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Role of Matrix Metalloproteinase Inhibitors in Preventing Abdominal Aortic Aneurysm

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Abstract

Abdominal aortic aneurysm (AAA) is a significant health problem in the United States with approximately 30,000 repair operations annually. Treatment of AAA is associated with more than 150,000 hospital admissions per year. The development of AAA is characterized by destruction of the elastic media of the aortic wall. A large body of evidence suggests that a group of enzymes called matrix metalloproteinases (MMPs) plays a significant role in the destruction of extracellular matrix in the aortic wall. MMP inhibition has, therefore, been viewed as an alternative pharmacotherapeutic approach to slow down the development and progression of small AAAs thus reducing the need for surgical intervention.

Keywords

Abdominal Aortic Aneurysm; Matrix Metalloproteinases; Matrix Metalloproteinase Inhibitors; Doxycycline; Small Molecule Inhibitors

Abdominal Aortic Aneurysm

Abdominal aortic aneurysm (AAA) represents a life-threatening condition associated with aging, atherosclerosis and progressive connective tissue destruction in the aortic wall.¹⁻⁴ Rupture of AAA is the 13th leading cause of death with approximately 15,000 deaths/year in the United States.^{2, 5} While currently an estimated 1-2% of the population harbor AAAs, the incidence of AAA is expected to increase with population aging, since AAAs are a complex late-age-at-onset disease.¹⁻⁵ This was shown clearly in a study in which the overall prevalence of AAA was found to be 9.5% in a cohort of 4,741 participants who were 65-90 years old with 6.2% (173/2785) of the women and 14.2% (278/1956) of the men found to have an AAA.⁶

In year 2000, about 30,000 open AAA repair operations were performed in the United States. Treatment of AAA is associated with more than 150,000 hospital admissions per year.⁷ At the present time, treatment of AAA consists of either surgical or endovascular repair.^{3, 4} Both options are effective in preventing deaths from AAA rupture. Surgical intervention, however, is recommended only to patients with AAAs larger than 5.5 cm in diameter.⁸

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Abdominal ultrasonography examination is a simple, non-invasive screening test for AAA, but most AAAs detected by ultrasonography are too small for an operation. It is this group of patients with small AAAs who need long-term imaging surveillance in the form of regular ultrasonography scans. From the patients' point of view the situation can be very threatening, since there is currently no effective treatment to slow down or prevent the growth of an AAA. Development of pharmacological therapies to prevent AAA expansion would be an attractive option for such patients. It has been estimated that inhibiting the growth of an AAA by 40%, could delay the onset of rupture by perhaps 5 years.^{9, 10, 11} A medical treatment for AAAs could be considered a "maintenance drug" for controlling the growth of AAAs and would provide the much needed treatment option for small AAAs.

Matrix Metalloproteinases and Their Role in the Pathophysiology of AAA

Extracellular matrix (ECM) proteins such as collagens, elastin and proteoglycans are important structural components in arteries and myocardium.¹²⁻¹⁴ Disturbances in the synthesis and degradation of these structural elements have been shown to occur in many disease conditions. In the arteries, in conditions such as atherosclerosis, cerebrovascular disease and AAAs, accumulation of ECM or increased degradation of it by matrix metalloproteinases (MMPs) are well established changes and contribute to the pathogenesis of these diseases.¹⁵ In the myocardium, similar events may lead to ventricular hypertrophy and dilatation, and it has been shown in animal studies that inhibiting MMP activity reduces left ventricular dilatation after myocardial infarction.¹² Also, the levels of many MMPs are increased during the remodeling process after myocardial infarction.¹²

AAAs are a common degenerative disease of the aortic wall.^{1, 2, 16, 17} They are characterized by signs of local chronic inflammation of the aortic wall,¹ decrease in the number of smooth muscle cells in the aortic media layer,¹⁸ and fragmentation of the ECM of the aorta at the site of the aneurysm.¹⁹ Studies on mRNA and protein have demonstrated increased local expression of proinflammatory cytokines and MMPs (Table I) as well as an imbalance between the MMP expression and the expression of their naturally occurring inhibitors, the TIMPs.^{16, 20} Furthermore, AAAs can be induced in a surgical experimental model in which elastases are infused into rat or mouse aorta.^{21, 22} Genetically-engineered mice lacking the MMP9 gene are resistant to AAA formation in this elastase infusion model supporting a role for MMPs in the development and rupture of AAAs.²²

MMPs are a family of enzymes whose main function is degradation of the ECM. These enzymes are present in normal healthy individuals and they play an important role in processes such as wound healing, pregnancy and parturition, bone resorption and mammary involution.^{23, 24} MMPs are defined by following characteristics: 1) they are proteinases that degrade at least one component of the ECM; 2) they contain a zinc ion and are inhibited by chelating agents; 3) they are secreted in a latent form, requiring activation for proteolytic activity; 4) they are inhibited by TIMPs; and 5) they share common amino acid sequences.

These zinc- and calcium-dependent endopeptidases degrade most components of the ECM and are involved in the remodeling and degradation of the structural ECM molecules, such as collagens, elastin, proteoglycans and glycoproteins.^{23, 24} MMPs share a common domain structure: 1) the pro-peptide, 2) the catalytic domain; and 3) the hemopexin-like carboxy-terminal domain which is linked to the catalytic domain by a flexible hinge region. MMPs are initially synthesized as inactive zymogens with a pro-peptide domain that must be removed before the enzyme is active. The pro-peptide domain is part of "cysteine switch" containing a conserved cysteine residue which interacts with the zinc in the active site and prevents binding and cleavage of the substrate keeping the enzyme in an inactive form. In most MMPs the cysteine residue is in the conserved sequence. Some MMPs have a prohormone convertase

cleavage site (furin-like) as part of this domain, which when cleaved, activates the enzyme. The active site of the catalytic domain is a 20 Å groove that runs across the catalytic domain. A catalytically important zinc ion is bound by three histidine residues in the active site. The gelatinases, such as MMP2, incorporate fibronectin-type II modules inserted immediately before in the zinc-binding motif in the catalytic domain.²⁵ The catalytic domain is connected to the carboxy-terminal domain by a up to 75 amino acids long flexible hinge or linker region. The carboxy-terminal domain has structural similarities to the serum protein hemopexin. It has a four bladed β-propeller structure providing a large flat surface which is thought to be involved in protein-protein interactions. This determines substrate specificity and is the site for interaction with TIMPs. The hemopexin-like domain is absent in MMP7, MMP23, and MMP26, as well as in the plant and nematode MMPs. MMPs are anchored to the plasma membrane through the hemopexin-like domain.

In healthy tissue, MMPs are tightly regulated by TIMPs.^{23, 24} Several studies have shown that aneurysmal tissue has imbalance between MMPs and TIMPs.^{10, 20, 26-29} Expression of members of MMP family, which are produced by macrophages, is upregulated in aneurysm formation.³⁰ Several members of the MMP family (Table I) have been implicated in AAA pathogenesis, including collagenase-1 (MMP1), stromelysin-1 (MMP3), gelatinase A (MMP2), gelatinase B (MMP9), macrophage elastase (MMP12), collagenase-3 (MMP13), and membrane type 1 MMP (MT1-MMP).^{30, 31}

It appears that the type of MMP present in aortic media dictates the size of developing aneurysm. Alternatively, different MMPs are expressed at different stages of the aneurysm development. MMP2 is expressed at high levels in small aneurysms, while MMP9 is found in medium-sized,²⁷ large³² or ruptured aneurysms. Recent studies have shown that MMP9 plasma levels decrease after successful exclusion of AAA from the circulation.^{33, 34} MMP9 has attracted particular interest because it is the most abundant elastolytic proteinase produced by human AAA tissues *in vitro*.³² It is also expressed abundantly *in situ* by aneurysm infiltrating macrophages located at the site of tissue damage.³² MMP inhibition has emerged as a potential pharmacotherapeutic approach to limit the development and progression of AAAs.^{9-11, 35}

Doxycycline as an MMP Inhibitor

The objective of developing inhibitors of MMPs and inflammation is to stabilize aneurysmal disease and prevent further expansion, thus delaying or eliminating the need of surgery. To date, there is no inhibitor that specifically inhibits MMP9, however there are inhibitors that inhibit all or most MMPs nonspecifically.

One of the nonspecific inhibitors of MMPs is doxycycline. Tetracyclines were discovered in 1948 as natural fermentation products of *Streptomyces aureofaciens*. The first chemically purified tetracycline was chlortetracycline.³⁶ Basic chemical structure consists of a tetracyclic naphthacene carboxamide ring system. Tetracyclines with antibiotic activity have a dimethylamine group at carbon 4 in ring A. Nonantibiotic properties of tetracyclines include inhibition of inflammation, proteolysis, angiogenesis and apoptosis.³⁷

Doxycycline inhibits MMPs via mechanism similar to that found with the endogenous inhibitors of MMPs.³⁸ Nonantibiotic, chemically modified tetracyclines have a similar efficacy to MMP inhibitors, which suggests that the MMP-inhibiting property of tetracyclines is unrelated to their antimicrobial activity.³⁶ Attempts in mice and men have been made to inhibit MMP expression to prevent AAA formation using doxycycline^{10, 39-46} (see Tables II and III). Prall et al¹⁰ showed that doxycycline inhibits MMP activity at doses (100 mg/kg) much higher than those required for its antimicrobial activity. The same study also showed that doxycycline has maximum inhibitory effect on MMP9 and MMP2. Pyo et al²² showed that in

the aortas of mice receiving doxycycline the diameter of AAA was less than in the aortas of mice not receiving doxycycline and that mice which received doxycycline were less prone to developing new aneurysms in the abdominal aorta. Direct inhibition of MMP activity, as measured by means of *in vitro* assays, may be only one of several mechanisms by which doxycycline prevents MMP-mediated ECM degradation.³⁶ For example, tetracyclines downregulate gene expression⁴⁷ and in the presence of doxycycline normal extracellular processing associated with activation of proMMPs may be altered, resulting in diminished activation, accelerated enzyme degradation and loss of enzymatic activity.⁴⁸

Other Potential Drugs as Treatment Options for AAA

In Table IV other agents tested in animal models to prevent development and growth of AAA are summarized. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID), and Holmes et al⁴⁹ tested it in the elastase-perfusion model in rats. The control group received saline, and the treatment group received 4 mg/kg/day of indomethacin for seven days. None of the animals in the indomethacin group developed AAAs; moreover the mean increase in the aortic diameter was less than half of that in the control group.

Moore et al⁵⁰ used a hydroxamate based MMP antagonist (RS132908) for 14 days in rats exposed to elastase perfusion. AAAs developed in all rats in the control group, but only in 62% of the rats treated with RS132908. Bigatel et al⁵¹ tested BB-94 (Batimastat) in the same animal model and reported that the increase in the aortic diameter was significantly less in the treatment group as compared to the control group. Boyle et al⁵² exposed porcine aortic segments to calcium channel blocker amlodipine, and showed that the treated segments had significantly reduced MMP9 activity. Curci et al⁴² tested doxycycline and four other tetracycline derivatives in the elastase-perfusion model and achieved inhibition of the development of AAA in a dose-dependent manner. Lawrence et al⁵³ used rapamycin, a potent immunosuppressant, in the same model, and showed that rapamycin significantly reduced the rate of aneurysm expansion.

Hydroxymethylglutaryl-coenzyme A reductase inhibitors, also known as statins, are widely prescribed for their lipid-lowering effects. They have been shown to inhibit the expression of inducible nitric oxide synthase (iNOS) and several MMPs, including MMP9, by smooth muscle cells and macrophages.⁵⁴ These effects of statins might be attributed to interference with the protein isoprenylation that mediates activation of upstream signaling pathways in inflammation.⁵⁵ Statins can directly modulate the biology of AAA wall in humans and can suppress MMP9 production by inhibiting the activation of neutrophils and macrophages, thus indicating that statin therapy could be an effective treatment for AAA patients.⁵⁶ Kalyanasudaram et al⁵⁷ showed that simvastatin reduces MMP9 protein levels and downregulates mediators of inflammation.

Parodi et al⁵⁸ used curcumin in the rat elastase-perfusion model and showed that the structural integrity of medial elastin was significantly greater in the curcumin-treated mice as compared to the control group. Diferloylmethane (curcumin) is a major component of turmeric, a widely used spice in oriental cooking, and it has been shown to have anti-inflammatory properties.

Targeted Approach for Developing New Specific MMP Inhibitors

Considering that doxycycline is not specific to any particular MMP, it has not been shown to be effective in randomized clinical trials at low doses⁴³ and that it also has other side effects, there is a need to develop more specific MMP inhibitors for AAA. Structure-based ligand design to discover small molecule inhibitors has been used recently to identify new drugs for cancer therapy by targeting key signaling pathways.⁵⁹ The same approach has potential to find putative inhibitors of MMPs to block their function by attempting to interfere with their

catalytic mechanism and their putative binding sites to cofactors or interacting molecules. Virtual screening of large chemical libraries can be performed fast *in silico*, and the best results are then tested for biological activity in experimental systems. The advantages of the use of small molecule drugs include: 1) convenient non-injectable dosing with greater patient compliance; 2) better tissue penetration; 3) specificity for one or few molecular pathways; 4) short half-life with reduced immunosuppression; 5) non-immunogenic; 6) easier and cheaper to manufacture; and 7) easy to combine with other drugs (cocktail of several small molecule drugs).⁶⁰

To find specific inhibitors for each of the MMPs, we propose a four-step strategy (Figure 1):

1. Identify novel MMP inhibitors using computational biology:⁵⁹ Docking computer programs can be used to find new potential MMP inhibitors. Ideal candidates would be small molecules, which differ from each other in their chemical structure and act at different sites of the molecular structure of MMPs.
2. Test the inhibitory effect of MMP inhibitors in *in vitro* assays: Commercially available recombinant MMPs can be used for the testing. The inhibitors will be added in different concentrations and combinations and the activity of MMP can be determined by commercially available gelatinase activity assay kits. The results of newly designed inhibitors can be compared to doxycycline. Those small molecule inhibitors showing inhibitory effect in *in vitro* tests will be used for step 3.
3. Test the inhibitory effect of new MMP9 inhibitors in cell culture systems: After the initial *in vitro* testing, a more functional test of the MMP9 inhibitors can then be carried out in cell culture-based systems and using the Boyden chamber⁶¹ to find out if migration and invasion of cells is affected. Inhibitors which show an effect *in vivo* will then be tested in step 4.
4. Use selected inhibitors in surgical animal models^{22, 62-64} for AAA. One of the experimental animal models for AAAs in rats uses elastase-perfusion to create dilatation in the abdominal aorta.^{21, 22} In another experimental model the aorta is exposed to calcium chloride for 15 minutes, and four weeks later the aorta shows signs of aneurysmal changes.⁶⁴ Yet another experimental model for AAA is to use mice that are genetically deficient for the apolipoprotein E gene and have been exposed to angiotensin II infusion for an extended period of time.^{62, 63} MMP inhibitors can be tested for their ability to prevent aneurysm formation and growth in these models as has been done in the previously reported experiments with doxycycline and other reagents (Tables II, III and IV).

Concluding Remarks

Despite considerable progress, there are several unresolved issues with regard to the basic mechanism(s) of action of MMP inhibitors in limiting MMP-mediated tissue destruction. For example, tetracyclines are relatively weak inhibitors of MMP activity *in vitro*, displaying inhibitory constants in the micromolar range in most assay systems.⁶⁵ Tetracyclines are frequently as effective as other MMP inhibitors *in vivo* and their beneficial influence on connective tissue destruction can often be achieved at remarkably low doses.⁶⁶ One explanation for this paradox may be better pharmacokinetics and tissue absorption compared to those of other currently available MMP inhibitors. Tetracyclines can also suppress the activation of iNOS in macrophages and other cell types through post-transcriptional mechanisms.⁶⁷ Because nitric oxide serves as a potent inflammatory mediator, tetracycline-induced suppression of iNOS may amplify any direct inhibitory effects on MMP activities *in vivo*. Finally, new evidence suggests that tetracyclines may also act to reduce steady-state levels of mRNA for at least some MMPs. This has been demonstrated most prominently for MMP2

in cultured human skin keratinocytes,⁴¹ and for MMP8 in rheumatoid synovial fibroblasts and endothelial cells,⁶⁸ but it remains to be determined how specific this effect is for different MMPs and for different cell types.

The targeted approach outlined in figure 1 using computational biology to design specific small molecule inhibitors⁵⁹ for MMP9 and other key players in the pathogenesis of AAA is likely to be the future of pharmacotherapeutics for cardiovascular diseases. Such “maintenance drugs” could prevent AAA development in individuals who are at increased risk of developing this lethal disease because of the genetic risk factors that they have in the blueprint of their genome⁶⁹⁻⁷¹ or because of the environmental risk factors that they are exposed to. Preliminary animal experiments⁷² show promising evidence that such drugs could also prevent the growth of already existing small AAAs and thereby either delay or completely eliminate the need for surgical intervention. It is expected that such inhibitors will be useful not only in the treatment of AAA, but also in the therapies for other chronic cardiovascular diseases such as atherosclerosis,^{12, 13, 73-76} cerebrovascular disease,^{77, 78} and left ventricular hypertrophy,^{14, 79} as well as other diseases including cancer,⁸⁰ and arthritis,^{81, 82} diseases in which MMPs also play a key role.

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Figure legend.

Outline of the steps needed to develop new MMP9 inhibitors.

Table 1
Summary of studies where MMP levels were measured in human aortic tissues or plasma

Study	No. of Samples	Method of Detection					MMP type											
		AAA	Controls	IH	IS	W	PCR	Z	N	E	1	2	3	8	9	12	13	MT1-MMP
Irizarry et al., 1993 ⁸³	8		4			+					+							
Newman et al., 1994 ⁸⁴	21		4			+							+		+			
Newman et al., 1994 ⁸⁵	10		2	+						+	+	+	+		+			
Newman et al., 1994 ⁸⁶	6		6			+		+		+		+	+					
Freestone et al., 1995 ²⁶	66			+		+				+				+				
McMillan et al., 1995 ³²	8		7		+			+				+		+	+			
Thompson et al., 1996 ³¹	10		10		+			+		+				+				
Patel et al., 1996 ⁸⁷	5		2			+		+			+			+				
Sakalihasan et al., 1996 ⁸⁸	10		6					+			+			+	+			
McMillan et al., 1997 ²⁷	19		4				+							+				
Tamarina et al., 1997 ²⁰	8		5				+			+				+				
Knox et al., 1997 ⁸⁹	18		7	+				+		+		+	+					
Curci et al., 1998 ⁹⁰	15		12	+	+	+	+	+			+				+			
Davis et al., 1998 ⁹¹	10		10	+			+	+			+			+				
Elmore et al., 1998 ⁹²	15		6			+	+				+			+				
McMillan et al., 1999 ⁹³	22		8						+	+				+	+			
Kamijima et al., 1999 ⁹⁴	17		3							+		+	+	+	+			
Crowther et al., 2000 ⁹⁵				+		+					+							
Hovsepian et al., 2000 ⁹⁶	25		5						+	+				+	+			
Sangiorgi et al., 2001 ³⁴	45		10									+	+	+	+			
Carrell et al., 2002 ⁹⁷	8						+			+	+	+	+	+				
Peterson et al., 2002 ⁹⁸	30		30						+	+	+	+	+	+	+			+
Annabi et al., 2002 ⁹⁹									+									
Lorelli et al., 2002 ³³									+	+								
Goodall et al., 2002 ¹⁰⁰									+	+		+	+	+				
Nishimura et al., 2003 ¹⁰¹	34		11	+		+			+	+		+	+	+			+	
Wilson et al., 2005 ¹⁰²	40		10	+														
Watanabe et al., 2006 ¹⁰³	53		9						+				+					
Tromp et al., 2004 ¹⁰⁴	36		20				+							+		+		
Crowther et al., 2000 ¹⁰⁵	4		4		+			+				+						+

Table II
Summary on studies where doxycycline was used in animal models for AAA

Study	Species	Route of Administration	No. of Animals	Doxycycline Group	Control Group	Dose used mg/kg/day
Petrinec et al., 1996 ¹⁰⁶	Rat	Subcutaneous	24	24		100
Curci et al., 1998 ⁴²	Rat	Subcutaneous	24	6		60
Pfall et al., 2002 ¹⁰	Mouse	Oral	30	10		100
Kaito et al., 2003 ¹⁰⁷	Rat	Subcutaneous	33	10		30
Manning et al., 2003 ⁴⁵	Mouse	Oral	32	30		30
Bartoli et al., 2006 ³⁹	Mouse	Oral	13	26		100
Bartoli et al., 2006 ³⁹	Mouse	Osmotic minipump	7-14/dose	20		0.25-1
Sho et al. 2004 ¹⁰⁸	Rat	Periaortic infusion	9	9		1.5

Table III

Summary on studies where doxycycline was used in human AAA patients

Study	Route of administration	No. of Patients	Doxycycline group		Control group	Dose used	Study design
Curci et al., 2000 41	Oral	8	36		7	200 mg/day	Prospective Trial
Baxter et al., 2002 40	Oral					200 mg/day	Prospective Trial
Mosorin et al., 2001 109	Oral	17	15		150 mg/day	Double Blind Randomized Control Trial	

Table IV
Suppression of AAA Development by Agents other than Doxycycline in Animal Models

Study	Species	Number of Subjects Treated Group	Untreated Group	Inhibitor
Moore et al., 1999 ⁵⁰	Rat	8	6	RS132908
Bigatel et al., 1999 ⁵¹	Rat	12	11	BB-94
Curci et al., 1998 ⁴²	Rat	22	6	Tetracycline Derivatives
Holmes et al., 1996 ⁴⁹	Rat	8	6	Indomethacin
Lawrence et al., 2004 ⁵³	Rat	20	18	Rapamycin
Armstrong et al., 2005 ¹¹⁰	Rat	8	8	Rofecoxib
Armstrong et al., 2005 ¹¹⁰	Rat	10	10	Indomethacin
Armstrong et al., 2005 ¹¹⁰	Rat	10	10	1400W
Parodi et al., 2006 ⁵⁸	Mice	36	31	Curcumin
Kalyanasundaram et al., 2006 ⁵⁷	Rat	19	19	Simvastatin
Nakahashi et al., 2002 ¹¹¹	Rat	-	-	Alpha-Tocopherol
Miralles et al., 1999 ¹¹²	Rat	82	73	Indomethacin