Chapter 4

The Role of Metalloproteinases in the Development of Aneurysm

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1. Introduction

Matrix metalloproteinases (MMPs) were first described by Gross fifty years ago. They are a family of zinc-dependent endopeptidases. They comprise a group of 25 enzymes. Metalloproteinases were first described as proteases degrading extracellular matrix (ECM) proteins such as collagens, elastin, proteoglycans and laminins, hence they were named matrix meatalloproteinases. MMPs were divided according to their substrate specificity into collagenases, gelatinases, stromolysins and matrilysins. This classification was later replaced by numbering the enzymes according to the chronology of their identification. Metalloproteinases are also called metalloproteases.

Four metalloproteases (MMP-14, MMP-15, MMP-16 and MMP-24) have a transmembrane and cytosolic domains. They constitute a subgroup of membrane-type metalloproteases (MT-MMPs) [1, 2].

2. Physiological role of metalloproteinases

MMP-1 (collagenase 1) hydrolyzes collagen types I, II, III, VII, VIII, X and XI, as well as gelatin, fibronectin, vitronectin, laminin, tenascin, aggrecan, links protein, myelin basic protein and versican. MMP-2 (gellatinase) degrades collagen types I, II, III, IV, V, VII, X and XI, gelatin, elastin, fibronectin, vitronectin, laminin, entactin, tenascin, SPARC and aggrecan, links protein, galectin-3, versican, decanin and myelin basic protein. One of the most important differences between theses two metalloproteinases is the possibility of the hydrolysis of elastin and collagen type IV by MMP-2, but not by MMP-1. Researches have also focused their interest on MMP-9 which can degrade collagen types IV, V, VII, X and XIV, fibronectin, laminin, nidogen, proteoglycan link protein and versican.

For a long time metalloproteinases have been vied solely as enzymes of matrix proteins breakdown. Results of researches performed in recent years indicate that there is a group of

non-matrix proteins which can be substrates for various MMPs. Metalloproteinases are involved in the activation of latent forms of effective proteins. For example, MMP-2, MMP-3 and MMP-9 can activate interleukin 1β (IL-1β). They can also act on active cytokines, IL-1β undergoes subsequent degradation catalyzed by MMP-3. Metalloproteinases can alter cell surface proteins such as receptors and act on microbial peptides.

Metalloproteinases are not indiscriminately released by cells. They are secreted to or anchored to cell membrane. MT-MMPs have a specific transmembrane domain placing them in a certain position. Other metalloproteinases can be bound by specific cell-MMP interactions. This phenomenon allows an exact localization of their proteolytic activity [1,2].

3. Activation of metalloproteinases

Metalloproteinases are encoded as inactive proenzymes, zymogens. They undergo proteolytic activation. This process can take place either intracellulary or extracellulary. One third of MMPs are activated by intracellular serin protease, furin. This process takes place in trans-Golgi network. A number of MMPs has a cleavage site for other metalloproteinases. MMP-3 activates proMMP-1 and pro-MMP-7. Some metalloproteinases have been described to be activated by kallikrein or plasmin.

In vivo studies indicate that reactive oxygen species (ROS) generated by neutrophils can both activate and subsequently inactivate MMPs. Hypochlorus acid (HClO) generated by neutrophil myeloperoxidase and hydroxyl radicals can activate proMMP-1, proMMP-7 and proMMP-9, whereas peroxynitrate can activate proenzymes of MMP-1, MMP-2 and MMP-9. This process enables a control of burst of proteolytic activity within an inflammatory setting.

Like some other proteases, activity of MMPs is controlled also by two other mechanisms, regulation of gene expression and specific inhibitors. MMP-2 is constitutively expressed and regulation of its activity occurs by either activation or inhibition. Expression of a number of metalloproteinases is up-regulated during various pathological conditions. Among them inflammation is the most studied setting. MMPs are inhibited by α-2 macroglobulin and tissue inhibitors of metalloproteinases (TIMPs). There are four TIMPs. Their secretion is also regulated and represents another point in a network of control of the activity of metalloproteinases. TIMP-3 is primarily bond to ECM and allows a regulation of MMPs' activity in the very site of their action. The network of the control of the activity of metalloproteinases is complex and very precise. Sometimes TIMP interacts with proMMP and inactivate other MMP, e.g. a complex of TIMP-1 and proMMP-9 inactivates MMP-3.

Protection from MMP degradation represents the next step in this sophisticated network of diverse interactions. Neutrophil gelatinase-associated lipocalin (NGAL) bounds to MMP-9 protecting this metalloproteinase from its degradation [1,2].

4. Localisation of metalloproteinases in a vascular wall

Metalloproteinases can be detected in all three layers of a vascular wall. Endothelium can produce MMP-1 and MMP-2. Smooth muscle cells (SMC) of both intima and media are the

next source of MMPs. They can secrete MMP-2 and MMP-9. SMC can also produce TIMP-1 and TIMP-2. Adventitia is the layer where MMP-9 can be synthesized. Apart from these most studied metalloproteinases some other MMPs can be detected in a vascular wall: MT1-MMP, MMP-3, MMP-8, MMP-10, MMP-12 and MMP-13. Metalloproteinases are found not only in a wall of arterial wall, but in veins as well.

The balance between the expression of MMPs and TIMPs plays a vital role in preserving the proper and health state of the vascular wall. This equilibrium between activation and inactivation of MMPs is a part of a balance between synthesis and degradation of collagen and elastin, two proteins which have various properties and functions in the arterial wall. Both proteins are crucial for a proper function of the arterial wall. An interruption of these two balances may lead to a development of various vascular pathologies including atherosclerosis, formation of aneurysm and inflammation [3-5].

5. Metalloproteinases and aneurysms

The most studied aneurysm is abdominal aortic aneurysm (AAA), far less research has been focused on aneurysms of cerebral arteries and thoracic aortic aneurysm. All aneurysms are characterized by the destruction of the structural integrity of the extracellular matrix proteins, mainly collagens and elastin. MMPs involved in this pathology can origin both from the cells that physiologically constitute the arterial wall and are stimulated to secrete MMPs, i.e. endothelium, SMC and cells that infiltrate the arterial wall in a response to various stimuli [1, 3, 6-9].

Many scientists points out that cells constituting an inflammatory infiltrate are the major source of metalloproteinases involved in the development of aneurysms. Studies of samples derived from patients undergoing surgery for AAA demonstrated that macropgages from the inflammatory infiltrate can express MMP-1, MMP-2, MMP-3, MMP-9 and MT-1MMP. Metalloproteinase-2 was often detected in cells physiologically constituting the arterial wall, but was absent in macrophages within aneurysms. The pathogenesis of AAA and aneurysms of cerebral arteries differs as these vessels present different types of arteries and there are some differences in the physical characteristic of blood flow in them. Recent experimental studies carried on animals confirmed the role of macrophage infiltration in the formation of intracranial aneurysms. A degranulation of mast cells induces the expression and activation of MMP-2 and MMP-9. Inhibitors of mast cell degranulation inhibited the development of cerebral aneurysms in experimental rats [10, 11].

Human studies confirmed that the expression of metalloproteinases within the AAA is greater than in other sites, remote from the dissection. Nishimura *et al.* observed a different profile of MMP activation in small size abdominal aortic aneurysms, less than 45 mm and large size AAA with diameter exceeding 45 mm. In small size AAA MMP-2 and MMP-9 presented greater gene expression whereas in large size AAA membrane type-1 metalloproteinase and MMP-9 had greater expression. The same study demonstrated also differences in the distribution of the metalloproteinases in the arterial wall. MMP-2 was detected mainly in the intima, whereas MMP-9 was present both in intima and adventitia.

Nishimura *et al.* also observed a significant correlation of the expression of MMP-2 and MMP-9 and between each of these metalloproteinases and TIMP-1 [6].

The degeneration of collagen and elastin leading to the development of aneurysms is a multifactorial process. Various factors may take part in the stimulation of both cells constituting the arterial wall and cells infiltrating it to produce MMPs. Aortic wall is subjected to cyclic stretching because of pulsative blood flow which is a normal physiological condition. AAA is often accompanied by an intraluminal thrombus. It causes that some cells within the aneurysm may be subjected to hypoxia. Experimental study of Oya *et al*. revealed that macrophages cultured in conditions subjected to cyclic stretching under normoxia and hypoxia which simulated the pulsative blood flow and hypoxia due to thrombus presented an increased MMP-9 production. These macrophages produced interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) leading to increased apoptosis of vascular smooth muscle cells. Hypoxia was also demonstrated to augment the expression of MMP-1, MT-1 MMP, MMP-2, MMP-7 and MMP-9 in SMC derived from human aorta [12, 13].

Nowadays many scientists focus their research on finding new factors which may augment the secretion of metalloproteinases by the cells present in aneurysms. Experimental studies confirmed that stenosis resulting in a turbulent blood flow can be the next factor increasing expression of MMP-2 and MMP-9 within abdominal aortic aneurysm. Interesting results were obtained by Stolle *et al*. Mice exposed to cigarette smoke and angiotensin II treatment had increased the incidence of AAA and higher gene expression of MMP-2, MMP-3, MMP-8, MMP-9 and MMP-12 in aorta and increased proteolytic activity of two most investigated metalloproteinaes, MMP-2 and MMP-9. Although each of this two factors alone induced minor changes, their combination accelerated the pathologic process. Exposure of the arterial wall to an increased concentration of angiotensin II represents conditions that may be observed in patients with arterial hypertension. This experiment demonstrates that coexistance of arterial hypertension and smoking augments the risk of a development of aneurysm [14, 15].

Studies of Zhang *et al*. demonstrated that human AAA tissues had elevated levels of advanced glycation end products (AGEs) and their receptor (RAGE). In experimental model this group of researchers observed that AGEs induce the production of MMP-9 by macrophages. An increased serum concentration of AGEs accompanies poorly controlled diabetes mellitus. These results indicate that such patients may be at a greater risk of a development of AAA [16].

Abdominal aortic aneurysm is characterized not only by the destruction of its structural integrity of the extracellular matrix protein and inflammatory infiltrate but also by intensive neovascularisation. These new blood vessels developing inside the arterial wall in a place of growing aneurysm are the next source of metalloproteinases degrading ECM. Immature neovessels express MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9 and MMP-12 [8].

Polish scientists have observed that the intraluminal thrombus occurring within AAA may modulate the activity of MMP-8, MMP-9, neutrophil elastase and TIMP-1. Specimens from patients with thin, less than 10 mm, thrombus-covered wall of AAA presented significantly higher activity of 3 evaluated metalloproteinases and lower TIMP-1 concentration than thick, exceeding 25 mm, thrombus-covered wall. The intraluminal thrombus may exert its pathological effect through trapping erythrocyte and neutrophils and monocytes. The exact mechanism of activation of MMP in these conditions has not been fully elucidated. Scientists consider various factors activating metalloproteinases, such as hypoxia caused by reduced blood flow or oxidative stress in trapped blood cells. This aspect needs further evaluation [17].

Japanese researchers compared the activity of MMP-2 and MMP-9 in ruptured and unruptured middle cerebral artery dissections obtained during neurosurgery in the same patient. Both metalloproteinases presented greater expression in the ruptured dissection [9].

6. Possible diagnostic markers

The results of scientific researches discussed so far concern the evaluation of either expression or activity of MMPs in the tissue obtained from aneurysms in humans or experimental animals. These measurements have the scientific importance but cannot serve as a diagnostic marker. A growing interest is focused on finding circulating predictors of risk of the development of aneurysm or plasma markers of existing, yet undiagnosed aneurysm. Plasma levels of MMPs are of great interest. MMP-2 and MMP-9 are the candidates for such markers. Polish researchers observed significantly higher plasma concentration of MMP-9 in patients with AAA and thin intraluminal thrombus than in patients with abdominal aortic aneurysm and thick thrombus. Hellenthal *et al.* points out that plasma level of MMP-9 may serve as a marker discriminating patients with and without endoleak within AAA [18-20].

Several polymorphisms of metalloproteinases have been discovered. Their role in the development of AAA is being studied. The polymorphisms of MMP-2 (1306C/T), MMP-3 (5A/6A) and MMP-13 (77A/G) may contribute to the pathogenesis of AAA. The studies of circulating markers of aneurysms give promising results but further research is still required [21].

7. Visualisation of metalloproteinases in aneurysm

Several studies have been aimed at imaging of matrix metalloproteinases and quantifying the inflammatory process that drives abdominal aortic aneurysm development. American scientists developed the MMP-activated probe, MMP Sense 18-20 (VisEn Medical, Woburn, USA) that was used for the *in vivo* and *ex vivo* macroscopic scale imaging. This method based on fluorochromes may be used intravascularly. A new magnetic resonance imaging contrast agent, P947, has been tested for its capabilities of targeting MMPs *in vivo* in

expanding experimental AAA. This method allows the detection of MMP activity within the inflammatory infiltrate within AAA and may become a potential non-invasive method to detect AAA at a high risk of rupture [22, 23].

8. Possibilities of pharmacological modulation of metalloproteinases activity

Patients with a high activity of MMPs within the aneurysm are at increased risk of its rupture leading to serious clinical consequences including death. It is of a great importance to find agents which can inhibit MMPs activity and reduce this risk.

Doxycycline, a tetracycline antibiotic, is a known inhibitor of metalloproteinases activity with a growing body of evidence of its beneficial effects observed in animal studies. However data from human studies comprising 6 controlled trials and 2 cohort studies gave conflicting results. The safety of long term use of doxycycline needs evaluation [24].

Statins are a well known group of drugs lowering plasma cholesterol level used to reduce the risk of a coronary heart disease. They have a pleiotropic mode of action reducing the progress of atherosclerosis. Experimental studies indicate that simvastatin can reduce the activity of MMP-2 and MMP-9 in AAA and suppress the development and expansion of abdominal aortic aneurysm. A study performed on samples derived from patients receiving atorvastatin and undergoing surgical treatment for AAA gave promising results demonstrating a significantly reduced activity of MMP-13. Another study with short term, 4 weeks, administration of atorvastatin preceding the operation did not show any differences in the activity of MMP-2, MMP-8, MMP-9, TIMP-1 or TIMP-2. These data indicate that statins may require a long term use to develop their beneficial influence on MMPs' activity [25-27].

Drugs which are administered for a long time focus the scientists' interest. Anti-hypertensive drugs have been studied in this aspect. Calcium channel blocker, azelnidipine, decreased the activity of MMP-2 and MMP-9. The similar influence of angiotensin II receptor blockers, olmesartan and losartan, was observed. The latter was also shown to act synergistically with doxycycline. Perindopril, an angiotensin converting enzyme inhibitor, is known as an anti-hypertensive agent with an ability to affect vascular wall remodeling. In an experimental study perindopril significantly reduced the activity of MMP-2 and MMP-9. In animal studies the activity of MMP-2 and MMP-9 were also decreased by edaravone, a scavenger of reactive oxygen species, resveratrol, a plant derived polyphenolic compound. Two inhibitors of cyclic adenosine monophosphate phosphodiesterase (PDE) were also shown to inhibit the activity of metalloproteinases. Cilostazol, the inhibitor of PDE-3 decreased the activity of MMP-2 and MMP-9, whereas ibudilast, which predominantly blocked PDE-4, decreased the expression of MMP-9 [28-37].

Although the experimental studies indicate that various drugs can reduce the expression and activity of metalloproteinases, their potential use in humans to protect from AAA or

inhibit its development requires further studies. Their efficacy and safety of a long term administration must be proven.

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