Efficacy, safety and novel targets in cardiovascular disease : advanced applications in APOE*3-Leiden.CETP mice

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Summary

Cardiovascular disease (CVD) is currently globally the major cause of mortality and morbidity, and 85% of all CVD deaths are related to the formation of atheromatous plaques in the vessels. Chronic exposure to cardiovascular risk factors, such as dyslipidemia, hypertension and diabetes, increases the rate and severity of atherosclerosis. Despite advances in treatment strategies, many patients remain at increased cardiovascular risk. This thesis described a variety of studies that aimed to reduce CVD risk by (I) evaluation of novel lipid-lowering interventions, (II) identification of cardiovascular side-effects of registered drugs and an environmental pollutant, (III) development of a novel animal model combining dyslipidemia and diabetes, and (IV) evaluation of the cytokine oncostatin M (OSM) as potential target for CVD. In all studies, the APOE*3-Leiden(.CETP) mouse model was used as translational model for human lipoprotein metabolism and atherosclerosis development.

In **Chapter 2** we evaluated the effects of a vaccine against proprotein convertase subtilisin/kexin type 9 (PCSK9), which induced an effective immune response against PCSK9 thereby decreasing plasma cholesterol levels, markers of systemic inflammation and atherosclerosis progression. However, as most patients at CVD risk are treated after development of atherosclerosis, therapies that regress pre-existent lesions are required. Therefore, **Chapter 3** evaluated whether pre-existent atherosclerotic lesions could regress by aggressive lipid lowering using a combination of antibodies against PCSK9 (alirocumab) and angiopoietin-like 3 protein (ANGPTL3) (evinacumab) on top of atorvastatin. This strategy decreased plasma non-HDL-C levels to 1 mmol/L and subsequently regressed atherosclerotic lesion size, improved lesion stability and diminished macrophage accumulation.

In **Chapter 4** we explored the etiology of reported toxic cardiovascular off-target effects of three generations tyrosine kinase inhibitors (TKIs), imatinib, nilotinib and ponatinib, respectively, that are used for the treatment of patients with chronic myeloid leukaemia (CML). The first generation TKI imatinib reduced atherosclerosis development, whereas the second and third generation TKIs, nilotinib and ponatinib, respectively, increased cardiovascular risk through induction of a prothrombotic state. In addition, imatinib and ponatinib decreased plasma cholesterol levels, by a mechanism that was investigated in **Chapter 5**. Imatinib was found to decrease plasma TC and TG levels by reduction of the very-low-density-lipoprotein (VLDL) particle production and cholesterol ester content of the VLDL particles, while ponatinib reduced plasma total cholesterol levels by lowering intestinal lipid absorption.

The dose effects of perfluorooctanoic acid (PFOA) on lipoprotein metabolism are presented in **Chapter 6**. Before being phased-out, PFOA has been widely used as an emulsifier in the manufacture of fluoropolymers, is extremely stable and therefore persists in the environment. Positive associations between serum PFOA levels and plasma cholesterol have been reported in environmentally and occupationally exposed adults,

though the observations are inconsistent. Using APOE*3-Leiden.CETP mice, we demonstrated that PFOA did not alter plasma lipid levels or lipoprotein metabolism at environmentally or occupationally relevant exposure levels. However, when mice were exposed to toxicologically relevant PFOA doses, non-HDL-C levels were decreased and HDL-C levels were increased. In the latter mice, PFOA decreased VLDL particle production and increased VLDL clearance. Moreover, HDL-C levels were increased through reduction of CETP activity and changes in gene expression of proteins involved in HDL metabolism. These data indicate that the reported associations observed in epidemiological studies are associative rather than causal.

Diabetes is an important risk factor for CVD and currently, novel anti-diabetic drugs have to demonstrate their cardiovascular safety before approval. Consequently, translational models are warranted for the evaluation of these drugs. In **Chapter 7** we described the characteristics of the APOE*3-Leiden.Glucokinase$^{+/-}$ (E3L.GK$^{+/-}$) mouse model, which was generated by cross-breeding the hyperlipidemic APOE*3-Leiden mouse with the hyperglycemic glucokinase knockout (GK$^{+/-}$) mouse. E3L.GK$^{+/-}$ mice had elevated plasma lipid levels as in E3L mice, and elevated plasma glucose levels as in GK$^{+/-}$ mice, leading to enhanced atherosclerosis progression which was predicted by glucose exposure. We propose that the E3L.GK$^{+/-}$ mouse is a promising novel diet-inducible disease model for investigation of the etiology and evaluation of drug treatment on diabetic atherosclerosis.

The role of cytokines in the initiation and progression of atherosclerosis is increasingly recognized and consequently, novel therapies targeting cytokines are being developed. In **Chapter 8** we evaluated the role of the cytokine OSM in the initiation of atherosclerosis and found that OSM induced endothelial activation *in vitro* using human endothelial cells from different vascular beds, and *in vivo* using APOE*3-Leiden.CETP mice. In **Chapter 9** we exposed APOE*3-Leiden.CETP mice for 16 weeks to OSM which increased plasma E-selectin levels and endothelial activation in the aortic root. However remarkably, we found a reduction in atherosclerotic lesion size, corresponding to our observation that higher serum OSM levels in humans are associated with post coronary heart disease and overall survival probability in the AGES Reykjavik Study, suggesting a protective cardiovascular effect. However, the confusing effects of the increased endothelial activation on the one hand, and reduced atherosclerosis on the other hand, need to be further elucidated.

In conclusion, we discussed several strategies that may contribute to further CVD risk reduction in the future. We described two novel lipid-lowering strategies, we unraveled (part of) the etiology of the cardiovascular safety issues of TKIs that are used for the treatment of CML, and we investigated the dose effects of PFOA on lipoprotein metabolism. Looking forward, we developed a novel mouse model that can be used for the study of diabetic macrovascular complications, and we evaluated the potential of OSM as novel target in CVD.