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Titel:

Regulation of Apoptotic Metabolic Pathways by Phospholipase A2 VIA and Protective Effects of Ursodeoxycholyl Lysophosphatidylethanolamide

Promotionsfach: Innere Medizin
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Abstract:

Background and aims: NAFLD is one of the worldwide health problems. However, its pathogenesis remains unclear and the current therapy for NAFLD is ineffective. It is well-known that iPLA2β mediates monocyte chemotaxis to remove apoptotic cells in inflamed tissues. iPLA2β also mediates assembly of very low density lipoprotein (VLDL) in hepatocyte cell lines in *vitro* as shown in recent studies. Additionally, iPLA2β has been reported to show its cytoprotection in heart, pancreatic islet B cells, and PC12 cells (Williams et al. 2002; Zhao et al. 2010; Ma et al. 2011). However, it remains unclear about the effect of iPLA2β on lipid metabolism, cellular apoptosis and its possible liver protection. To address this, we provided analyses of diverse serum lipids and hepatocellular apoptosis in iPLA2β knockout (iPLA2$\beta^{-/-}$) mice. Besides that we compared UDCA-LPE with other current drugs in mice hepatocytes to find a new active agent.

Methods: Whole body iPLA2$\beta^{-/-}$ and age-matched wild-type mice were fed with chow diet. Hepatocytes were isolated from these mice and C57/BL6 mice for apoptosis induction by 300 uM palmitate. Pro-apoptosis cleaved PARP-1, cleaved caspase3, and Bim proteins were measured by Western blot. Caspase 3 activity was measured by luminescence. Triglyceride and cholesterol in serum and lipid extracts were measured by using kits. Gene expression was performed by quantitative TaqMan RT-PCR.

Results: We demonstrated that the body, hepatic and visceral fat weight as well as fasting serum, hepatic triglyceride and cholesterol levels were significantly decreased in iPLA2$\beta^{-/-}$ mice. Moreover, SREBP1 mRNA expression was obviously decreased while mRNA levels of FAS, SCD1, ACC1 and fatty acid elongase Elovl6 were slightly decreased. In contrast, mRNA levels of CPT1a, DGAT1 and ATGL were obviously increased in iPLA2$\beta^{-/-}$ mice.

Compared with wild-type mice, upon tyloxapol exposure, serum chylomicron, VLDL, and LDL levels in the triglyceride and cholesterol fractions as well as serum apoB protein levels were significantly decreased in mutant mice. And also, inflammatory markers including TNF-alpha, NF-κB, IL-6, MCP-1, CCl4, RANTES (CCl5), VCAM-1, and CC-chemokine receptor 2 were increased in iPLA2$\beta^{-/-}$ mice livers.

In vitro experiments, more apoptotic hepatocytes were found in iPLA2$\beta^{-/-}$ group and palmitate induced an increase of apoptotic rate. In vivo, more apoptotic hepatocytes were found in iPLA2$\beta^{-/-}$ group and LPS induced a further increase of apoptotic rate. In both vitro and vivo experiments, cleaved caspase-3 and Poly-ADP-ribose polymerase (PARP-1) were signicficantly increased in iPLA2$\beta^{-/-}$ mice.

In our study, apoptosis of hepatocytes isolated from iPLA2β-/- or wild-type mice could be reversed by UDCA-LPE. UDCA-LPE inhibition of lipoapoptosis was associated with an increase in TG upon Pal and UDCA-LPE co-treatment of mice. TG level was drastically increased. UDCA-LPE co-treated with Pal increased expression of stearoyl-CoA desaturase-1 (SCD-1) mRNA and protein at earlier time point prior to significant apoptosis induction. We demonstrated that UDCA-LPE inhibition of lipoapoptosis was SCD-1 dependent. Adenosine 3',5'-cyclic monophosphate was shown to be involved in UDCA-LPE's abilities to regulate SCD-1 and inhibit lipoapoptosis.

Conclusion: Our results suggest that iPLA2β is essential for maintaining liver homeostasis, and that inactivation of iPLA2β may render predisposition for liver disease, particularly liver cirrhosis. UDCA-LPE exhibits its effectiveness and elicits hepatocellular protection. Our research has revealed that iPLA2β promotion of liver homeostasis and UDCA-LPE promotion of hepatoprotection are associated with alterations of hepatic lipid metabolism. These findings are novel and that pathways in modulating lipid metabolism may be used as a new perspective for therapy of liver disease including liver cirrhosis and NAFLD. In this thesis, strong evidence has been presented for potential use of UDCA-LPE for treatment of inflammation in NASH.