Abstract

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Title of doctoral thesis:

Influence of inflammation modulation on excretory mechanisms during intrahepatic cholestasis

Intrahepatic cholestasis accompanies several systemic diseases, and can be induced by several drugs. All of its forms are associated with a certain degree of inflammation. The aim of this research was therefore to study changes in hepatic and renal elimination pathways during different forms of cholestasis, especially endotoxin-induced, and to characterize their modulation by administration of currently used or potential anti-inflammatory agents.

One of the most significant alteration of excretory mechanisms develops during sepsis. The status induces acute renal injury through activation of immune response activated by lipopolysaccharides (LPS) on their surface. In this study, we examined the possibilities to prevent such damage by two potent anti-inflammatory drugs, dexamethasone and anakinra, an IL-1 receptor antagonist. Biochemical and molecular signs of renal impairment were observed in rats administered the LPS from *Salmonellatyphimurium*, after pre-treatment with saline, dexamethasone or anakinra. In untreated endotoxemic rats characteristic symptoms of renal damage appeared within 10 hours - such as reduced glomerular filtration, microalbuminuria and reduced tubular secretion of azithromycin, prototype substrate for the transporters Mdr1

and Mrp2. Pre-treatment by both immunosuppressants alleviated all of these hallmarks typical for AKI and returned tubular secretion of azithromycin back to the control level. This effect was associated with up-regulation of basolateral transporters for organic anions. Application of both substances paradoxically reduced apical Mdr1 and Mrp2 transporters. Furthermore, dexamethasone increased renal excretion of bile acid through downregulation of transporter for reabsorption, Asbt. Both agents decreased the plasma concentrations of cytokines involved in the inflammation and decreased the concentration of NO in response to the reduction of iNOS expression in the kidneys and liver. Dexamethasone and anakinra were able to alleviate the symptoms of acute kidney injury and modulate changes in expression of transporters involved in renal excretion of drugs which were imposed due to administration of endotoxin. In this work, we have demonstrated the important role of IL-1 beta in the development of renal impairment during sepsis.

The next step was to elucidate the changes in billiary excretion of drugs during sepsis. We evaluated the protective effect of the clinically available iron chelators in the development of acute liver injury after administration of endotoxin, where lipopolysaccharide was administered, or following pre-treatment with dexrazoxane (DEX) or deferoxamine (DFO). Although both compounds reduced the iron content in the liver, only DFO demonstrated a protective effect against liver damage.

Further, we analyzed the potential choleretic effect of boldin, a natural choleretic agent in healthy and cholestatic rats. Boldin caused up-regulation of Bsep and increased biliary clearance of substrates and bile acids. Furthermore, we described the ability of boldin to stimulate FXR, a transcriptional regulator of Bsep. We confirmed this mechanism in the following study in which cholestasis was induced by high sucrose diet (HSD) applied to rats with hereditary hypertriglyceridemia (HHTg). Boldin alleviated the negative influence of HSD-induced liver steatosis on the biliary excretion of bile acids and glutathione. The data support positive effects of FXR agonists in the therapy of non-alcoholic fatty liver disease.