General introduction and scope of this thesis

GENERAL INTRODUCTION AND SCOPE OF THIS THESIS

Cardiovascular diseases are a major cause of mortality worldwide.[1] Cardiovascular disease includes all diseases of the heart and circulation, including coronary diseases, such as angina and myocardial infarction and other diseases like stroke, heart failure, hypertension (high blood pressure), and aortic aneurysm formation. The underlying mechanisms vary depending on the disease in question. In this thesis, the role of DNA damage, atherosclerosis and the renin angiotensin system, factors that modulate cardiovascular damage and disease, are investigated and discussed.

DNA damage and aging

Aging is an inevitable part of life and unfortunately also a major risk factor for health complications and disease. As such, the prevalence of cardiovascular disease increases tremendously with age.[2] During aging, diverse detrimental changes in cells and tissues occur and manifest differently in each cell type. These series of structural, architectural and compositional modifications also take place in the heart and the vasculature with age, which will eventually affect cardiovascular performance and sets the stage for the onset of cardiovascular disease including heart failure, myocardial infarction, hypertension, stroke and aneurysm formation.

One of the principal causes of aging is the accumulation of DNA damages over time. Both endogenous (internal) and exogenous (external) agents can affect our DNA, resulting in irreversible DNA damage. Normally, these damages are recognized and can be repaired by different DNA repair systems. However, if DNA repair is hampered this can have serious consequences for cells such as cell cycle arrest, cellular senescence and apoptosis, processes which are known to be involved in the development of cardiovascular disease. One of these DNA repair systems is the Nucleotide Excision Repair (NER) pathway.[3] Four important steps in this system are damage recognition, helix unwinding, dual incision, and repair ligation. Key proteins that are involved in the dual incision step are the DNA endonucleases ERCC1/XPF and XPG.

Ercc1 mouse model of accelerated aging

The ERCC1 (Excision Repair Cross Complementation group 1) protein, along with its binding partner XPF (Xeroderma Pigmentosum group F), forms an endonuclease that is involved in different DNA pathways; NER, interstand crosslink repair and homologous recombination repair. The ERCC1-XPF protein complex is responsible for excision of various types of DNA lesions at the 5'end of the damage. The XPF protein contains the endonuclease catalytic activity, and ERCC1 is necessary for DNA binding. Human patients and mouse mutants with mutations in the ERCC1-XPF complex can exhibit xeroderma

pigmentosum, severe Cockayne syndrome, XFE progeroid syndrome, Cerebro-oculo-facio-skeletal syndrome and/or Fanconi anaemia features.[4]

The *Ercc1*$^{d/-}$ mutant mouse is one of the most widely studied mouse models of accelerated aging, containing one knockout allele and one protein truncating mutation, via which the last seven amino acids at the C-terminus of the Ercc1 protein are deleted. These mice have a severely compromised, but not completely inactive, DNA repair capacity and exhibit premature death (with a lifespan of ~24 weeks).[5] During their life they experience a remarkably wide range of pathological, physiological and behavioral features related to accelerated aging such as progressive neurodegeneration (e.g. dementia, ataxia, priapism, hearing and vision loss), osteoporosis, kyphosis, sarcopenia and retarded growth. Moreover, *Ercc1*$^{d/-}$ mutant display accelerated age-dependent vasodilator dysfunction, increased vascular stiffness, increased blood pressure and vascular cell senescence.[6] Thus, the *Ercc1*$^{d/-}$ mouse model can be used to study the aging process due to endogenous DNA damage and its effects on the heart and vessels.

Fat deposition (e.g. atherosclerosis)

A fatty streak is the first, by eye, visible lesion in the development of atherosclerotic disease. These fatty streaks, also called plaques, are caused by accumulation of fat, cholesterol and other substances. The build-up of these plaques in and around the vasculature is called atherosclerosis. These plaques can cause thickening and stiffening of the vessel wall. Over time, these plaques can become so thick that they can block the inside of the artery and interfere with normal blood flow. Some of the diseases that could develop as a result of this plaque build-up include coronary heart disease, carotid artery disease, peripheral artery disease and chronic kidney disease. Moreover, it is thought that atherosclerosis and aneurysms are highly associated, as atherosclerosis is frequently observed in the aortic wall of patients with abdominal aortic aneurysms. It should be noted that many of the risk factors for aortic aneurysms are similar to those for atherosclerosis, including smoking, hypertension, inflammation and family history. However, in some patients atherosclerosis leads to aortic narrowing, while in others it leads to aortic dilatation; consequently, there is much debate as to whether atherosclerosis is a causative factor in aneurysm formation.

Aneurysmal Fibulin-4 mouse model

One of the mouse models that can be used to study the relation between atherosclerosis and aneurysms formation is the Fibulin-4 mouse model. Fibulin-4 is one of the seven members of extracellular matrix proteins that play an important role in elastic fiber and collagen assembly and function. Mice with a reduced expression of Fibulin-4 (indicated as 'Fibulin-4^{R}', where R stands for reduced expression) display defects in the aortic wall, which could lead to aneurysm formation. Hence, mice with only 25% expression of Fibuline-4 (homozygous Fibulin-4$^{R/R}$ mice) develop aortic aneurysms, while mice with a 50%

expression of Fibulin-4 (heterozygous Fibulin-$4^{+/R}$ mice) develop minor aortic abnormalities.[7] These heterozygous Fibulin-$4^{+/R}$ mice do not yet develop aneurysms spontaneously but are susceptible to develop aneurysms upon exposure to different stressors such as age and high fat diet, and thus are a good model to test the effect of risk factors on aneurysm formation.

The renin-angiotensin system

The renin angiotensin system (RAS) has emerged as one of the most important links in the pathophysiology of many types of cardiovascular diseases.[8] Besides its classical regulatory effects on blood pressure and sodium homeostasis, the RAS is involved in the regulation of vascular tone and remodeling of the vessel wall. Dysregulation and overproduction of the RAS hormone angiotensin II (Ang II), the main peptide of the RAS, is believed to contribute to the initiation and progression of several cardiovascular diseases. Historically, Ang II in circulating blood was seen as a regulatory hormone involved in the regulation of blood pressure, aldosterone release and sodium reabsorption. Yet, now there is also ample evidence that locally produced Ang II promotes cell proliferation, apoptosis, fibrosis, oxidative stress and inflammation, processes known to contribute to remodeling of the vasculature.[9] It is generally believed that the local production of Ang II is involved in the pathogenesis and progression of atherosclerosis and aneurysm disease, and that inhibition of the RAS has beneficial therapeutic effects on the vasculature, possibly even on aortic aneurysms. Moreover, since Ang II signaling affects the aging process, while many vascular diseases are age-related, it is important to understand how the RAS is regulated during normal aging. Although we know that the systemic RAS is suppressed during aging, the activity of tissue RAS in the elderly is not fully understood yet and needs to be further explored.

Scope of this thesis

Cardiovascular diseases are life-threatening and their occurrence increases with age. Most often there is not one cause for disease, but instead several risk factors are involved that increase the risk of development and progression of disease. In this thesis, important factors are explored that play a role in cardiovascular damage and disease, such as DNA damage, atherosclerosis and the RAS. **Chapter 2**, part I, starts with describing the general structure and cell biology of the vessel wall. In **Chapter 3** the focus lies on the components that are under the influence of the RAS and that contribute to the development and progression of vascular disease; e.g. extracellular matrix defects, atherosclerosis and aging.

In part II, we studied the effect of two risk factors, atherosclerosis and increased RAS signaling, on the development and progression of aortic aneurysms. In the clinic it is known that in some patients atherosclerosis leads to aortic narrowing, while in others it leads to

aortic dilatation. Hence, a better understanding on the differences in pathogenesis leading to both atherosclerosis and aneurysm formation is required. Therefore, in **Chapter 4** the relation between atherosclerosis and aortic aneurysms formation was investigated, with the intention to find molecular pathways and markers that differentiate these two diseases. Furthermore, it has been well established that activation of the RAS plays an important role in the physiology and pathophysiology of the cardiovascular system and it has been suggested that over activation of the RAS promotes the development of aortic aneurysms. In **Chapter 5** the therapeutic potential of the RAS blocker losartan, an angiotensin II type 1 (AT_1) receptor, on aneurysm progression was examined.

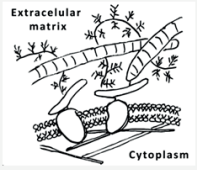
In part III, the effect of defective DNA repair and the consequential aging process on the development of cardiovascular damage is investigated. **Chapter 6** discusses the effect of accelerated aging on vascular function and morphology, as well as the effect of dietary restriction, known to induce an anti-aging response, on the vasculature. In **Chapter 7**, the effect of aging on the heart was characterized and the use of fluorescent molecular markers for the early detection of cardiovascular disease was tested.

In part IV, the role of the RAS was investigate under various conditions. Though it is suggested that changes in the reactivity and/or responsiveness of the systemic RAS occur with aging, little is known about the regulation and activity of the RAS within local tissues during aging. Yet, this knowledge is required to successively treat and/or prevent renal disease in the elderly. In **Chapter 8** the use of the renin activatable near-infrared fluorescent probe ReninSense680™ was tested to facilitate non-invasive imaging of renin activity *in vivo*. In addition, the intrarenal renin activity was determined in accelerated aging *Ercc1*$^{d/-}$ mice with age-related kidney pathology. Besides the traditional role of the RAS in blood pressure regulation, it is hypothesized that certain RAS components are synthesized in the brain and that this so-called brain RAS is relevant in the regulation of the cardiovascular system. However, the concept of a brain RAS has been controversial and this controversy continues to this day. Therefore, in **Chapter 9** the occurrence of (pro) renin in the brain was re-evaluated. Inhibition of the RAS with aliskiren, a potent renin inhibitor, is hampered by diarrhea at high doses and thus no maximum effect of renin inhibition in humans has been established. Accordingly, in **Chapter 10** the use of VTP-27999, a novel renin inhibitor -without major side-effects at high doses- was examined in order to establish the maximum effect of renin inhibition, focusing on the kidney.

Part II Aortic aneuryms: ECM defect & atherosclerosis

Chapter 4
Atherosclerosis and ECM defect

Chapter 5
ECM defect and RAS blockade in aneurysms

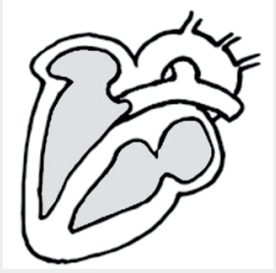


Part III Cardiovascular aging & defective DNA repair

Chapter 6 Vascular aging

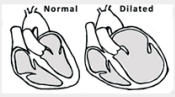
Chapter 7 Molecular imaging of cardiac aging

Cardiovascular damage and disease
This thesis



Aortic aneurysm

Ventricular dilatation



Vascular dysfunction

Part I Introduction

Chapter 2 Structure & cell biology of the vessel wall

Chapter 3 RAS and vascular disease

Part IV The renin-angiotensin system

Chapter 8 RAS changes during aging

Chapter 9 Brain (pro)renin

Chapter 10 VTP-27999 as RAS inhibitor

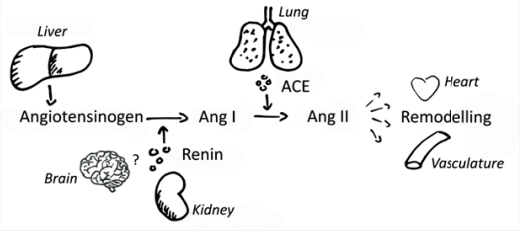


Figure 1. Schematic overview of topics and relationship discussed in this thesis.

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