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Pharmaceutical stabilization of abdominal aortic aneurysms : changing its natural history

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Chapter

SUMMARY AND GENERAL DISCUSSION

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SUMMARY AND GENERAL DISCUSSION

Abdominal aortic aneurysms (AAAs) are potentially lethal due to rupture. Rupture occurs mainly in AAA greater than 55mm and acute repair still results in mortality over 30%. Although the results of elective treatment have significantly improved over the years and mortality is low (<3%), there is a considerable risk of morbidity. AAAs are prevalent mostly in elderly patients and generally only progress slowly in size. Therefore, treatment that slows aneurysm growth would allow patients to avoid aneurysm repair, in particularly elderly patients. Insight into the pathophysiology of the disease has improved over the past few years and continuing research has led the focus towards finding pharmaceutical means to inhibit or even abrogate aneurysm growth.

The aim of this thesis was to identify new possible targets for pharmacological treatment of AAAs and to apply this insight to the development of new therapies in a preclinical setting. Besides, understanding the cause of AAA progression can help identify secondary prevention strategies aimed at slowing down expansion.

The pathophysiological development of AAA is currently thought to arise through a complex interaction among the structural properties of the infrarenal aortic wall and various risk factors. Several risk factors for AAA disease have been described in epidemiological screening studies. Amongst others, a history of high plasma cholesterol, hypertension and smoking history, have been associated with AAA disease. These associations have led to various clinical studies evaluating the potential of pharmaceutical strategies, such as anti-hypertensive agents and statins, to inhibit AAA growth rate. A systematic review on current conducted human studies evaluating these pharmaceutical strategies is described in **Chapter 2**. This chapter highlights lack of high quality human studies evaluating the potential of pharmacological inhibition of AAA growth. The majority of the studies had little scientific value because of the retrospective design and small sample size. Moreover, the interpretation of the studies was hindered by lack of standardized measurements and inappropriate statistical analysis. Currently, no pharmaceutical therapy can be recommended for the stabilization of AAA disease.

Chapter 2 also describes the effect of pharmacological cardiovascular risk management on AAA growth. It has long been thought that AAA disease is closely related to atherosclerosis disease. Both diseases are found in the arteries, they have both similar predisposing risk factors and they exhibit similar immune cells at the lesion site. However, after the unsuccessful testing of cardiovascular risk management for human AAA disease the resemblance is less alike than initially thought. Beside the apparent stenotic and occlusive nature in atherosclerosis and dilatation in AAA disease there are more differences. Only 10% of the AAA cases reveal atherosclerotic plaques at the side of AAA formation and aneurysms rarely occur at atherosclerotic prone arteries. It is proposed that the risk factors of both diseases can promote atherosclerosis and AAA disease separately, but the risk factors cannot be defined as causative. For example smoking has a much stronger promoting effect on AAA disease than on atherosclerosis¹.

In a large population screening study smoking accounted for >70% of all AAAs². The strong association between smoking and AAA has been described as early as 1958³ and underlined by Lederle in 2011 who described a possible specific link between declining age adjusted AAA mortality and a reduction in annual adult per capita cigarette consumption⁴. Therefore, smoking cessation should be pursued regardless of other therapies.

Another potential risk factor for AAA disease is a history of pulmonary emphysema. Several parallels have been observed between AAA disease and chronic obstructive pulmonary disease (COPD). Reduced lung function occurs commonly in AAA patients and is associated with an increase in risk of AAA rupture⁵. In both diseases there is a strong association with smoking and this might explain the association between lung function and aneurysm development and growth. However, **Chapter 3** reveals that the increased prevalence of COPD in AAA patients is independent from smoking. This suggests that this relationship only reflects a common susceptibility. This is supported by the appearance of increased inflammation in both diseases with in particular converging pathways including the matrix-metallo protease 9 (MMP9) and neutrophil elastase pathway^{6,7}. In aneurysm disease, increased expression of pro-inflammatory cytokines, such as MMP9, induces degradation of the extracellular matrix and therefore enhances vascular dilatation⁸. Higher levels of circulating pro-inflammatory cytokines, causing e.g. proteolysis, have been found in AAA patients compared to controls without AAA disease^{9,10}. Besides, pathological studies of end stage human AAA biopsies and those performed in animal models have demonstrated the importance of inflammation, proteolysis and vascular smooth muscle cell loss in AAA¹¹⁻¹³. Therefore, insight into immune responses in AAA disease is important for the discovery and application of new therapies.

Several studies identify the vitamin D receptor (VDR) as a potent immunoregulatory factor¹⁴. Reports on the potential of VDR antagonists are diverse. There are several reported mechanisms such as quenching NFkB, MAPK and interference with macrophage activation. In **Chapter 4** it is demonstrated that in AAA disease the effects of VDR activation through a vitamin D analogue are restricted to the T-helper cell content (CD4+) and the expression of cysteine proteases cathepsin K and L. It is unclear if the effects of VDR activation on T-helper cell content will influence AAA disease. The role of T-helper cells in AAA disease remains unclear because results from animal studies are inconclusive and clinically accelerated aneurysm progression during intensive immune suppression has been reported¹⁵⁻¹⁸. Also the effect of inhibiting cysteine proteases on AAA growth remains uncertain. Collagen is the primary matrix component of the media and adventitia of the aortic wall and a prominent target of cysteine proteases. Therefore, interference with cysteine proteases has great potential in pharmaceutical stabilization of AAA growth. In **Chapter 5** the importance of cysteine proteases by quantitative collagen degradation assays assessing the general cysteine protease and MMP mediated collagen degradation is established. Although the cysteine proteases: cathepsin K, L and S have separately been positively linked to AAA progression, inhibiting selectively cathepsin K and S with statins did not result in effective growth reduction or stabilization of AAAs^{8,19-21}. Due to this, in Chapter 5 the effect of a broader spectrum, multiple cysteine proteases inhibitor (E64) on AAA growth was tested. The inhibition of cysteine proteases with the E64 effectively blocked aneurysm formation and preserved collagen in two different murine models of AAA disease. Neutrophils play a crucial role in the vascular inflammation seen in AAA disease^{22,23}. They appear to be responsible for reduced cystatin C levels, the primary endogenous inhibitor of several cysteine proteases. They are also known to produce several serine proteases as well as MMP8 and MMP9, all known to promote degradation of the extracellular matrix. One of the main activators of neutrophils is interleukin 8 (CXCL8). Abundant expression of CXCL8 is a hallmark of human AAA. It is responsible for the ongoing inflammatory response in the aortic wall⁶. Through binding of the CXCR1/2 receptors CXCL8 causes chemotaxis and activation of neutrophils. Moreover, CXCL8 is also the putative receptor for CXC-mediated angiogenesis, another key feature of human

AAA disease^{24;25}. **Chapter 6** describes the CXCL8/neutrophil axis in human AAA and the effect of blocking the neutrophil receptors on the aneurysm formation in the elastase model. Interference with the CXCL8/neutrophil axis resulted in fully abrogating experimentally treated mice compared to control mice. Currently phase 2 trials for several CXCR2 antagonists are being conducted. The first, a phase 2 trial on the effect of a CXCR2 antagonist in COPD is highly promising and there is a significant improvement in lung function and a reduction in inflammation. There has been no negative effect on bone marrow functions in healthy subjects and no increase in general infections. This suggests that blocking neutrophil chemotaxis and activation has great potential in reducing human AAA growth^{26;27}. Currently the way to impair neutrophil chemotaxis, is through colchicine. This compound has been used over decades in the treatment of gout. **Chapter 7** addresses the inhibiting effect of colchicine on aneurysm formation in the murine elastase model of AAA disease. Chapter 7 also proposes two different mechanisms in which colchicine has an effect on human neutrophils in the aneurysm wall. The first direct inhibition of neutrophil chemotaxis is by inhibiting the effect of TNF α and by reducing adhesion of neutrophils to the endothelial cells. Furthermore colchicine has an indirect effect mediated via the NALP3 inflammasome. The NALP3 inflammasome activates several interleukines via caspase-1 and activates the production of e.g. CXCL8. We describe the prominent activation of the NALP3 inflammasome and its pathway in the human AAA tissue. Several mechanisms leading to the activation of the inflammasome have been proposed. One of the most plausible explanations is the activation through the mitochondrial reactive oxygen species (ROS) induced by smoking; a major risk factor of AAA disease. There are ongoing concerns about the therapeutic margin between the side-effects of colchicine. A recent study on colchicine preventing the post-pericardiotomy syndrome in patients after cardiac surgery and a study on colchicine preventing pericarditis revealed no difference in side-effects between placebo and colchicine. However, the effect in AAA patients still needs to be investigated. Another inflammatory hallmark of AAA disease, besides abundant CXCL8 expression, is increased expression of interleukin 6 (IL6) in the human AAA wall. Also a polymorphism in the IL6 gene has been identified as a remarkable strong and independent risk factor for AAA disease²⁸. For years, IL6 has been described as a pro-inflammatory cytokine²⁹⁻³¹. However, recent studies also prescribe IL6 anti-inflammatory properties with roles in tissue protection and regeneration³²⁻³⁴. The two roles of IL6 are thought to reflect two different signaling routes: the *classical* IL6 receptor route being responsible for the anti-inflammatory/tissue regenerative activities, and the non-classical, *trans-signaling* route via the soluble IL6 receptor being responsible for the pro-inflammatory activities^{29;31;35}. A role for IL6 in AAA disease is investigated in **Chapter 8**. The IL6 receptor CD126 was modestly present in human AAA tissue samples in contrast to the soluble receptor, which was abundantly present in both atherosclerotic samples and AAA samples. The phosphorylation of STAT 3 in AAA human tissue samples indicates activity of the IL6/STAT3 axis in aneurysm disease. However, this does not differentiate between the pro- or anti-inflammatory routes. Therefore the properties of anti-IL6 treatment in the murine elastase model were tested. Unexpectedly, inhibiting interleukin 6 with an anti-IL6 antibody resulted an aneurysm rupture in ~50% of the treated animals. Delayed treatment did not result in a decrease in aneurysm formation. These observations suggest a protective role for IL-6 in the acute phases of injury. However, with modest expression of the classical signaling route, the observations do not indicate a major role for IL-6 in aneurysm diseases.

Strong clinical and molecular associations exist between AAA and popliteal artery aneurysms (PAA). Yet, while the natural history of AAA is that of rupture, the primary concern in PAA's is thrombosis and rupture of PAA is rare³⁶. A patho-histological (re-)examination of human AAA samples and popliteal aneurysm wall samples, in **Chapter 9**, revealed adipogenic degeneration of the adventitial layer of the AAA wall. Moreover, enrichment of adipocyte-related genes and pathways in ruptured AAA versus non-ruptured controls implied an association between the extent of fatty degeneration and AAA rupture.

CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis underlines the importance of chronic inflammation and proteolytic activity in AAA formation. Investigating human aneurysm tissue and using the elastase mouse model of aneurysm disease, several potential targets for pharmacologically stabilizing aneurysms were revealed.

Mouse models of human disease pathogenesis have become a central part of biomedical research. This is because the laboratory mouse provides the most experimentally accessible mammalian model, sharing e.g. organ systems and systemic physiology with humans.

Currently the translation from the murine models to the clinical trials is challenging. This might question the validity of the existing mouse models of AAA disease. Differences in physiology, as well as variations in homology of molecular targets between mice and humans, may lead to translational limitations. Therefore, the clinical impact of the pharmacological targets investigated in this thesis remains unclear. An ironic shortcoming of the ability to assess the usefulness of the murine models is the lack of knowledge of the biochemical and cellular characteristics of initiating factors in the human disease. Nonetheless, the pathological studies of end stage human biopsies have demonstrated several similarities between the cellular and biochemical characteristics of the mouse models and human AAA disease. Besides, the mouse models, as described in Chapter 1, reproduce adequately the hallmark features of human AAA disease. Consequently, can mimic the disease pathology and be utilised to provide a genuine insight into the pathogenesis of AAA. In particular the elastase model of AAA disease has enabled more detailed investigations on the cellular and molecular mechanisms of the disease in a controlled manner. Even though murine models remain a unique source of *in vivo* information, in the near future other emerging translational alternatives will complement or may eventually replace the link between *in vitro* studies and clinical applications. In the past years a wide range of alternatives to animal-based preclinical research has emerged. These include epidemiological studies, autopsies, computer modeling and phase 0 studies. These approaches towards clinical disease are promising and might have a great impact on the field of human AAA disease.

It has been generally accepted that the natural history of an AAA is progressive aortic wall degradation, ultimately culminating in loss of structural integrity and aortic rupture. The segmental weakening of the artery and profound matrix changes, with increased intra-molecular collagen cross linking in the AAA wall, are a hallmark of AAA disease. A complete loss of normal collagen architecture was found. Besides, the connections that normally allow the tissue to behave as a coherent network are missing in AAA disease³⁶. These changes in the aortic wall do not necessarily reflect the primary cause of AAA formation. More likely, these changes reflect inappropriate

collagen deposition in a setting that is characterized by ongoing inflammation and activation of multiple proteolytic pathways.

This thesis highlights the importance of chronic inflammation in the abdominal aortic aneurysm disease and designates multiple inflammatory pathways as potential targets for pharmaceutical stabilization of the expansion of the vessel wall. Especially the neutrophil-receptor pathway has been indicated as great value in the formation of AAA. The surprising complete abrogation of aneurysms in the elastase mouse model with the neutrophil-receptor inhibitor, DF2156A, together with the high expression of the CXCL8-axis components in human AAA disease, indicates a great significance of this pathway. Since human AAA development is typically an inflammatory process diffused over a large number of years, disrupting the ongoing inflammatory response by blocking the activation loop in this pathway yields highly promising prospects for clinical stabilization of the aneurysm wall. DF2156A is currently under investigation in phase 2 trials for COPD. Therefore, the translation into a phase 2 trial investigating the effect of DF2156A on AAA disease should be feasible in the near future. The results of this trial are highly awaited.

Besides DF2156A, this research has yielded several other new therapeutic targets applications (such as colchicine and E64) that could be translated into the clinic. Since colchicine is a registered drug for gout, the translation into a clinical trial to treat AAA disease could be relatively quick and easy. E64, the broad-spectrum cathepsin inhibitor, still needs additional investigation into its efficacy and safety profiles before clinical application could be initiated.

Currently, as depicted in Chapter 2, the number of randomized controlled trials is low and the scientific quality of the available literature is limited. Patient drug compliance is difficult to assess and a major challenge. Another challenge for randomized controlled trials is the slow growth rate of the AAA. The mean aortic diameter increases between 1-2.5 mm/year and these changes are within the inter-observer measurements error reported for ultrasound³⁷⁷. Therefore, careful planning, standardization of measurements and quality control of the clinical trials needs to be ensured. Finally, it is becoming more and more apparent that AAA disease is highly multi-factorial, with several risk factors, genetic predisposition and actual mechanical difference of the aneurysm itself. Therefore, a number of very large, carefully planned RCTs is required that correct for the large amount of potential bias.

This thesis investigates the pathophysiology of abdominal aortic aneurysms and proposes several pharmaceutical inflammatory targets to manipulate its growth. Hopefully, the data will elicit further research and translation of the compounds to clinical trials. Ultimately, to get closer to pharmaceutically stabilizing AAA growth.

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