitive signaling pathways that lead to pathologic aortic wall remodeling.

Abbreviations: ECM, extracellular matrix; TAAD, thoracic aortic aneurysm and dissection; BAPN, beta-aminoproprionitrile; PGG, pentagalloyl glucose; EPCG, epigallocatchin gallate.

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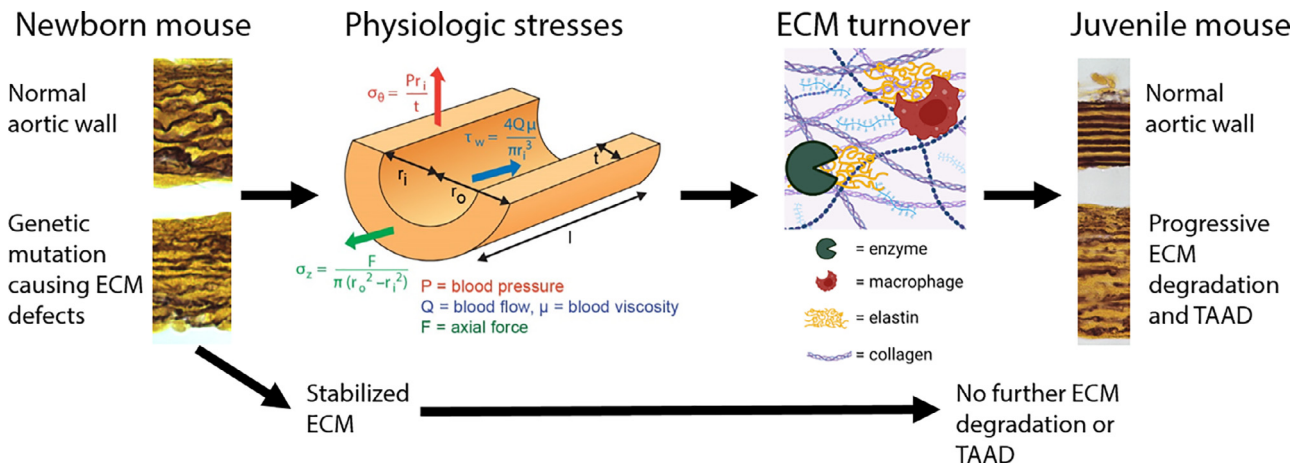

Fig. 1. Initial defects in ECM assembly may lead to progressive ECM degradation with exposure to physiologic stresses and ECM turnover. Cross-sections of newborn mouse aorta are shown on the left. The normal aortic wall is from a wild-type mouse, while the genetic mutation example is from a mouse model that does not produce fibulin-4, an elastin associated protein, in the smooth muscle cells and develops TAAD with development and aging [1]. Cross-sections of juvenile mouse aorta are shown on the right with the progressive ECM degradation visible in the mutant model. Physiologic wall stresses in the circumferential direction (σ_θ) due to pressure, the axial direction (σ_z) due to tethering forces, and luminal shear stress (τ_w) due to volumetric blood flow contribute to failure of the defective ECM fibers. Turnover of ECM proteins due to circulating and cell-secreted enzymes, as well as activation of inflammatory cells such as macrophages, facilitates breakdown of the defective ECM fibers. The normal aortic wall ECM is able to withstand physiologic stresses and resist or balance ECM turnover. Stabilizing the defective ECM would theoretically protect it from progressive degradation, retain structural integrity of the aortic wall, and prevent pathological remodeling that contributes to TAAD and eventual failure. Figure partially made with Biorender.com. Images of aortic wall cross-sections courtesy of Hiromi Yanagisawa, University of Tsukuba.

3. Prevent the release of ECM protein fragments that activate inflammatory pathways and changes in the accessibility and activity of sequestered growth factors, such as TGFβ, that contribute to disease progression.

ECM stabilization is not a new science. Animal hides, which are composed primarily of collagen with some elastin, have been stabilized for centuries so that humans can use them for warmth, protection, and shelter. Plant based tanning of hides to keep them from getting hard or rotting began around 5000 BCE and used tannins naturally occurring in the bark and leaves of plants. Tannins bind to ECM proteins in the hide and coat them, causing them to become less water soluble and more resistant to degrading enzymes and bacteria. With the industrial revolution, advancements in technology and chemistry led to chromium, aldehyde, and synthetic tanning agents.

Chemical agents that stabilize the ECM are currently used for preservation of xenograft replacement tissues, such as heart valves. Biologic pig and cow replacement valves are treated with glutaraldehyde before implantation to stabilize the ECM, fix the tissues, and prevent degradation. ECM stabilization with glutaraldehyde has also been used to strengthen and prolong deterioration of tissue-engineered replacement organs with *in vitro*-derived ECM. While glutaraldehyde is an effective stabilization agent, it is cytotoxic and can make the tissues susceptible to calcification. Alternatives that have been investigated to replace or supplement glutaraldehyde stabilization include, but are not limited to, tannic acid, pentagalloyl glucose (PGG), genipin, proanthrocyanidin, epigallocatchin gallate (EPCG), neomycin trisulfate, and carbodiimide [6].

For TAAD treatment, an ECM stabilization agent must be nontoxic, targeted to ECM in the affected area, and prevent additional degradation without adverse effects on the aortic wall mechanical behavior. Nontoxic stabilizing agents include PGG and EPCG. Targeting methods include antibodies to degraded elastin [7], localized delivery through stents or balloons, rechargeable stents, microbubbles, nanoparticles that diffuse into disrupted ECM, and nanoparticles activated by low shear stress in the dilated aorta. Many of these approaches take advantage of the common histopathology of degraded ECM and or abnormal aortic geometry and hemodynamics to deliver the treatment at the site of interest. Conveniently, the targeting should scale with disease severity in these approaches.

The best characterized stabilization agent to date for aneurysmal disease is PGG, which is a derivative of tannic acid and has been used extensively to prevent and reverse abdominal aortic aneurysms [8]. In addition to stabilizing ECM, PGG is a polyphenol that has beneficial effects including reduction of inflammation, matrix metalloproteinase activity, and TGFβ signaling. It is challenging to separate out these multiple effects *in vivo*. Hence, we performed *in vitro* studies on the mouse carotid artery [9] and ascending aorta [10] to evaluate PGG's efficacy in stabilizing the ECM and maintaining mechanical behavior in conjunction with ECM degradation and arterial dilation caused by enzymatic elastase treatment. In both arteries, we found that preventative PGG treatment (before elastase) partially prevents the structural and mechanical changes associated with ECM degradation. Restorative PGG treatment (after elastase) was only investigated in the ascending aorta, but it partially reverses the structural and mechanical changes, although it was not as effective as the preventative treatment. These studies demonstrate that ECM stabilization is a viable short term treatment strategy to prevent arterial dilation associated with ECM degradation.

As these were *in vitro* experiments and the cells were no longer alive, the changes were due to non-specific binding of PGG to the ECM and protection from load- and enzyme-induced degradation and were not due to any signaling pathways activated or suppressed by PGG. However, PGG upregulates lysyl oxidase expression, which may further stabilize newly deposited ECM through crosslinking [8]. Since ECM deposition occurs primarily during postnatal growth in mice and humans, this may provide an additional benefit to young individuals with TAAD. The importance of developmental timing for ECM crosslinking is shown by the TAAD mouse model where BAPN delivery from 4 to 8 weeks of age (during maturation) caused 75% of male wild-type mice to die of aortic dissection and/or rupture, while BAPN delivery from 8 to 12 weeks of age (after maturation) caused no mortality [3]. Increased crosslinking and/or stabilization of the ECM during maturation may be critical for TAAD prevention.

Our short term, *in vitro* studies with PGG [9,10] must be followed up with *in vivo* studies in TAAD models (such as BAPN delivery or genetic mouse models of Marfan Syndrome) to determine safe and effective PGG delivery methods, dosages, and timelines for ECM stabilization, as well as to investigate the additional *in vivo* benefits of PGG activity [8]. Preventative treatment was more effective than restorative in our *in vitro*

studies and we know that maturation is a critical time for ECM deposition and crosslinking, so it will be important to identify individuals early in disease progression, which is possible with modern genetic screening. ECM stabilization will address the root of the problem in TAAD, preventing progressive ECM degradation that occurs as incorrectly assembled or crosslinked ECM fibers are exposed to solid and fluid wall stresses with each cardiac cycle and are more susceptible to degrading enzymes and inflammatory cells (Fig. 1). Because the stabilized ECM will degrade more slowly, the mechanical integrity of the aortic wall will be maintained, preventing material failure; the mechanical environment of the cells will be maintained, preventing mechanosensitive signaling activity; and the physical integrity of the ECM fibers will be maintained, preventing the release of pathological peptides or alterations in growth factor sequestration and activation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jessica Wagenseil reports financial support was provided by National Institutes of Health. Jessica Wagenseil reports financial support was provided by The Marfan Foundation. Jessica Wagenseil reports financial support was provided by American Heart Association.

Data Availability

No data was used for the research described in the article.

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