Summary and Future Perspectives

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Impaired endothelial function is recognized as one of the earliest events of atherogenesis.[1, 2] In **Part I, *chapter 1***, we discussed the clinical value of the different techniques to evaluate endothelium-dependent vasomotor function. We also reviewed the efficacy of both angiotensin converting enzyme inhibitors and the HMG-CoA reductase inhibitors on improving vascular function. Despite the extensive experimental evidence, and some clinical trials studies using quinapril, the beneficial effects of other angiotensin converting enzyme inhibitors in general on vascular, endothelial function in humans are inconsistent. The currently available studies involving statin therapy are suggesting that treatment of hypercholesterolemia can restore endothelial dysfunction. In ***chapter 2***, we demonstrated that in vitro resistance of internal thoracic arteries to exogenous angiotensin II is independently related the long-term risk of cardiovascular events, including cardiac death, myocardial infarction, percutanous coronary intervention, re-coronary artery bypass grafting, stroke, and vascular surgery, even after adjustment for blood pressure and other cardiac risk factors. A surprisingly high percentage of 78 percent of all cardiovascular events occurred in patients with in vitro angiotensin II resistance. In addition, angiotensin II resistance was associated with a higher blood pressure an a trend to an increased serum and local angiotensin converting enzyme activity. However, from this study, we cannot deduct the exact molecular mechanims. It might be due to decreased angiotensin type 1 receptor expression, or decreased intracellular signalling. Receptor regulation is a complex process, which is balanced by factors downregulating the receptor and upregulating the receptor. Angiotensin II itself is a well-known factor inducing receptor downregulation; commonly refereed to as agonist induced receptor downregulation. It is tempting to speculate that increase of in vivo levels of angiotensin II results in decrease of in vitro vascular response to exogenous angiotensin II.

Since renal function is strongly associated with decreased cardiovascular outcome, we assessed whether renal function impairment related to systemic alterations of vascular responsiveness to angiotensin II, endothelial function or inflammatory markers (C-reactive protein and soluble intercellular adhesion molecule 1) in ***chapter 3***. We studied in vitro vascular segments of the internal thoracic artery of patients undergoing coronary artery bypass grafting. In contrast to a previous study in patients with hypertension,[3] we could not establish an association between endothelial function (vasorelaxation in response to methacholine) and renal function. In addition, we could not establish an association between renal function and vascular responsiveness to angiotensin II or serum angiotensin converting enzyme activity, or the serum inflammatory markers C-reactive protein and soluble intercellular adhesion molecule 1. Thus, it seems that the relationship between systemic vascular dysfunction and mild renal insufficiency might be more complicated than previously thought. In ***chapter 4***, we analyzed the preoperative predictive value of the inflammatory markers C-reactive protein and soluble intercellular adhesion molecule 1 levels on long term incidence of cardiovascular events in patients undergoing coronary artery bypass grafting. In contrast to soluble

intercellular adhesion molecule 1, cardiovascular event rate was significantly increased in patients with a preoperative C-reactive protein level above the median of 1.9 mg/l. Increased preoperative C-reactive protein was associated with a three fold increased risk for cardiovascular events, even after adjustment for conventional risk factors. In vitro exposure of C-reactive protein also can lead to upregulation of the angiotensin type 1 receptor, and is related to increased neointimal formation in the rat carotid artery angioplasty model.[4] In addition, in vitro exposure of vascular smooth muscle cells to cholesterol can also markedly augments angiotensin type 1 receptor mRNA and protein expression.[5, 6] Therefore, we assessed the effects of both C-reactive protein and cholesterol on the human vascular responsiveness to angiotensin II in ***chapter 5***. We observed an increased human vascular responsiveness to angiotensin II in patients with increased cholesterol levels and in patients with increased C-reactive protein levels. Interestingly, analyzing cholesterol and C-reactive protein jointly revealed that both increased cholesterol and increased C-reactive protein together was most strongly associated with increased vascular responsiveness to angiotensin II. These results are suggesting that increased C-reactive protein and increased cholesterol levels are interacting to increase human vascular responsiveness to angiotensin II. Since chronic heart failure is a condition characterized by neurohormonal activation, and increased levels of angiotensin II, an indicator of an activated renin-angiotensin system we determined in ***chapter 6*** the length of telomeres from the circulating leukocytes (inflammatory cells). Angiotensin II is a major oxidative stress inducing factor[7, 8] and oxidative stress in its turn is a major factor associated with telomere shortening and biological aging.[9-11] Increased inflammatory status, and increased leukocyte turnover is another factor decreasing telomere length. Since cardiac failure is in general also an age-related disease associated with increased oxidative stress and inflammation, we hypothesized that patients with chronic heart failure have decreased telomere length. We demonstrated that telomere length is indeed shorter in 620 patients with chronic heart failure compared to 183 age- and sex-matched controls, and that telomere length was related to the severity of the disease. This was even more highlighted in patients with chronic heart failure who had concomitant vascular, atherosclerotic disease. However, this research was cross-sectional in nature, thus we cannot conclude causality from this study. Nevertheless, this study opens a new avenue for cardiovascular research and is one of the most noteworthy observations of this thesis.

In **Part II**, we explored the effects of 3-hydroxy-3-methylglutaryl co-enzyme-A inhibitors (statins) in the presence of an activated renin-angiotensin system or inflammation. Recent evidence demonstrates that intensive lipid-lowering therapy with high dose statin provides significant clinical benefit beyond moderate lipid lowering therapy.[12, 13] However, dose-dependent effects of short-term statin therapy on vascular function have not been demonstrated. In ***chapter 7***, we therefore studied endothelial function and, more importantly, vascular responsiveness to angiotensin II in coronary artery diseased patients randomized to low or high dose statin treatment (10 or 80 mg atorvastatin), or placebo. Endothelium dependent vasodilatation was improved with statin therapy, but was significantly further improved with high dose as compred to

low dose treated patients. Endothelium improvement was accompanied by reduced vascular response to angiotensin II. This seems to be in contrast with our previous observation (***chapter 5***), but might be due to statin induced downregulation or to cholesterol associated downregulation. We might also have to acknowledge that a certain balance between factors modifying receptor expression exists, ultimately resulting in increased or decreased vascular responsiveness to angiotensin II. These findings obviously require additional research. Nevertheless, the improvement of endothelial function might provide a potential mechanism for the clinical benefit of intensive lipid lowering treatment in coronary heart disease. Considering the effects of statin on inflammation, it was known that statins effectively reduce inflammatory parameters such as C-reactive protein. However, whether these are long lasting changes remained to be determined. In ***chapter 8*** we studied patients who were not in need of a statin for ethical reasons. We assessed the consequences of withdrawing statin therapy on serum cholesterol and C-reactive protein levels. Patients who discontinued statin therapy after 4 years of treatment had cholesterol levels and C-reactive protein levels increased up to pre-treatment levels within 3 months. Interestingly, this increase was not related to the increase of cholesterol. These data might provide mechanistic support for the acute increase in cardiovascular risk associated with statin discontinuation.

Besides reducing cholesterol and C-reactive protein, evidence suggests that there exists a plethora of potential relevant beneficial effects of statins[14] to improve vascular function. We hypothesized statins might be useful to intervene in the pathophysiological process of instent restenosis. Therefore, we studied in ***chapter 9*** the anti-restenotic potency of statin therapy. We used the rat abdominal stent model and tested whether supraphysiological levels of angiotensin II would be associated with increased vascular injury as measured by in vitro vasomotor function and neointimal formation. Angiotensin II is well known to increase oxidative stress and exaggerate neointimal formation after vascular injury.[15] Indeed, angiotensin II infusion increased in-stent restensosis and decreased endothelial function. In both the presence and absence of co-administration of angiotensin II, statin treatment attenuated in-stent restenosis and in parallel improves endothelial function. The observation that, even in a hostile environment with high levels of angiotensin II, statin therapy is still effective in reducing neo-intimal formation is important and warrants further research.

These findings also indicate that in patients with an activated renin-angiotensin system, statins might be beneficial. Indeed, chronic heart failure patients have a highly activated renin angiotensin system. Therefore, we searched for the available clinical evidence existing in literature in ***chapter 10***. Since statins were developed as cholesterol lowering drugs, we started to search for studies evaluating the association between cholesterol and outcome in patients with chronic heart failure. Surprisingly, most studies observed that lower, rather than higher serum cholesterol levels, were consistently associated with increased mortality rates in patients with chronic heart failure. We retrieved as many as 47 clinical trials evaluating the efficacy of statin treatment compared to placebo in numerous patient categories, involving more than 100,000 patients in total. However, patients with chronic heart failure have been systematically excluded from these trials. Therefore, we feel that the effect of statin therapy in these

patients remains to be established. Few studies focussed on the efficacy of statin treatment in patients with chronic heart failure and none of them reported mortality data. Most of the surrogate endpoints reported were favourable for the use of statins. Next, we aimed to gain a better understanding of the possible effects of statin therapy in patients with chronic heart failure and provided an balanced overview of the relevant potential beneficial and detrimental consequences of statin therapy we should take in consideration. On the one hand, three lines of evidence points towards a harmful effect of statin treatment in chronic heart failure. First, as mentioned, in patients with chronic heart failure, lower cholesterol levels have been repeatedly related to worse outcome, possible related to the scavenger function of cholesterol for cardiodepressive and harmful endotoxins. Second, statins might interfere with the enzymatic isopentenylation of Sec-tRNA, resulting in selenoprotein deficiency. Third, statins in chronic heart failure might adversely affect mitochondrial function through inhibition of ubiquinone. On the other hand, evidence is accumulating pointing towards beneficial effects of statin treatment chronic heart failure. First, most animal experimental data suggest beneficial effects of statins on left ventricular function. Second, beneficial effects of statins demonstrated in non-chronic heart failure conditions, e.g. on vascular function, atherosclerosis, and left ventricular hypertrophy might also be beneficial in chronic heart failure. Third, clinical knowledge on statin treatment in chronic heart failure is generally favourable, but is primarily based on retrospective post-hoc studies performed in large-clinical trials or small prospective studies with surrogate endpoints. Currently, we are awaiting the outcome of two large clinical trials evaluating the role of statins in patients with chronic heart failure.[16, 17]

Reference List

1. Libby P. Inflammation in atherosclerosis. *Nature* 2002 December 19;420(6917):868-74.
2. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999 January 14;340(2):115-26.
3. Perticone F, Maio R, Tripepi G, Zoccali C. Endothelial Dysfunction and Mild Renal Insufficiency in Essential Hypertension. *Circulation* 2004 August 2.
4. Wang CH, Li SH, Weisel RD, Fedak PW, Dumont AS, Szmitko P, Li RK, Mickle DA, Verma S. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation* 2003 April 8;107(13):1783-90.
5. Li Y, Zhu H, Kuppusamy P, Roubaud V, Zweier JL, Trush MA. Validation of lucigenin (bis-N-methylacridinium) as a chemilumigenic probe for detecting superoxide anion radical production by enzymatic and cellular systems. *J Biol Chem* 1998 January 23;273(4):2015-23.
6. Nickenig G, Sachinidis A, Michaelsen F, Bohm M, Seewald S, Vetter H. Upregulation of vascular angiotensin II receptor gene expression by low-density lipoprotein in vascular smooth muscle cells. *Circulation* 1997 January 21;95(2):473-8.
7. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 1997 February 4;95(3):588-93.
8. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. Angiotensin II-mediated Hypertension in the Rat Increases Vascular Superoxide Production via Membrane NADH/NADPH Oxidase Activation . Contribution to Alterations of Vasomotor Tone. *J Clin Invest* 1996 April 15;97(8):1916-23.
9. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A* 2004 December 7;101(49):17312-5.
10. Von Zglinicki T, Saretzki G, Docke W, Lotze C. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res* 1995 September;220(1):186-93.
11. Von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002 July;27(7):339-44.
12. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM, the Pravastatin or Atorvastatin Evaluation and Infection Therapy - Thrombolysis in Myocardial Infarction. Comparison of Intensive and Moderate Lipid Lowering with Statins after Acute Coronary Syndromes. *N Engl J Med* 2004 March 8;NEJMoa040583.
13. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, Gotto AM, Greten H, Kastelein JJ, Shepherd J, Wenger NK. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med* 2005 April 7;352(14):1425-35.
14. Liao JK. Effects of statins on 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition beyond low-density lipoprotein cholesterol. *Am J Cardiol* 2005 September 5;96(5A):24F-33F.

15. Daemen MJ, Lombardi DM, Bosman FT, Schwartz SM. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991 February;68(2):450-6.
16. Kjekshus J, Dunselman P, Blideskog M, Eskilson C, Hjalmarson A, McMurray JV, Waagstein F, Wedel H, Wessman P, Wikstrand J. A statin in the treatment of heart failure? Controlled rosuvastatin multinational study in heart failure (CORONA): study design and baseline characteristics. *Eur J Heart Fail* 2005 October;7(6):1059-69.
17. Tavazzi L, Tognoni G, Franzosi MG, Latini R, Maggioni AP, Marchioli R, Nicolosi GL, Porcu M, on behalf of the GISSI-HF investigators. Rationale and design of the GISSI heart failure trial: a large trial to assess the effects of n-3 polyunsaturated fatty acids and rosuvastatin in symptomatic congestive heart failure. *Eur J Heart Fail* 2004 August;6(5):635-41.