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**Title:** Innovative pharmaceutical interventions in experimental atherosclerosis : focusing on the contribution of non-HDL-C versus HDL-C

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Summary

Cardiovascular disease (CVD) is the leading cause of death worldwide despite the successful development of several pharmaceutical interventions of which statin therapy is the dominating lipid-lowering treatment option. Atherosclerosis, a chronic inflammatory disease of multifactorial origin, is a dominant contributor to the development of CVD. The research described in this thesis investigated the effects of innovative pharmaceutical interventions in experimental atherosclerosis, targeting hypertension and high blood cholesterol, more specifically high low-density lipoprotein-cholesterol (LDL-C) and low high-density lipoprotein-cholesterol (HDL-C), as risk factors for CVD.

In view of the fact that hypertension is a leading risk factor for CVD and associated with the development of atherosclerosis, we investigated the anti-atherosclerotic effects of aliskiren, the first commercially available, orally active, direct renin inhibitor approved for the treatment of hypertension in chapter 2. In this study in APOE*3Leiden.CETP mice, we demonstrated beneficial effects of aliskiren on atherosclerosis development and plaque stability when administered alone and in combination with atorvastatin, possibly via a mechanism involving T cells. These results suggest a potential benefit of using aliskiren in a clinical setting, particularly in combination with statin treatment.

Cholesterol contained in LDL particles is well recognized as a primary causal risk factor for coronary heart disease (CHD) as evidenced by experimental, epidemiological and genetic data and intervention trials. However, despite the fact that epidemiological studies consistently reported an inverse association between HDL-C and CHD risk, the benefits of raising HDL-C remain less defined. In Chapter 3 to 6, we investigated the effects of novel lipid-modifying treatment strategies, i.e. LDL-C-lowering and/or HDL-C-raising compounds on atherosclerosis development in the APOE*3Leiden.CETP mouse model, since these mice respond to both LDL-C-lowering and HDL-C-raising compounds in a human-like manner.

The benefits of niacin on plasma lipids were first described in 1955 and led to the development of niacin for therapeutic purposes. In chapter 3, we aimed to address the discrepancy between the beneficial effects of niacin in initial clinical trials and the lack of effect of niacin on top of statin treatment on the reduction of CVD events in the large AIM-HIGH and HPS2-THRIVE clinical trials by evaluating the effects of niacin alone and in combination with simvastatin on atherosclerosis development. We showed that niacin decreases atherosclerosis development mainly by reducing non-HDL-C with modest HDL-C-raising and additional anti-inflammatory effects. The additive effect of niacin on top of simvastatin was mostly dependent on its non-HDL-C-lowering capacities. These data suggest that clinical beneficial effects of niacin are largely dependent on its ability to lower LDL-C on top of concomitant lipid-lowering therapy and may explain the failure of niacin in the AIM-HIGH and HPS2-THRIVE trials in patients subjected to aggressive LDL-C-lowering treatment.
In 1989, markedly increased HDL-C led to the discovery of the first mutation in the cholesteryl ester transfer protein (CETP) gene in two Japanese subjects. CETP facilitates the transfer of cholesteryl esters from atheroprotective HDL to atherogenic V(LDL) and has become a target to increase HDL-C. This has led to the development of several small molecule CETP inhibitors. In chapter 4, we investigated the effects of a broad dose range of the novel CETP inhibitor, anacetrapib on atherosclerosis development and we examined possible additive/synergistic effects of anacetrapib on top of atorvastatin. In our study, anacetrapib dose-dependently reduced CETP activity, thereby decreasing non-HDL-C and increasing HDL-C. Moreover, anacetrapib dose-dependently reduced atherosclerosis development. This effect was mainly ascribed to a reduction in non-HDL-C despite a remarkable increase in HDL-C and without affecting HDL functionality. In addition, anacetrapib improved lesion stability and added to the anti-atherogenic effects of atorvastatin. We further explored the mechanism by which anacetrapib reduces (V)LDL-C and whether this effect is dependent on the inhibition of CETP activity in chapter 5. In this study, we showed that anacetrapib reduces (V)LDL-C by increasing hepatic remnant uptake via two mechanisms: (i) inhibition of CETP activity, resulting in remodelled VLDL particles that are more susceptible to hepatic uptake; and (ii) a CETP-independent reduction in plasma proprotein convertase subtilisin/kexin type 9 (PCSK9) level that has the potential to increase LDL receptor (LDLR)-mediated hepatic remnant clearance.

In 2003, Abifadel et al. identified two French families with autosomal dominant hypercholesterolemia caused by mutations in PCSK9. PCSK9 is a serine protease responsible for LDL receptor (LDLR) degradation by preventing the recycling of the receptor to the cell membrane after internalization. The upregulation of the LDLR after statin treatment is accompanied by an upregulation of PCSK9 which in turn promotes LDLR degradation. Inhibition of PCSK9 is, therefore, a potential novel strategy in the treatment against CVD, especially in combination with statin treatment. We, therefore investigated the effects of 2 dosages of the fully human, monoclonal antibody against PCSK9, alirocumab alone and in combination with atorvastatin on atherosclerosis development in chapter 6. In this study, alirocumab dose-dependently increased hepatic LDLR protein levels and consequently decreased plasma cholesterol levels and reduced the development of atherosclerosis. Moreover, alirocumab improved lesion morphology and enhanced the beneficial effects of atorvastatin. The anti-atherosclerotic effect was strongly dependent on the reduction of plasma TC levels, indicating that the majority of the effect was brought about by cholesterol lowering leaving limited/no space for other potential (pleiotropic) effects. This is the first study to show that a monoclonal antibody to PCSK9 reduces atherosclerosis development.

In chapter 7, we reviewed the effects of established and novel treatment strategies, specifically targeting HDL, other than statins on inhibition of atherosclerosis development in preclinical studies in animals expressing CETP, a crucial gene involved in HDL metabolism.
and implicated in the mechanisms by which most therapies modulate HDL. In addition, we conducted a meta-analysis to evaluate the potential effects of these treatment strategies on the prevention of clinical events in randomized controlled trials. We focused specifically on the contribution of non-HDL-C/ LDL-C-lowering versus HDL-C-raising on inhibition of atherosclerosis and the prevention of CVD. According to results from our systematic review and meta-analysis, it is evident that the protective role of lowering LDL-C and non-HDL-C is well-established. The contribution of raising HDL-C on inhibition of atherosclerosis and the prevention of cardiovascular disease remains undefined and may be dependent on the mode of action of HDL-C-modification. Nonetheless, treatment strategies aimed at improving HDL function and raising apolipoprotein A-I may be worth exploring.

In conclusion, the research described in this thesis provides evidence for anti-atherogenic effects of several innovative pharmaceutical interventions that are currently being investigated in clinical trials, specifically targeting hypertension and hypercholesterolemia as risk factors for CVD. Our results further support additional benefit of these treatment strategies in combination with statin treatment which is currently the ‘gold standard’ therapy for the treatment of CVD. Most of these lipid-modifying treatment strategies affect both LDL-C and HDL-C and we demonstrate that the beneficial effects of these treatment strategies predominantly derive from their non-HDL-C/ LDL-C-lowering abilities. Nonetheless, results from preclinical studies and clinical trials support the notion that treatment strategies aimed at improving HDL function and raising apolipoprotein A-I may also inhibit the development of atherosclerosis and reduce the prevalence of CVD.