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Side-chain Oxidized Oxysterols as Metabolic Regulators in Liver and Brain

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ABSTRACT
Oxysterols are oxygenated derivatives of cholesterol characterized by very short half-life and ability to pass lipophilic membranes easily, thus are considered as important intermediates in cholesterol excretion pathway and its conversion to bile acids.

The two major oxysterols in the circulation of human and mouse are 24S-hydroxycholesterol (24S-OH) and 27-hydroxycholesterol (27-OH), which are formed by cytochrome P450 enzymes cholesterol 24S-hydroxylase (CYP46A1) and 27-hydroxylase (CYP27A1), respectively. The two oxysterols 27-OH and 24S-OH are both strong inhibitors of cholesterol synthesis and activators of LXR *in vitro*. However, their role as physiological regulators under *in vivo* conditions is controversial.

The overall aim of this thesis was to investigate the regulatory role of side chain oxidized oxysterols as metabolic regulators *in vivo*. In particular we have studied the role of 24S-and 27-hydroxycholesterols (24S-and 27-OH) as regulators of cholesterol synthesis and activators of LXR. We used mouse models with increased levels of 27-OH (CYP27A1 transgenic mice and Cyp7b1 knock-out mice (Cyp7b1-/-) as well as a mouse model with no detectable levels of 27-OH in their circulation, Cyp27 knock-out mice (Cyp 27-/-). Cyp 27-/- mice were treated with cholic acid to compensate for the reduced formation of bile acids.

In **Paper I**, we studied a possible regulatory role of 27- and 24S-hydroxycholesterol in the brain using human CYP27A1 transgenic mice and Cyp27 knock-out (Cyp 27-/-) mice. The levels of 27-OH were increased about 12-fold in the brain of CYP27A1 transgenic mice while the levels of 24S-OH was decreased by about 25%, most probably due to increased metabolism by the CYP27A1 enzyme. The mRNA levels of HMG-CoA reductase and HMG-CoA synthase in the brain were increased. In accordance with increased cholesterol synthesis, most of cholesterol precursors were also increased. The increased cholesterol synthesis is likely due to reduced inhibition by 24-OH. 27-OH is an activator of LXR. In spite of this, there was no upregulation of the LXR-target genes in the brain of the transgenic mice. In contrast, some of the genes were downregulated. In Cyp27 -/- mouse brain, cholesterol synthesis was slightly increased with increased levels of cholesterol precursors. The increased synthesis is probably the consequence of the absence of an inhibitory effect of the flux of 27-OH into the brain. The results of this study are consistent with the possibility that both 24OH and 27OH have a suppressive effect on cholesterol synthesis in the brain. Since there was no activation of the LXR-target genes in the brain of the transgenic mice, we concluded that 27OH is not a general activator of LXR in the brain.

In **Paper II**, we studied a possible regulatory role of 27-hydroxycholesterol in the liver using the above three mouse models. In the liver of CYP27 transgenic mice we found a modest increase of the mRNA levels corresponding to the LXR target genes Cyp7b1, and Abca1. There was no effect on a number of other LXR-regulated genes. There were no significant effects on cholesterol synthesis at the transcriptional level and with the exception of a modest decrease in T-MAS the levels of cholesterol precursors were not affected. In the liver of the Cyp7b1 knock-out mice, there were also no effects on cholesterol synthesis neither at the transcriptional level nor in the levels of cholesterol precursors, with the exception of increase in desmosterol. If the high levels of 27-OH are important, the same effects would be expected in the two mouse models. In the liver of the Cyp27 knock-out mice there was a modest activation of some LXR- regulated genes, Abcg5, Abcg8, Fas and Srebp1c. If 27-OH is of importance as a normal activator of the above genes a suppressing effect would be expected. The overall results do not support the contention that 27-OH is an important regular of cholesterol homeostasis or an activator of LXR-regulated genes under basal conditions in the liver.

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