Intervention in hepatic lipid metabolism : implications for atherosclerosis progression and regression

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Chapter 9

English summary
Nederlandse samenvatting
List of abbreviations
List of publications
Curriculum Vitae

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ENGLISH SUMMARY

Atherosclerosis is the underlying cause of most cardiovascular diseases. It is a progressive inflammatory disease which has a clinical onset by vascular obstruction from the deposits of plaque, subsequently developing into athero-thrombosis and abnormal blood flow. The evidence to support a cholesterol-atherosclerosis link has been revealed in the past three decades. There is a growing consensus that therapeutic lowering of plasma VLDL- and LDL-cholesterol levels and raising of HDL-cholesterol level will reduce the risk of cardiovascular incidence. This dissertation is dedicated to the regulation of lipid metabolism pathways, both in plasma and liver, and its subsequent effects on atherosclerotic lesion progression and regression. In **Chapter 1**, established mechanisms underlying lipid metabolism and atherosclerotic pathology are reviewed, including the important players involved in plasma and hepatic lipoprotein metabolism pathways and the atherosclerotic plaque progression / regression process.

1. Hepatic lipid metabolism and pharmaceutical interventions in the liver

The first part of the thesis focuses on the hepatic lipid metabolism and the pharmaceutical interventions in the liver.

In **Chapter 2**, we used real-time quantitative PCR to compose the hepatic cell type-specific expression profile of nuclear receptors (NRs), providing the most complete quantitative assessment of NRs distribution in liver reported to date. We have identified several liver-enriched orphan NRs as potential novel targets for pharmaceutical interventions. Specifically, members of the orphan receptor family COUP-TFs showed distinguished distributions in liver. COUP-TF3 was abundantly and exclusively expressed in liver parenchymal cells, while the other two members, COUP-TF1 and COUP-TF2, were expressed exclusively in non-parenchymal cells. Despite the moderate to high expression level in the liver, the physiological functions of COUP-TFs have not been fully exploited, and the ligand for COUP-TFs has not been identified. Our study suggests a physiologic important role of COUP-TF3 in hepatocytes, and that of COUP-TF2 in endothelial cells and macrophages.

In **Chapter 3**, we have determined the hepatic expression profile of NAFLD-related gene PNPLA3. To gain insight into the potential function of PNPLA3 in liver, we have determined the effect of diet change on the hepatic expression profile of PNPLA3 in mice, using microarray and real-time PCR analysis. Cellular distribution analysis revealed that PNPLA3 is expressed in hepatocytes but not in liver endothelial or Kupffer cells. Microarray-based gene profiling showed that the expression level of PNPLA3 is highly influenced by the intra-hepatic lipid status. Furthermore, our study suggests an essential function of PNPLA3 in hepatic lipogenesis.

In addition to lipid-lowering therapies, HDL has become the next promising therapeutic target. The anti-dyslipidemic drug niacin, also known as nicotinic acid, not only lowers plasma levels of pro-atherogenic lipids/lipoproteins, but also is the most effective agent available to increase HDL-cholesterol. Recently, it has been shown that niacin also reduces the hepatic expression and plasma levels of the pro-atherogenic CETP. In **Chapter 4** we investigated the mechanism underlying the CETP-lowering effect of niacin. We propose that the primarily reduced hepatic

cholesterol accumulation via the lipid-lowering effect of niacin leads to attenuated hepatic inflammation, and thus less macrophage infiltration into and/or increased macrophage emigration out of the liver. The decreased amount of hepatic macrophages, which are significant contributors of CETP, leads to an overall reduction in hepatic CETP expression and a lower plasma CETP level. In conclusion, we have shown that niacin does not directly alter macrophage CETP expression, but attenuates the liver inflammation and macrophage content in response to its primary lipid-lowering effect, which leads to a decrease in hepatic CETP expression and the plasma CETP mass.

The clinical use of niacin has been limited due to the cutaneous flushing effect, which is mediated by the niacin receptor GPR109A in the skin. Therefore, in **Chapter 5**, we assessed the properties of two partial agonists for GPR109A and compared them to niacin. We show that these two GPR109A partial agonists of the pyrazole class are promising drug candidates to achieve the beneficial lipid-lowering effects while they successfully avoid the unwanted flushing side effect.

2. Atherosclerotic lesion regression and mouse models

The second part of the thesis focuses on the concept of atherosclerotic lesion regression, shedding insights in the role of LXR activation and application of mouse models in regression studies.

While numerous studies have been dedicated to inhibit the progression of atherosclerosis, recent attention has been focused on reversing atherosclerosis, which is the regression of existing atherosclerotic plaque. In previous studies, a dramatic regression of large advanced lesion was achieved with or without LXR agonist by utilizing surgical aorta transplantation into wildtype mice. This procedure leads to a rapidly improved milieu for the atherosclerotic lesions. In both **Chapter 6** and **Chapter 7** we investigated other mouse models to explore technically easier and more efficient approaches for plaque regression.

In **Chapter 6** we first used LDLr$^{-/-}$ mice with high-fat high-cholesterol Western-type diet (WTD) for 6 weeks to develop atherosclerotic plaques. Subsequently, a group of mice was sacrificed to obtain baseline data, whilst the rest of the mice were switched to a low-fat cholesterol-free chow diet without or with LXR agonist T0901317 supplementation for 3 weeks. In addition, we fed C57BL/6 mice with cholate-containing cholesterol-enriched atherogenic diet for 16 weeks to induce atherosclerotic plaque development and perform regression study with LXR agonist T0901317. In **Chapter** 7 we used bone marrow transplantation technique, reconstituting ApoE$^{-/-}$ mice with bone marrow from wildtype mice, to restore apoE function in macrophages and normalize plasma lipoprotein profiles. This is a novel mouse model with chow diet feeding, providing an alternative model to investigate atherosclerotic plaque regression without robust surgical measures. We performed this regression study in parallel on initial and advanced lesions. In conclusion, both of these two chapters, focusing on novel mouse models to induce atherosclerotic lesion regression, demonstrate that 1) intact LDL receptor function is crucial to overcome LXR-induced hyperlipidemia and to achieve plaque regression; 2) ApoE$^{-/-}$ mice reconstituted with bone marrow from C57BL/6 mice represents a promising mouse model with chow diet feeding to study the regression of atherosclerosis, providing an alternative model to investigate plaque regression without robust surgical measures; and 3) rapidly optimized plasma lipoprotein profiles, combined

with LXR agonist treatment, induced favorable gene expression profiles, leading to regression of both initial and more advanced atherosclerotic plaques.

In **Chapter 8**, the results obtained from all the experiments mentioned above are summarized and discussed with respect to the implications of these studies for future investigations.