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Direktor: Professor Dr. med. Thomas Unger

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Impact of Renin-Angiotensin- and Kallikrein-Kinin Systems in Aneurysm Formation and Possible Therapeutic Implications

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Dr. med. Elena Kaschina

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Dekanin: Prof. Dr. med. Annette Grüters-Kieslich
1. Gutachter: Prof. Bernhard Schiefer/Medizinische Hochschule Hannover
2. Gutachter: Prof. Heikki Ruskoaho/ University of Oulu, Finland

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1. Introduction

1.1. Vascular Remodeling

The arterial wall is an organ capable of remodeling in response to hemodynamic, mechanical, and biochemical stimuli [1]. The direction and scale of remodelling are coordinated by endothelial production of growth factors, proteases, and cellular adhesion molecules in response to sensed changes in blood flow. Moreover, changes in vascular structure are not solely determined by hemodynamic forces, and a role for inflammatory responses and changes in extracellular matrix components has been suggested [2]. An aneurysm is defined as a permanent dilation of the arterial wall, which is characterized by outward vessel remodelling, both in vessel dimension and vascular structure.

1.2. Abdominal Aortic Aneurysm

Clinical relevance

Abdominal aortic aneurysm (AAA) is a permanent localized dilatation of the abdominal aorta encompassing all three layers of the vessel wall that exceeds the normal diameter by 50% [3]. It is estimated to be the tenth most common cause of mortality and accounts for 2% of all deaths; up to 8% of men over 60 years are now affected [4]. Present treatment options include endovascular stents or open surgery, but these procedures are not appropriate for all patients. Furthermore, invasive procedures provide no therapeutic advantage for AAA <5.5 cm diameter. The value of early endovascular intervention, for aneurysms of 4 to 5.4 cm, is currently under investigation [5].
Although most aneurysms remain asymptomatic and undiagnosed, risk of rupture increases dramatically when diameters exceed 5.5 cm. The prognosis of ruptured AAA remains poor, and the overall mortality remains high (80% to 90%) [6].

Pathomechanisms of Aneurysm Formation

The pathogenesis of AAA formation is complex and not fully understood. There are, however, well defined risk factors, such as male sex, cigarette smoking, hypertension, advanced age, atherosclerosis, and a genetic predisposition [4]. Connective tissue disorders (e.g. Marfan- and Ehlers Danlos syndrome) have also been strongly associated with AAA. Other causes of the development of AAA include: infection (*Chlamydia pneumoniae*), arteritis, trauma und cystic medial necrosis.

Aneurysm formation involves a complex process of destruction of the aortic media and supporting lamina through degradation of elastin and collagen [7,8]. This leads to a decrease in tensile strength in the aortic wall which can then lead to aneurysm formation. Recent evidence has confirmed the significance of a chronic inflammatory process, proteolysis and extracellular matrix degradation [9].

Inflammation

Chronic inflammation of the aortic wall plays an important role in the pathogenesis of AAA. Studies of human AAA tissue have shown extensive inflammatory infiltrates containing macrophages and lymphocytes in both the media and adventitia, and increasing aneurysm diameter was associated with a higher density of inflammatory cells in the adventitia [10]. In AAA, inflammatory cells (polymorphonuclear neutrophils, T cells, B cells, macrophages, mast cells, NK cells, etc.) percolate through all layers of the wall (Fig.2). These infiltrating cells secrete various inflammatory factors, including cytokines, chemokines, leukotrienes, reactive oxygen species, and immunoglobulins. The vessels of the *vasa vasorum* form the pathways by which inflammatory cells access the aortic intima and media. Medial neovascularization and decreased vascular smooth muscle cells also characterize AAA lesions. T helper cell type 2 (Th2) express IL-4, -5, -8, and -10 and TNF-alpha for the regulation of the local immune response [11]. These cells also release Fas ligand and FAP-1, leading to the apoptosis of VSMC [12]. Activated macrophages are the main cells secreting MMPs, leading to the disruption of the orderly lamellar structure of the aortic media. Mast cells synthesize and release chymase and cathepsin G as well as pro-inflammatory cytokines and growth factors [13]. In various experimental models of aneurysm we also found an inflammatory response. For example, in kininogen-deficient animals with AAA changes in plasma cytokines were compatible with inflammatory vascular damage, i.e., upregulation of IFN-γ and downregulation of GM-CSF [14]. In the elastase rat model of AAA we observed inflammatory infiltrates in the adventitia and found the increased MCP-1, MPO levels in serum, an up-regulation of NFkB as well as an increase in cytokines TNF-alpha and TGF-1 beta [15]. Furthermore, TNF-alpha was the most important factor contributing to aortic dilatation by obese mice [16]. Activation of NFkB by uremic toxins followed by inhibition of the elastin and collagen genes transcription contributed to vascular remodelling during mild uremia (Kaschina et al., not published data). Thus, inflammatory response consistently followed aortic dilatation by all remodelling models, although the expression of inflammatory markers was not always the same.

Proteolysis of Extracellular Matrix Proteins

The degradation of tunica media by means of proteolytic process seems to be the basic pathophysiologic mechanism of the AAA development. Understanding of proteolytic processes suggests potential areas for therapy.
MMPs are considered to be the predominant proteinases [17]. Several MMPs are known to degrade elastic fibres (MMP-2, -7, -9, and -12), several degrade interstitial collagen (MMP-1, -2, -8, -13, and -14), and others degrade denatured collagen (MMP-2 and -9) [18]. Particularly, MMP-2 and MMP-9 have attracted interest in the process of AAA development [19]. Patients with AAA have elevated MMP-2 and MMP-9 protein levels in the vasculature remote from the aorta, and the increase in these proteins was correlated with aneurysm diameter [20, 21]. The activation of MMPs is tightly regulated by tissue inhibitors of metalloproteinases (TIMPs), and mRNA levels of TIMPs were decreased in AAA tissue [22, 23] . We could find an activation of pro-MMP-2, MMP-3, MMP-9, pro-MMP-12 in the aneurysmal as compared to healthy

aortic tissues. Consistently with the MMPs up-regulation, the active form of TIMP-4 was down-regulated [24]. In animal model of AAA, by kininogen deficient Brown Norway Katholiek rats (BN/Ka) aneurysm formation was associated with an enhanced elastolysis, increased expression of MMP-2, MMP-3 and down-regulation of TIMP-4 [14].
Other proteases are also reported to contribute to the initiation and progression of AAA [25]. Cathepsins are members of cysteine proteases and are regulated by the inhibitor cystatin C. We found an activation of cathepsins D, L, H in the aneurysmal as compared to healthy aortic tissues. In elastase induced model of AAA we could detect an up-regulation of both MMP-3 and cathepsin D proteins [15]. Moreover, aortic dilatation in mice experimental obesity model was associated with an up-regulation of MMP-3, cathepsin B and cathepsin D in the media and in the adventita of aorta [16].

1.3. The Role of Renin-Angiotensin system (RAS) in AAA

Angiotensin II and vascular remodeling

There is evolving evidence that angiotensin II (Ang II) participates in the initiation and propagation of AAAs [26]. Animal studies have consistently demonstrated the ability of Ang II to promote the formation of AAAs, although the mechanisms of this effect have not been defined [27].
The presence of angiotensinogen, angiotensin-converting enzyme (ACE) and the angiotensin receptors in peripheral tissues strongly suggest that Ang II can be both generated and perform its effects locally [28]. Local RAS has been implicated in vascular remodelling. The formation of Ang II by peptidases which are different from ACE has been known as alternative Ang II - generating pathway (Fig.1). Renin and cathepsin D convert angiotensinogen to Ang I, cathepsin G, tissue plasminogen activator and tonin convert angiotensinogen directly to Ang II, chymase and cathepsin A convert Ang I to Ang II [29]. Some of these proteases may be brought to the diseased site within a tissue by infiltrating cells such as leukocytes (e.g. cathepsin D and cathepsin G is secreted by monocytes and neutrophils), T-cells (also secreting cathepsin D and cathepsin G) or mast cells (an important source of chymase, cathepsin D and cathepsin G and even renin) [30]. These alternate Ang II-formation pathways may be more important for the formation of Ang II on a tissue level and therefore in the development of vascular disease. Ang II also activates MMPs [31]. Moreover, the increase of the proteolytic activity of cathepsins leads to extracellular matrix degradation, activation of MMPs, elastin hydrolysis and apoptosis [32]. These combined actions make RAS a strong contributor to aneurysm formation.
In human studies, the data about the role of RAS in aneurysm formation are limiting and still controversial. While some authors have shown that human aneurysmal tissue possesses an increased ability to generate Ang II compared to normal tissue [33,34], the comparison of the aneurysmal tissue from ruptured and unruptured cerebral aneurysm suggested that a decreased expression of local RAS components plays a role in the pathogenesis of disease [35]. In our study, where we compared the expression of different RAS components in human healthy, atherosclerotic and aneurysmatic aorta, most of RAS components showed a significantly stronger expression in AAA [36]. Interestingly, almost all RAS components were found in high

abundance in cells of the inflammatory infiltrate, which was mainly localized in the adventitia. Thus, an activated local RAS within the vessel wall may not only attract the inflammatory cells by stimulating the expression of cytokines and chemokines, but the infiltrate itself may serve as a kind of secondary local RAS.

The AT1 Receptor Antagonists

Ang II can bind to AT1- or AT2 receptors mainly localized within the adventitia and vascular smooth cells and the AT2 receptor preferably localized on endothelial cells [37]. Ang II, through its AT1 receptor, participates in vascular remodelling, endothelial dysfunction and inflammation. Ang II activates NF-kappa B via the production of superoxide, regulate cell adhesion molecules ICAM-1,VCAM-1, E-selectin and the cytokines IL-6, IL-6, IL-8, MCP-1 and TNF-alpha [38,39], which, in turn, mediate the adhesion and of monocytes, lymphocytes and leukocytes to the vessel wall, their migration, activation of T-cells, differentiation of B-cells etc. [40].

Evidence for an unfavourable of the AT1 receptor in AAA has been provided by our and other groups showing that AT1-blockade could slow AAA progression in different animal models, such as in a mouse model of atherosclerosis [41], in the mouse with Marfan syndrome [42] as well as in rat model of aneurysm [15].

AT1 receptor blockade is known to have beneficial effects on the vasculature via different protective mechanisms, e.g. anti-inflammatory, anti-oxidative, anti-atherosclerotic [43,44].

ACE inhibitors suppressed the development of elastase-induced AAAs in the rat [45]. Furthermore, treatment with ACE inhibitors in a population-based case-control study was associated with a reduced risk of ruptured abdominal aortic aneurysm [46].

Recently, we studied the effect of the AT1 receptor antagonist telmisartan in different models of aneurysm. Telmisartan treatment significantly reduced aneurysmal size, independently from blood pressure reduction [15]. Pro-inflammatory factors TNF-alpha, TGF-1 beta and NFkB, proteases MMP3 and cathepsin D as well as apoptotic markers caspase 3, p53 and Fas ligand proteins, were significantly downregulated in aortic tissue under telmisartan compared to vehicle treatment. Furthermore, telmisartan abolished the obesity-induced aortic dilatation in mice by inhibiting proteolytic dysregulation in perivascular adipose tissue [16]. This effect was due to attenuated cytokine-induced expression of cathepsin B, cathepsin D, MMP2 and MMP3 in adipocytes. Also in the VSMCs telmisartan prevented IL-1 alpha induced secretion of MMP2 and MMP9 [47]. Whether this antiproteolytic effect is primary or only secondary to the anti-inflammation requires further investigations.

The AT2 Receptor Agonists

Recent investigations have established a role for the AT2 receptor in cardiovascular, brain and renal function as well as in the modulation of various biological processes involved in development, cell differentiation, tissue repair and apoptosis. Although the AT1 receptor is dominant in the adult organism, an increase of AT2 receptor expression has been observed under pathological conditions, such as vascular injury, myocardial infarction and congestive heart failure, renal failure, brain ischemia and sciatic or optic nerve transsection [44, 48, 49].

The AT2 receptors are localised on the vascular endothelial cells [50] as well as on the cells involved in the inflammatory and immune reactions, such as monocytes [51], mast cells [52], T-

cells [53] and C-kit +cells [54]. Therefore, the AT2 receptor stimulation may be vascular protective due to its anti-inflammatory effects, which has been recently demonstrated in the model of myocardial infarction [55]. Treatment with the AT2 receptor non-peptide agonist compound 21 decreased up-regulated levels of monocyte chemoattractant protein-1 and myeloperoxidase as well as cardiac IL-6, IL-1beta, and IL-2 expression. AT2 receptor–coupled signaling leading to reduced IL-6 levels involved inhibition of nuclear factor κB, activation of protein phosphatases, and synthesis of epoxyeicosatrienoic acid [56]. Thus, it could be hypothesized that a direct activation of the AT2 receptor may be beneficial by AAA. This pharmacological approach requires further investigation.

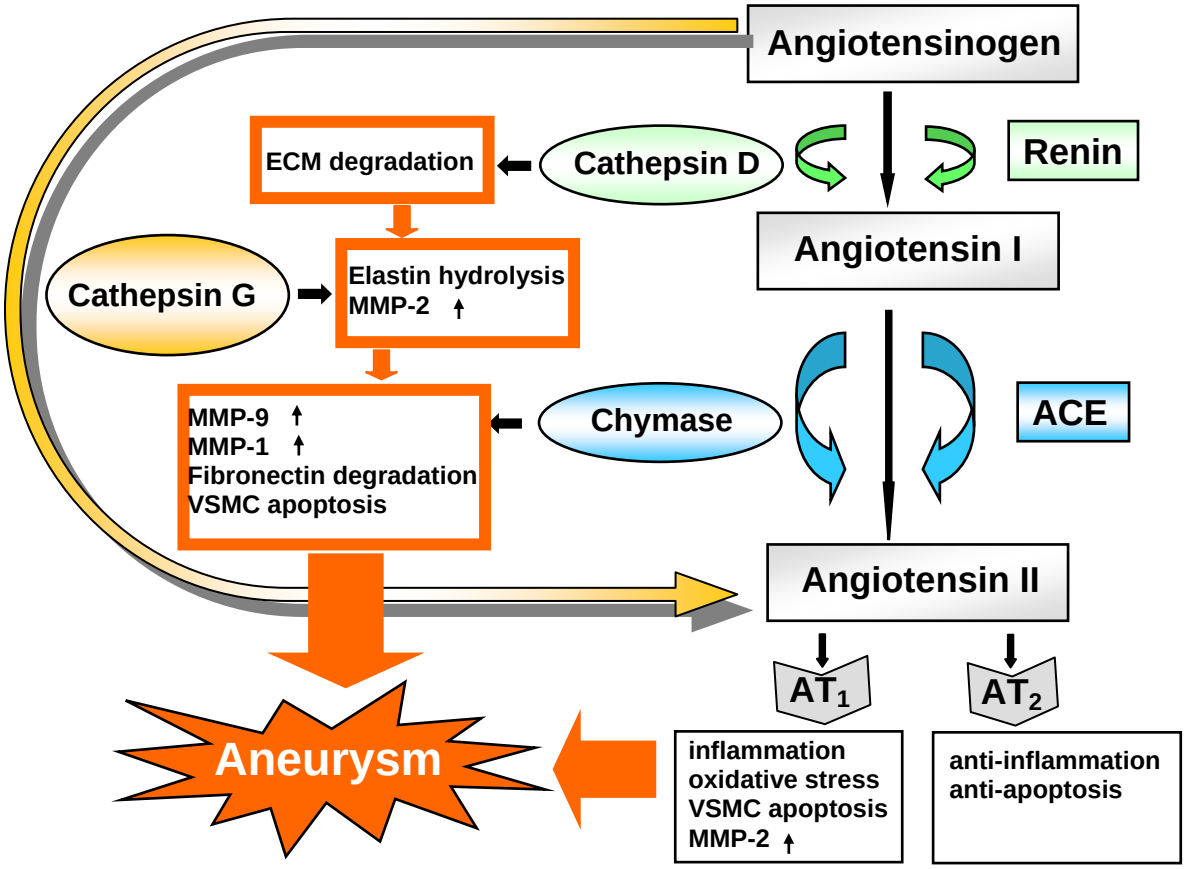

Figure.1 The role of different RAS components in aneurysm formation

1.4. The Role of Kallikrein-Kinin System

Kallikrein-Kinin System (KKS)

Kallikrein-kinin system is known to counterbalance RAS. Kinins (bradykinin and lysyl-bradykinin) have been implicated in the regulation of renal function, blood flow, and blood pressure. Kinins are generated from kininogens by tissue and plasma kallikreins [57], and their

pharmacological effects are mediated by B1- and B2 subtypes of kinin receptors. Kininogen, also known as alpha-2-thiol proteinase inhibitor is a secreted plasma glycoprotein that is involved in the generation of kinins, modulation of platelet aggregation, angiogenesis and acute phase response [58]. Kininogen is present in the vascular wall [59]. High molecular weight kininogen (HMWK) consists of 6 domains and is processed by plasma kallikrein to release bradykinin and yield HKa. HKa is a disulfid-linked dimer containing heavy chain (domains 1 to 3) and light chain (domains 5 and 6).

Kininogen in the Regulation of MMPs

We could demonstrate for the first time that kininogen deficiency by Brown Norway Katholiek (BN/Ka) rats contributes to aneurysm formation and this process is associated with proteolysis, increased release of inflammatory cytokines and FasL- and capase-3 dependent apoptosis [14]. These findings suggest that genetic kininogen deficiency renders vascular tissue prone to aneurysmatic but not to atherosclerotic lesions. Further studies demonstrated that kininogen affects the regulation of MMPs in vascular smooth muscle cells (VSMCs). Using an *in vitro* model of VSMCs, cultured from the rat aorta, we found that the cleaved form of high molecular weight kininogen (HKa) affected the expression of MMP-9 and MMP-2 and their tissue inhibitors (TIMPs). Treatment of VSMCs with HKa reduced in a concentration-dependent manner IL-1alpha-induced release of MMP-9 and MMP-2 associated with decreased MMP enzymatic activity levels [60]. Our findings have been recently confirmed by Wu et al. [61], who demonstrated the down-regulation of MMP-2 by HKa in endothelial cells. HKa has been also shown as a potent agent in other studies: It inhibits migration and invasion of prostata cancer cells [62] as well as it inhibits angiogenesis [63].

Kininogen in the Regulation of Apoptosis

Kininogen like cystatins is a potent inhibitor of cysteine proteases (cathepsins) [64]. Cathepsins are released from macrophages during inflammation and possess highest elastinolytic and collagenolytic potential [65]. Therefore, one of the additional possible protective pathways of kininogen, similar with cystatins, may include cysteine protease inhibition. Interestingly, cystatin deficiency increases elastic lamina degradation and aortic dilatation in apolipoprotein E-null mice and correlates inversely with increased aortic diameter in human [25]. The findings highlight a potentially important role for imbalance between cysteine proteases and their inhibitors in arterial wall remodelling. Moreover, cathepsins induce apoptosis [32]. Therefore, we investigated the role of kininogen in the apoptosis of VSMCs [66]. High molecular weight kininogen (HMWK) concentration-dependently prevented aortic VSMC from entering apoptosis which was associated with a down-regulation of apoptotic index, cleaved caspase 3 and 9, decreased caspase 8 activity and reduced release of cytochrome C and cathepsin B into the cytosol. Consistent with these results, the expression of the anti-apoptotic protein Bcl-X_L and phospho-42/44 MAPK was increased by HMWK. These results were confirmed by rescue of VSMC transfected with an HMWK expression vector.
All these findings raise the possibility that alterations of the arterial kallikrein-kinin system may play an important role in the pathogenesis of vascular disease. Other findings, e.g, association of kininogen deficiency in human with vertebral artery dissection [67], development of dilatative cardiomyopathie in kallikrein deficient mice [68] and association of low kallikrein gene activity with the brachial artery inward remodeling [69] also confirm the protective role of KKS.

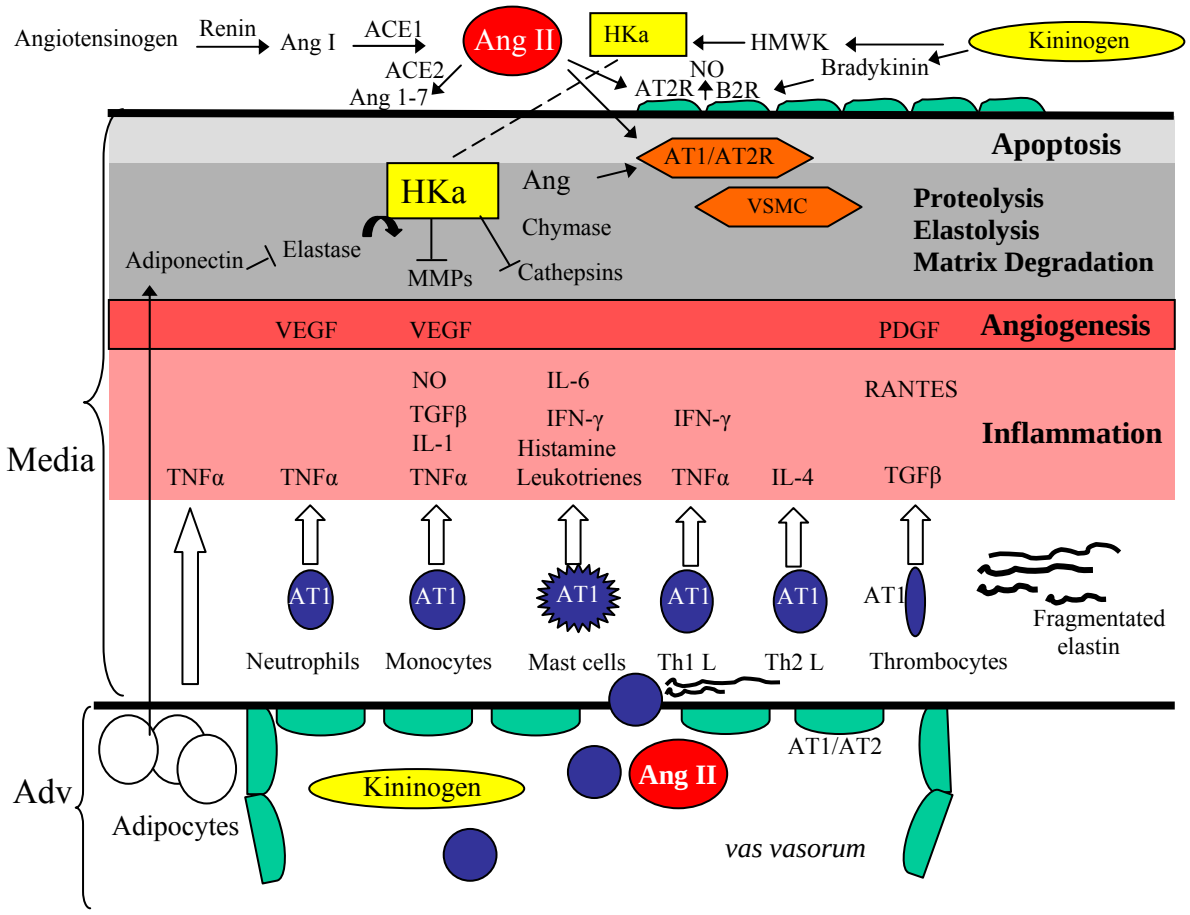

Figure 2. Molecular pathways of aneurysm formation. Implication of RAS and KKS.

1.5. Other treatment strategies for AAA

Apart from RAS- and KKS-associated medications, a number of pharmacological therapies have also the potential to limit AAA progression. Among them, statins [70], antibiotics (roxithromycin) [71] and anti-inflammatory agents [72] appear to inhibit the AAA growth rate in humans. However, the sample size and follow-up period of studies were limited. Therefore, a large randomized study with long-term follow-up of small AAA should be performed to clarify the effect of these agents.

Based on molecular mechanisms of AAA formation, numerous new strategies have been proposed to prevent AAA development. One of them is c-Jun N-terminal kinase (JNK) regulation. Inhibitor of JNK not only prevented AAA formation, but also caused regression of established AAA in mouse models [73]. Furthermore, a combined treatment using a JNK inhibitor and decoy ODNs against NFkB and ets has been reported to regress AAA in animal models [74].

Another new strategy is the inhibition of mast cell degranulation. Application of disodium cromoglycate, an inhibitor of mast cell degranulation, reduced aortic expansion by 40% in an elastase-induced mouse AAA model, accompanied by the inhibition of recruitment of mast cells and macrophages [75]. Similarly, Tsuruda T. et al. [76] demonstrated that treatment with another inhibitor tranilast attenuated AAA progression in a $CaCl_2$-induced rat AAA model. Potential targets for AAA treatment could be summarised as follows: inhibition of proteolytic activity, inhibition of inflammatory response, and upregulation of synthesis of extracellular matrix proteins.

2. Objectives

Abdominal aortic aneurysm is a complex vascular disorder which causes significant mortality [4]. AAA is characterized by a chronic inflammatory reaction, matrix degradation and outward vascular remodelling [7,8,9].

Animal studies have demonstrated the ability of Ang II to promote the formation of AAAs, although the mechanisms of this effect have not been defined [27]. In human studies, the data about the role of RAS in aneurysm formation are limiting and still controversial [33,34,35].

The kallikrein-kinin system is known to counterbalance RAS. Kininogen is involved in the generation of kinins, inhibition of proteases and acute phase response [58]. The vascular effects of kininogen by aneurysm have not been investigated.

In this work, the role of different components of renin-angiotensin- and kallikrein-kinin-systems in aortic remodelling process were studied. The therapeutic targets were identified and their effects were analysed in various experimental models.

The research was focused on:

- The role of different RAS components in the transition from atherosclerosis to aortic aneurysm in humans (3.1., paper 1).
- The effect of the AT1 receptor antagonist telmisaratan in the treatment of aneurysm in the rat (3.2., paper 2).
- The pharmacological effects of AT2 receptor stimulation in experimental model of myocardial infarction (3.3., paper 3).
- The role of kininogen in aneurysm formation in genetic model (3.4., paper 4).
- The regulation of MMPs by kininogen in vascular smooth muscle cells *in vitro* (3.5., paper 5).

This study aims for a better understanding the molecular mechanisms underlying vascular remodelling during aneurysm formation. In particular, the study characterizes angiotensin receptors and proteases of the renin-angiotensin systems as well as high molecular weight kininogen in the context of outward vascular remodelling. The investigations are also focused on *in vivo* effects of RAS-associated medications, e.g. AT1 receptor blockers and AT2 receptor agonists.

3. Results

3.1. Transition from atherosclerosis to aortic aneurysm in humans coincides with an increased expression of RAS components.

Kaschina E, Scholz H, Steckelings UM, Sommerfeld M, Kemnitz UR, Artuc M, Schmidt S, Unger T.

Atherosclerosis; 205(2):396-403, 2009

3.2. Telmisartan prevents aneurysm progression in the rat by inhibiting proteolysis, apoptosis and inflammation.

Kaschina E, Schrader F, Sommerfeld M, Kemnitz UR, Grzesiak A, Krikov M, Unger T. *Journal of Hypertension*; 26:2361-73, 2008

3.3. Angiotensin II type 2 receptor stimulation: a novel option of therapeutic interference with renin-angiotensin-system in myocardial infarction.

Kaschina E*, Grzesiak A*, Li J, Foryst-Ludwig A, Timm M, Rompe F,Sommerfeld M, Kemnitz UR, Curato C, Namsolleck P, Tschöpe C, Hallberg A, Alterman M, Hucko T, Paetsch I, Dietrich T, Schnackenburg B, Graf K, Dahlöf B, Kintscher U, Unger T, Steckelings UM.

*Both authors contributed equally to this work

Circulation; 118(24):2523-32, 2008

3.4. Genetic kininogen deficiency contributes to aortic aneurysm formation but not to atherosclerosis.

Kaschina E, Stoll M, Sommerfeld M, Steckelings UM, Kreutz R, Unger T.

Physiol Genomics; 19:41-49, 2004

3.5. Cleaved high molecular weight kininogen, a novel factor in the regulation of matrix proteinases in vascular smooth muscle cells.

Vosgerau U, Lauer D, Unger T, **Kaschina E.**

Biochem Pharmacol; 15;79(2):172-9, 2010

4. Discussion

There is evolving evidence that RAS is widely involved in the aneurysm formation. Ang II has direct effects at the cellular level and influences aortic remodelling through the AT1 receptor (papers 1 and 2). The presence of angiotensinogen, the angiotensin receptors and other RAS components such as chymase, cathepsin G and cathepsin D in aorta strongly suggest that Ang II can be both generated and perform its effects locally (paper 1). The converting proteases contribute to aneurysm formation both by generation of Ang II and by additional mechanisms of extracellular matrix degradation.

The AT1 receptor antagonist, telmisartan, prevents AAA progression independently of blood pressure reduction by inhibiting proteolysis, apoptosis and inflammation in aortic tissue (paper 2). Telmisartan acts anti-inflammatory by down-regulation of various cytokines as well as by reducing MCP-1, a chemokine which strongly contributes to aneurysmal vascular degeneration by leukocyte recruitment. Apoptosis of vascular smooth cells is also closely related to extracellular matrix degradation. Whether the anti-apoptotic properties of telmisartan are primary or secondary to the anti-inflammatory mechanisms, for example, down-regulation of TNF-α, requires further investigation.

Evidence for a favourable effect of AT1 receptor antagonism obtained in the experimental studies should be confirmed in a large randomized study with long-term follow-up of AAA. Further investigations should also be focused on the anti-inflammatory properties of the AT2 receptor (paper 3) in AAA as well as on the new treatment strategy using AT2 receptor agonists.

Some components which take part in Ang II generation, namely chymase, cathepsin G and cathepsin D, additionally possess features such as chemotaxis or proteolysis which may accessorily contribute to AAA formation (paper 1). Thus, other possible pharmacological targets are RAS-associated proteolytic enzymes and their selective inhibitors.

The link of AAA with known risk factors for the disease, such as smoking, atherosclerosis, and hypertension could help us in the understanding of pathomechanisms. For example, a comparison of molecular pathways between AAA and atherosclerosis, which we have used, studying the role of RAS components, could be one of possible study approaches in further investigations (paper 1). Our data suggest that in humans RAS activation is not just a key player in the pathogenesis of atherosclerosis, but that a further increasing activation may be involved in the transition from atherosclerosis to AAA.
The main pathomechanisms of AAA formation, such as inflammation, proteolysis and the degradation of exracellular matrix have been established (papers 1, 2, 4, 5). At the same time, the factors that initiate and maintain the abnormal intracellular signalling pathways, such as inflammation, are not yet clear. Also, the interaction of mediators and signalling pathways is not understood. If these points could be clarified, the most effective molecular target would be identified, leading to important new discoveries and therapies for small AAA.

The kallikrein-kinin system, which is known to counterbalance the RAS, is also implicated in the AAA (papers 4 and 5). Our group was first to demonstrate the protective action of KKS in aneurysm (paper 4). We have also provided an explanation of this effect showing the regulation of proteases by high molecular weight kininogen (paper 5). The cleaved form of high molecular weight kininogen affected the expression of MMP-9 and MMP-2 and their tissue inhibitors (TIMPs). These cellular effects could be mediated through uPAR-receptor or other yet unknown receptor. The determination of kininogen-domain which exerts inhibitory action requires further investigations.

Taken into consideration that there are close interactions between RAS and KKS, e.g. in the signalling between the AT2 receptor and KKS, research should be also focused on the interaction mediators. Moreover, a combined approach based on the inhibition of RAS and activation of KKS may become increasingly important in the treatment of AAA.

4. Summary

The overall prevalence of aortic aneurysms has strongly increased in the last 30 years due to an ageing population. Present treatment options such as endovascular stents or open surgery procedures are not appropriate for all patients. The risk of death from aneurysm rupture remains a significant clinical problem. Better understanding of aortic aneurysms is important for development of new pharmacological treatments.

In this dissertation, different aspects of AAA were investigated in the context of renin-angiotensin- and kallikrein-kinin-systems. In the first study we have found that most RAS components were significantly stronger expressed in human AAA when compared to atherosclerotic lesions (paper 1). Some components which take part in Ang II generation, namely chymase, cathepsin G and cathepsin D, additionally possess features such as chemotaxis or proteolysis which may accessorily contribute to AAA formation. Therefore, in humans increasing of RAS activation may be involved in the transition from atherosclerosis to AAA.

The next study (paper 2) was designed to elucidate protective mechanisms of AT1 receptor blockade by AAA formation. We studied telmisartan, the AT1 receptor antagonist with long duration of action and high lipid solubility. Telmisartan prevented aneurysm expansion in an experimental model of AAA independently of blood pressure reduction. Several mechanisms of telmisaratan underlie vascular protection: inhibition of proteolysis, reduced production of proinflammatory cytokines and prevention of apoptotis in the aorta.

The AT2 receptors which are known to afford tissue protective actions may be exposed to enhanced Ang II levels after AT1 blockade. The effects of the AT2 receptor stimulation were investigated using a specific AT2 receptor agonist compound 21 (paper 3). In the model of myocardial infarction, compound 21 reduced infarct size and improved heart function. These protective effects were associated with the anti-inflammation and anti-apoptosis.

The implication of kallikrein-kinin system in aneurysm formation was discovered in a genetic animal model (paper 4). In this study we reported that Brown Norway Katholiek rats, which feature a deficiency of plasma kininogens, develop severe abdominal aortic aneurysm. A genetically determined kininogen deficiency promoted the formation of AAA but not atherosclerosis and was associated with enhanced elastolysis, FasL- and caspase-3-mediated apoptosis, changes in plasma cytokines and the induction of the MMPs associated proteolytic cascade. Next *in vitro* study has revealed a molecular basis for these effects (paper 5). HKa affected the regulation of MMP-9 and MMP-2 and their tissue inhibitors TIMPs in VSMCs as demonstrated by a negative regulation of cytokine-induced MMP expression and activity. This study suggested that HKa might contribute to prevent the extracellular matrix from excessive degradation in the context of physiological and pathophysiological vascular remodeling.

Thus, pharmacological interference with various components of renin-angiotensin- and kallikrein-kinin-systems is a promising approach for the treatment of AAA.

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8. Statement

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