Vascular Aneurysms: A Perspective

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Aneurysms develop as a result of chronic inflammation of vascular bed, where progressive destruction of structural proteins, especially elastin and collagen of smooth muscle cells has been shown to manifest. The underlying mechanisms are an increase in local production of proinflammatory cytokines and subsequent increase in proteases, especially matrix metalloproteinases (MMPs) that degrade the structural proteins. The plasminogen system: urokinase-type PA (u-PA), tissue-type PA (t-PA) and plasminogen activator inhibitor-1 (PAI-1) and the MMPs system-MMPs and TIMPs contribute to the progression and development of aneurysms. Recent studies suggest that aneurysms may be genetically determined. To date, most observable candidate genes for aneurysm (elastin, collagen, fibrillin, MMPs and TIMPs) have been explored with little substantiation of the underlying cause and effect. Recently, overexpression of the MMP-2 gene has been suggested as an important phenomenon for aneurysm formation. Along with MMPs, matrix formation also depends on JNK (c-Jun N-terminal kinase) as its activation plays important role in downregulating several genes of matrix production. Under stress, activation of JNK by various stimuli, such as angiotensin II, tumor necrosis factor-\alpha and interleukin-1 \beta has been noted significantly in vascular smooth muscle cells. Several therapeutic indications corroborate that inhibition of MMP-2 and JNK is useful in preventing progression of vascular aneurysms. This review deals with the role of proteases in the progression of vascular aneurysm.

\textbf{Keywords:} Aneurysm, Proteinase, Antiproteinase, Plasminogen system.

\section*{Introduction}

Vascular aneurysm, often asymptomatic, is a major disease generally of the adult aorta caused by progressive medial degeneration of the aortic wall. Aneurysm is a complex multi-factorial disease with life threatening implications. About 50,000 patients undergo surgical repair of only abdominal aortic aneurysm and another 15,000 patients die from the ruptured aneurysms each year in USA\textsuperscript{1,2}. There is accumulating evidence that the incidence of aneurysms is increasing worldwide despite a general decline in other forms of atherosclerotic cardiovascular diseases\textsuperscript{3}. Recent estimates suggest that aneurysms affect about 15\% of the US population over 65 yrs of age\textsuperscript{4}.

The biochemical and histopathological changes established in aneurysms have been extensively characterized. Aneurysms demonstrate arterial dilatation, wall thickening and a reduction in the elastin and collagen ratio of the extracellular matrix (ECM)\textsuperscript{5}. These structural changes are accompanied by a wide spread inflammatory infiltrate, a rich cytokine milieu and excessive local concentrations of a number of matrix metalloproteinases (MMPs), such as MMP-2 and -9. These MMPs have been considered responsible for the widespread degradation and remodeling of the ECM matrix that is demonstrated in established and expanding aneurysms\textsuperscript{5}.

Proinflammatory stimuli increase expression of MMPs through activation of transcription factor activating protein-1, which is frequently a downstream target of c-jun N-terminal kinase (JNK)\textsuperscript{6,7}. The levels of activated JNK are high in...
aneurysmal tissues than in atherosclerotic lesions and activation of JNK is demonstrated to be relatively exclusive in comparison to other protein kinases, such as p38 MAPK and ERK1/2. Epidemiological reports have demonstrated a familial tendency to aneurysm formation and evidence now exists through segregation analysis of multigenerational pedigree that aneurysm susceptibility is controlled by a single gene defect. Recent studies suggest that the genes for elastin, collagen, fibrillin, TIMP1 and TIMP-2 have modest forthright implication to aneurysm, whereas MMP-2 gene is regarded as an important candidate for the progression of aneurysm. This review deals with the role of proteases, especially MMPs in the progression of vascular aneurysm.

Pathophysiology of aneurysmal degeneration

Although the cause of aneurysmal degeneration is not clearly known, it is widely recognized that aneurysms are closely associated with chronic inflammation and destruction of connective tissue proteins within the outer arterial wall. The development of aneurysmal dilatation is primarily attributed to the depletion of medial and adventitial elastin, whereas rupture of the aneurysm is generally thought to involve the additional degradation of adventitial collagen. Because the biochemical events occurring within the aneurysm wall are superimposed upon the constant tensile strength associated with arterial blood pressure, hemodynamic forces also contribute to aneurysmal degeneration, as well as the risk of rupture itself. Indeed, pathophysiology of aneurysms operates through a gradual imbalance between factors acting to weaken the aortic wall and a compensatory “wound healing” response acting to resist tensile wall stress.

Role of proteases in aneurysm

Aneurysmal degeneration of arterial wall appears to be a process that is predominantly localized to the media and adventitia. Histological feature of aneurysms have revealed fragmentation and relative decrease in medial elastin. One of the primary differences between aneurysm and atherosclerotic tissue is the fragmentation and destruction of elastin tissue in the arterial media of the former. This elastolytic process is observed early in aneurysm formation and may be the crucial step in aneurysm pathogenesis.

The fibrillar collagen network of the arterial wall recognizes a marked cellular inflammatory response. The inflammatory infiltrate in aneurysms is composed of lymphocytes and macrophages. These immune cells are thought to play a causative role through their ability to produce cytokines that induce resident mesenchymal cells to produce MMPs. The proinflammatory cytokines secreted from macrophages have been shown to enhance production of MMPs in vascular smooth muscle cells (VSMCs).

The ECM plays an essential role in the integrity of vascular systems. Under normal conditions, elastin and collagen fibres resist spontaneous breakdown. Upon activation, SMCs migrate to the extracellular space and direction of movement is controlled by released chemoattractants, for example, platelet-derived growth factor (PDGF) that have attached to intima damaged by protease(s). This is achieved by proteases released by SMCs. During inflammation, proteolytic balance is altered and the resultant net proteolysis causes erosion, fragmentation and dissolution of elastic laminae and collagen bundles, leading to aneurysm. The pattern of events may occur in myocardial infarction or more chronically during atherosclerosis.

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degradation of elastin and collagen tissues within the aortic media\textsuperscript{33}. In vivo, the principal activator of MMP-2 is membrane type-1 matrix metalloproteinase (MT1-MMP), which contains a transmembrane domain within its structure\textsuperscript{34}. MT1-MMP binds the inactive proMMP2-TIMP2 complex at the cell surface and cleaves the proMMP-2, thereby activating the enzyme and localizing the collagenase activity at the cell membrane\textsuperscript{35}. The mechanism of activation of proMMP-2 by stimulants, such as cytokines and chemokines in SMCs has been shown to occur via PKC-\(\alpha\)-dependent and NF\(\kappa\)B-MT1MMP-mediated signaling pathway. The increase in MT1-MMP protein and mRNA expression by stimulants diminishes tissue inhibitor of matrix metalloproteinase-2 (TIMP-2) expression in SMCs. Activation of PKC-\(\alpha\) is responsible for inhibitory kappa B kinase (IKK) activation, I\(\kappa\)B-\(\alpha\) phosphorylation/degradation and subsequent NF-\(\kappa\)B activation. The activated NF-\(\kappa\)B, in turn, binds to the MT1-MMP promoter facilitating its increased expression, leading to proMMP-2 activation in combination with TIMP-2, which is also regulated by PKC-\(\alpha\) at the surface of SMCs\textsuperscript{36} (Fig. 2).

### 5-Lipoxigenase (5-LO) mediated aortic aneurysms

Activation of 5-LO pathway leads to the biosynthesis of proinflammatory leukotriene lipid mediators, which have been demonstrated to be mediated via manifestation of 5-LO activating protein for manifestation of vascular diseases, such as myocardial infarction and stroke\textsuperscript{37}. 5-LO positive macrophages localize in the diseased arteries in areas of neoangiogenesis and these cells constitute a main component of aortic aneurysms induced by an atherogenic diet\textsuperscript{38}. 5-LO deficiency markedly attenuates formation of aneurysms and is associated with reduced MMP-2 activity and diminished plasma macrophage inflammatory protein-1\(\alpha\) (MIP-1\(\alpha\) a.k.a CCL3), but only minimally affects the formation of lipid rich lesions\textsuperscript{39}. The leukotriene LTD\(_4\) strongly stimulates expression of MIP-1\(\alpha\) in macrophages and also MIP-2 (a.k.a CXCCL2) in endothelial cells\textsuperscript{40,41}. The
macrophage 5-LO cascade generates leukotrienes, which act on neighboring endothelial cells and also T cells, as well as on macrophages themselves, thereby activating the release of CCL3 and CXC chemokines and through indirect mechanisms activates proteases, for example, MMP-2. Thus, amplifying signals are generated to initiate cycles of inflammation and arterial wall remodeling in which 5-LO plays a regulatory role.

Role of JNK in aneurysm formation

Various stimuli have been linked to chronic inflammation observed in aneurysms, including mechanical stress, oxidative stress, angiotensin II and TNF-α. Most, if not all, of these stimuli activate JNK in VSMCs, which synthesize ECM and secrete MMPs. In macrophages, JNK participates in the secretion of proinflammatory cytokines and MMPs. JNK is known as a proximal signaling molecule in the pathogenesis of vascular aneurysms. Recent studies have shown that macrophages-derived MMP-9 and interstitial cell-derived MMP-2 work in concert toward the development of vascular aneurysms.

In VSMCs, JNK upon activation downregulates expression of several genes involved in matrix production, including those encoding lysyl oxidase (LOX) and prolyl 4-hydroxylase (P4H). As these enzymes are required for maturation of elastin and collagen fibres, respectively, activation of JNK might diminish cellular production of these structural proteins. Formation of vascular aneurysms is attenuated by increased local expression of LOX. Importantly, when mice with established abdominal artery aneurysms are treated with the pharmacologic JNK inhibitors, for example, SP600125 reduces inflammation of arterial wall and reduced expression of MMPs and considerable decrease in arterial diameter is observed (Fig. 4). Moreover, the artery diameters in treated mice were smaller at the end of therapy than at the start, indicating that aneurysms regressed during therapy.

Inhibition of NF-κB prevents the development of experimental abdominal aortic aneurysm. JNK has been suggested to play a role in the molecular events after these interventions. NF-κB synergistically regulates the expression of MMP-9/MMP-2 with AP-1, which is the major target of JNK pathway (Fig. 4).

Familial and genetic aspects

Aneurysms are known to occur with some degree of familial tendency and about 20% of patients with aneurysms report the disease in a first degree relative. Familial abdominal aortic aneurysms occur in an autosomal dominant fashion with an age-related, low degree of penetrance, which may be due to single-gene abnormality (polymorphism). Also, familial aneurysms appear to have a higher rate of rupture at smaller diameters than those occur spontaneously. The above facts reinforce that familial patterns need to be sought for all patients with aneurysms and ultrasound screening or precisely of first degree relatives over age of 55 yrs should be strongly recommended.

Therapeutic implications of aneurysm

Abdominal aortic aneurysms arise from chronic, irreversible destruction of connective tissue. A promising pharmacologic approach is not only to suppress development of aneurysms, but also to induce its regression.

In animal models of aortic aneurysms, genetic and pharmacologic inhibition of MMPs, especially MMP-2 and MMP-9 can suppress development of aneurysms. Clinical studies using doxycycline as an inhibitor of MMPs has been shown to markedly prevent development of aneurysms. Notably, all drug therapies proposed for aneurysms, directly or
indirectly inhibit MMP-9/MMP-2 and decrease degradation of elastin and collagen. Thus, pharmacological therapy could prevent degradation of connective tissue in arterial aneurysms. As doxycycline can suppress JNK as well as MMPs, inhibition of JNK may even be a mechanism by which doxycycline suppresses progression of aneurysms. Promoting production of ECM by blocking activation of JNK, therefore, suggests JNK may be a target along with MMPs and other proteinases for individuals with vascular aneurysms.

Propranolol treatment prevents progression of vascular aneurysms. It is unclear from these studies, however, whether this is due to a reduction in blood pressure or other hemodynamic effects of β-adrenergic blockade. Indeed, propranolol suppresses abdominal aortic aneurysms in a mouse model system. However, β blockers other than propranolol are found ineffective, indicating that propranolol-mediated enhancement of connective tissue cross-linking, rather than reduction in blood pressure appears to be the key mechanisms for regression of aneurysms alone.

An increase in inflammatory cell expression of MMPs has been demonstrated to be mediated by signaling pathways involving prostaglandin E2 in some systems. To delineate whether prostaglandin production might also participate in the induction of MMP expression, Holmes et al. examined rats treated with indomethacin, a pharmacologic cyclooxygenase inhibitor and found a marked reduction in aortic dilatation and aneurysm formation. Importantly, aortic tissues extracted from indomethacin-treated animals have been found to

Fig. 3—Model of 5-lipoxygenase (5-LO) pathway participation in leukocytes and macrophages recruitment and aneurysm formation [(1) Leukotriene D4 (LTD4) stimulates the production of MIP-α in macrophages through CysLT1R in an autocrine fashion, (2) MIP-α has a role in the recruitment of T cells in a paracrine fashion, (3) LTD4 stimulates the production of MIP-2 in endothelial cells, (4) MIP-2 participates in the recruitment of leukocytes and macrophages, (5) Cells in 5-LO dependent granuloma tissue may release ECM degrading factors (e.g., MMP-2), which break down the elastic lamina of the vascular wall, and (6) Lipids accumulate in aneurysmal macrophages. Step 1-6 does not necessarily represent the temporal sequence of events. Taken from ref. 39 with permission from the publisher]
produce substantially less MMP-2 and MMP-9 than those from control animals.

Conclusion and future direction
Population-based screening studies have revealed that about 15% of individuals over the age of 65 possess an unsuspected vascular aneurysm. Although the vast majority of these lesions are small and asymptomatic, their natural history is characterized by progressive growth and eventual rupture. Recognition of the involvement of different MMPs in the pathobiology of aneurysms suggest the possibility that recruitment of inflammatory cells into the adventitia and subsequent elaboration of cytokines and MMP-2 and MMP-9 are important cellular processes underlying the transformation of a slowly growing small aneurysm to a dangerous, fast-growing aneurysm.

Large aneurysms are at much higher risk of rupture than the smaller aneurysms. This description of the differences between two aneurysms highlights the possibility that recruitment of inflammatory cells into the adventitia and subsequent elaboration of cytokines and MMP-2 and MMP-9 are important cellular processes underlying the transformation of a slowly growing small aneurysm to a dangerous, fast-growing aneurysm.

An important question that could arise is whether therapeutic use of proteinase inhibitors causes harmful side effects? Proteinases participate in reproduction, wound healing and angiogenesis, among many other essential processes and there is every possibility that inhibitors could perturb the said processes. In addition, prolonged administration of proteinase inhibitors can promote cardiac failure by impairing healing and therapeutic angiogenesis after infarction. Despite this concern, it has been suggested that intermittent treatment with protease inhibitors, especially MMP-2 inhibitors may prove clinically useful in blocking progression of vascular aneurysms.

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References
extremity arterial evaluation in the vascular laboratory. J Vasc Surg 22, 417-421


33 Murphy G, McAlpine CG, Poll CT & Reynolds JJ (1985) Purification and characterization of a bone metalloproteinase that degrades gelatin and types IV and V collagen. Biochim Biophys Acta 831, 49-58


