Silver Lining in the Dark Cloud of Aneurysm Disease

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Abstract

An ascending aortic aneurysm is a common and very much unwelcome diagnosis that has never been associated with anything positive. We believe, however, that there actually is a silver lining to this disease: aortic root and ascending aortic aneurysms actually protect against atherosclerosis. We have found that patients with ascending aneurysms have both decreased arterial calcification and carotid intima-media thickness, late and early indicators of atherosclerosis, respectively. In addition to these clinical data, we also found data regarding molecular mechanisms, genetic studies, and pharmacologic evidence that corroborate our clinical findings, in particular, evidence regarding matrix metalloproteinases and transforming growth factor- β pathways. In this article, we lay out the evidence that has been accruing for the protective effect of ascending aneurysms against atherosclerosis.

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Introduction

Since its discovery, aortic aneurysm disease has been nothing but a black cloud for those afflicted with the disease, the number of whom has been steadily increasing

over the past decades. In 2007, aortic aneurysms accounted for 12,986 deaths in the US, making aneurysm the 19th most common cause of death in the country [1]. The disease is a nefarious one, as it is silent, with the first presenting symptom often death or an acutely life-threatening aortic dissection or rupture. Even when incidentally identified in advance of complications, the only effective intervention for large aneurysms involves major surgery [2].

There may, however, be a silver lining to this black cloud of the disease. Specifically, evidence is accumulating that aortic root and ascending aortic aneurysms seem to have to a *protective effect against atherosclerosis*.

Clinical Observations and Studies

We first noticed the association between ascending aortic aneurysms and decreased atherosclerosis in the operating room when, after operating on hundreds of ascending aneurysms, we noticed that the patients often had no visible atheromas in any segment of the aorta or in the femoral artery, which is often exposed for cannulation. In fact, the femoral artery was commonly soft and pliable, like that of a child or young adult, even without the fatty streaks that are known to begin at an early age in American males [3].

We proceeded to investigate this association from two different perspectives.

First, we compared the total body calcium score, a late indicator of atherosclerosis, in 64 patients with ascending

Fig. 1. Aneurysm patients have lower IMT and total body calcium score compared to control groups. Age is depicted as a 10-year interval. Body mass index (BMI) is depicted as a 10-unit interval. Note the marked similarity between the IMT values and total body calcium scores when compared to controls. DM = Diabetes mel-

litus; Smoke = smoking; AAE = annuloaortic ectasia; Dis. = dissection; Dyslip. = dyslipidemia; FH = family history (at least one family member with aneurysm disease); G = male gender; HTN = hypertension. * $p < 0.5$, and ** $p < 0.01$. Reproduced with permission from Hung et al. [5].

aneurysms or type A dissections to 86 normal controls. The score was based on CT scans, with numerical allocations to each of the following: right coronary artery, left anterior descending coronary artery, left circumflex coronary artery, ascending aorta, aortic arch, descending thoracic aorta, and abdominal aorta. We found a negative association between atherosclerosis and ascending aneurysm and dissection. Both the coronary artery calcium score and total body calcium score were significantly lower in ascending aneurysm and type A dissection patients, independent of the major risk factors of atherosclerosis. The total body calcium scores for the ascending aneurysm group, type A dissection group, and control group were 6.73, 6.34, and 9.36, respectively ($p = 0.03$ and $p < 0.0001$, respectively) [4].

Secondly, we wished to look at an early indicator of atherosclerosis. We chose carotid intima-media thickness (IMT), measured via well-documented ultrasound techniques. Fifty-two patients with ascending aortic aneurysms were compared with a spousal control group of 29 who had no known aneurysm. We found that patients with ascending aortic aneurysms had 0.131 mm lower carotid IMT values than controls ($p = 0.0002$) independent of atherosclerotic risk factors. Mean IMT was 0.50 ± 0.13 mm in the aneurysm group and 0.60 ± 0.11 mm in the control group [5]. These observations further strengthened the association between ascending aortic aneurysms and decreased atherosclerosis.

Thus, in ascending aneurysm patients, we had evidence of significant protection from atherosclerosis, both early and late (fig. 1).

While our studies provided clinical evidence supporting a protective effect of ascending aneurysm against atherosclerosis, we were curious as to whether there was a molecular basis for such a protective effect and what the mechanism(s) might be. What could be causing, in affected patients, both pro-aneurysmal and anti-atherogenic effects? We found two general categories of plausible mechanisms supported by the experimental literature: one related to the impact of matrix metalloproteinases (MMPs) and the other to transforming growth factor- β (TGF- β) pathways.

Molecular Mechanisms

There is a large body of evidence for an abnormally *proteolytic* balance of MMPs versus the tissue inhibitors of MMPs (TIMPs) in the pathophysiology of aneurysm disease. Silence et al. [6] used murine atherosclerotic models that were either apolipoprotein E (ApoE)-/-:TIMP-1+/+ or ApoE-/-:TIMP-1-/- . So, these mice were prone to atherosclerosis due to their ApoE deficiency. Mice that were TIMP-1 deficient (with increased MMP activity) manifested a reduction in atherosclerotic lesion

Fig. 2. Mechanism for anti-atherogenic and pro-aneurysmal effects of MMPs. Up-regulation of MMPs recruits macrophages, which in turn can also upregulate various MMPs. Adhesion to matrix components including collagen, fibronectin, and tenascin-C upregulates MMP-9 secretion. Pro-atherogenic cytokines and growth factors, such as IL-1 β , TNF- α , macrophage colony-stimulating factor (M-CSF) and platelet-derived growth factor (PDGF), upregulate MMP-14 and -16. Immune activation via CD40 ligand upregulates MMP-1, -3, -8, -9, and -11 [12]. Increased MMP activity leads to ECM degradation, which reduces atherosclerotic plaque size and at the same time causes thinning of vessel walls leading to aneurysms.

size, as well as a higher incidence of aortic aneurysms. Data in this study also showed that in the TIMP-1-deficient mice, there was an increase in macrophages/foam cells, leading Silence et al. [6] to suggest that the reduced atherosclerotic plaque sizes may be related to the accumulation of macrophages, which contribute to higher secretion levels of MMPs. This, in turn, leads to enhanced matrix degradation, reducing plaque size. Consistent with this interpretation are studies in ApoE-null mice that overexpressed MMP-1 in macrophages, which showed a reduction in plaque size, lipid deposition, and collagen content [7].

As for the pro-aneurysmal effects that Silence et al. [8] found in the TIMP-1-deficient mice, this confirmed their previous studies showing that in murine atherosclerotic models, stromelysin-1 (MMP-3), which activates pro-MMP-9, contributes to plaque size reduction along with promotion of aneurysm formation. A possible mechanism for the pro-aneurysmal effect of MMPs is the degradation of elastin and collagen, leading to matrix necrosis, which in turn causes thinning of the vessel wall [9, 10].

Simply put, these molecular biologic studies provide support for the general thesis that MMPs, while pro-aneurysmal, are also anti-atherogenic (fig. 2).

Another promising answer to the question of what mechanisms could be both anti-atherogenic and pro-aneurysmal, which has recently been garnering more attention, concerns the TGF- β pathways [11]. It is well accepted that TGF- β upregulation is a major contributing factor in aneurysm formation [12]. In terms of atherosclerosis,

studies have shown that TGF- β exerts anti-atherogenic effects in two ways. First, TGF- β protects against the initial formation of fatty streak lesions through certain functions in maintaining normal blood vessel architecture; namely, TGF- β inhibits vascular smooth muscle cell (VSMC) proliferation [13–15] and migration [16] and promotes extracellular matrix (ECM) formation [17, 18]. Second, TGF- β protects against the development of unstable plaques by maintaining the balance between leukocytes and VSMCs in the atherosclerotic plaque via its immunoregulatory role in the suppression of leukocyte recruitment [19, 20] and its powerful profibrogenic effects [21, 22].

One molecular mechanism that has been proposed for this dual anti-atherogenic effect has to do with the different TGF- β receptor profiles expressed by VSMCs in atherosclerotic lesions versus normal vessel walls. McCaffrey et al. [23] showed that in normal vessel walls, VSMCs predominantly expressed the type II TGF- β receptor, whereas in diseased (arteriosclerotic) vessel walls, VSMCs predominantly expressed the type I TGF- β receptor. Grainger [11] investigated this further and found that the difference in receptor profiles also led to functional differences. In cells that predominantly expressed the type II TGF- β receptor, there was increased contractile protein expression and low VSMC migration and proliferation. In cells that predominantly expressed the type I TGF- β receptor, however, there was decreased contractile protein expression and increased ECM expression, leading to stable plaque formation. The shift in receptor profiles has been

Fig. 3. Model for the role of TGF- β in the regulation of atherosclerosis. In VSMCs of normal vessel walls (left cycle), latent TGF- β is activated (black arrows), which then signals to cells high in type II TGF- β receptor concentration to perform the functions that maintain healthy vessel wall structure. If something occurs to diminish TGF- β activity (bold red arrows; 'first hit'), then the receptor profile of VSMCs changes (right cycle), and active TGF- β now signals to cells high in type I TGF- β receptor concentration. This leads to the upregulation of ECM expression and the formation of a stable plaque. If TGF- β activity is further diminished (thin red arrows; 'second hit'), inflammatory processes are upregulated and loss of ECM occurs, leading to unstable lesions and plaque rupture. Reproduced with permission from Grainger [11].

shown to be due to a decrease in TGF- β . When TGF- β is sufficiently decreased, there is increased inflammation and a loss of ECM at the plaque sites, which then leads to plaque rupture [11]. Although what exactly is causing a decrease in TGF- β is not completely understood, this difference in receptor profiles provides a model for the role that TGF- β plays in the initiation and progression of atherosclerotic lesions (fig. 3). To further support this, studies have shown that mutations in the type II TGF- β receptor are associated with atherosclerotic and restenotic vascular cells [24].

Increased TGF- β expression is therefore another possible mechanism through which ascending aneurysms protect against atherosclerosis.

Genetic Studies

Studies have demonstrated that polymorphisms in the human MMP-3 promoter, which lead to decreased expression of this gene, have been correlated with a more rapid progression of angiographically documented coronary atherosclerosis, providing further support for the role of MMPs in reducing atherosclerotic plaque size [25]. Interestingly, genetic mapping studies have shown that the gene for MMP-3 maps to chromosome 11q23, and a very similar region (11q23.3–q24) was identified as a locus for familial aortic aneurysms, providing genetic support for the role of MMPs in aneurysm formation [26].

Genetic studies have also shown an association between polymorphisms that decrease TGF- β levels and patients who have had acute myocardial infarctions [27]. A study in the Japanese population has also shown that a polymorphism that decreases TGF- β is a risk factor for acute myocardial infarctions [28], providing further genetic support for the anti-atherogenic role of TGF- β .

Pharmacologic Evidence

There are not only molecular and genetic studies that provide evidence that support and explain the protective effect that ascending aortic aneurysms have against atherosclerosis, but also pharmacologic studies that add to the evidence behind this phenomenon.

Studies on statins have shown that activation of TGF- β receptors and their signaling proteins, Smads, plays a significant role in the anti-atherogenic effects of statins. Vecerova et al. [29] found that atorvastatin increases the expression of TGF- β receptors and Smad proteins in ApoE/LDLR (low-density-lipoprotein receptor)-knock-out mice, leading to the conclusion that TGF- β /Smad pathways play an important role in the non-lipid-lowering anti-atherogenic effects of atorvastatin. Another study on statins had multiple findings that pointed to the TGF- β /Smad pathway being essential for statin-dependent actions in VSMCs [30]. The authors found that statins not only enhance the Smad pathway in cultured

VSMCs, but also that the TGF- β /Smad pathway mediates statin-induced VSMC death. They also found that statins increase type II TGF- β receptor levels and TGF- β expression in cultured VSMCs, as well as the expression of TGF- β -dependent ECM-regulatory proteins, all of which have been shown to play important roles in anti-atherogenesis [11, 30]. Yet another finding in this study is that atorvastatin upregulates the type II TGF- β receptor and the Smad pathway in VSMCs, leading to elevated ECM protein deposition and amelioration of atherosclerosis, suggesting a novel mechanism of statins involved in plaque stability [30].

Not only has TGF- β been found to play a key role in the anti-atherogenic effects of statins, TGF- β activity has also been found to be essential for the anti-atherogenic effects of estradiol [31]. Experiments done on ApoE- and LDLR-knockout mice have shown that estradiol increases TGF- β expression and that without a functional TGF- β signaling pathway, estradiol does not exert the effects on anti-fatty streak deposition that it has when the TGF- β signaling pathway is intact [31].

We would like to point out some limitations in the deliberations put forth in this paper. The molecular studies on MMP activity in TIMP-1-deficient mice that showed a reduction in atherosclerotic plaque size did not address the issue of how the increased proteolytic activity can lead to thin fibrous caps, which while nonocclusive, can lead to rupture and myocardial infarction. Also, the studies that are described here using rodent models that provide a molecular basis for the protective effect that ascending aortic aneurysms have against atherosclerosis have not been definitively studied in humans.

Conclusion

From our initial observations in the operating room, we have come to find a convincing body of evidence that points to ascending aortic aneurysms having a protective effect against atherosclerosis, with mechanisms likely involving MMPs and TGF- β . This body of evidence consists of a wide array of studies, including clinical, molecular, genetic, and pharmacologic investigations, providing an impressive amount of data that support and explain anti-atherosclerotic effects of ascending aortic aneurysms. However, this body of evidence is far from complete and more studies are needed to fully confirm and understand this phenomenon. There are many gaps in our knowledge of the involvement of MMPs and TGF- β in atherosclerosis, as well as in aneurysm pathogenesis. For example,

since the aneurysms in our patients are local (in the proximal ascending aorta) and the protection against arteriosclerosis is generalized throughout the patients, the mechanism remains to be shown. As well, the mean patient age in each of our two clinical studies, calcium score and carotid IMT, was 61 years; therefore, we cannot say if the protection against atherosclerosis extends into old age. Furthermore, it is becoming increasingly apparent that there is structural, biochemical, and even embryological heterogeneity of different segments of the aorta (above and below the ligamentum arteriosum) [32]; accordingly, the animal studies identified as supportive of our thesis may need further enhancement in the future, with confirmation of findings both above and below the ligamentum. Also, while the anti-atherosclerotic effects of TGF- β are well supported in the literature, we would like to point out that studies have also found TGF- β to be involved in atherogenic mechanisms [33], and further studies are needed to more clearly understand the role that TGF- β plays in the complex disease of atherosclerosis.

Understanding the protective mechanism that is the topic of this report could lead to the development of novel therapies to protect against atherosclerosis; some investigations are already under way. For example, triphenylethylene-derived drugs that increase TGF- β levels, such as tamoxifen and raloxifene, have been proposed as potential therapies for coronary artery disease [34, 35]. These investigations, however, are far from complete.

At present, it appears that we can share with our aneurysm patients a bit of good news in their otherwise negative aneurysm diagnosis: they are likely to be protected against atherosclerosis.

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