Introduction

In this thesis the regulation of the bilirubin-UDP-glucuronosyltransferase was investigated in rat livers. Bilirubin is the product of the heme catabolism. The glucuronidation of bilirubin is of vital importance with several pathological implications.

Glucuronide formation is catalyzed by uridine diphosphate glucuronosyltransferases [EC 2.4.1.17] (UGTs). Their substrate specificity is broad and overlapping. They are expressed mainly in hepatocytes but also in kidneys, intestine, skin and olfactory mucosa, too. They convert different, lipophilic compounds into more hydrophilic and thereby more readily excretable metabolites.

Main objectives

The regulation of bilirubin-UDP-glucuronosyl transferase in the liver was investigated in diabetes mellitus, starvation and alcoholism. Under these conditions acetonemia is a common feature. It is also well known that there is an induction of CYP2E1 - a member of the cytochrome P450 superfamily – upon administration of acetone, and an induced CYP2E1 in hepatocytes is also characteristic in the above mentioned states. CYP2E1 also has a heme oxygenase activity. The question arises, how is glucuronidation of bilirubin changed in these states; what is the molecular mechanism of these alterations? Do the inductions of CYP2E1 and UGT1A1 (the two crucial biotransformation enzymes in heme catabolism) occur independently, or they are coordinated processes?

Glucuronidation of bilirubin occurs in hepatocytes. However, several studies have shown that Kupffer cells play a central role in various liver injuries induced by ethanol. Therefore, the participation of Kupffer cells in the ethanol-induced alterations of bilirubin glucuronidation was also investigated using gadolinium chloride, a known Kupffer-cell-depleting agent.

In vivo experiments were planned in diabetic rats, acetone-treated rats, rats starved for 96 h, and in ethanol treated rats. BioBreeding/Worcester (BB/Wor) male rats were chosen as the animal model of human insulin-dependent (Type I) diabetes. Effect
of Kupffer cell depletion was studied after in vivo administration of gadolinium chloride to ethanol treated animals.

**Background**

The induction of enzymes in the 1\textsuperscript{st} and 2\textsuperscript{nd} phase of biotransformation is of great importance. In the 1\textsuperscript{st} phase mainly cytochrome P450 enzymes are induced. The UGTs in the 2\textsuperscript{nd} phase are also inducible by several 1\textsuperscript{st} phase inductors (methylcholantrene, phenobarbital, chlofibrate, etc.). The induction of UGTs is about 2-4-fold, in contrast to 1\textsuperscript{st} phase enzymes, where the induction can be up to 100-fold. 2\textsuperscript{nd} phase enzymes are induced under physiological conditions. Bilirubin is an end product of heme catabolism. In adults, 250-350 mg of bilirubin is produced daily, which results in a plasma concentration between 10 and 17 µM. Several glucuronidation reactions are catalyzed by UGT1A1. Bilirubin is not the only endogenous substrate of this enzyme.

The bilirubin catabolism occur mainly in the liver. However UGT1A1 is present beside the liver, in intestines, kidneys, in the prostate and gonades. Fasting leads to an increased bilirubin-UGT activity. In starvation and during the neonatal period the serum bilirubin concentration is elevated.

There are contradictory results in publications on bilirubin levels in diabetes mellitus as the used models and species are different.

Waltman et al. found that the serum bilirubin concentration is decreased in newborns from alcoholic women. Several reports have indicated that ethanol enhances the clearance of bilirubin and hepatic heme turnover, resulting in beneficial effects both in Gilbert's disease and neonatal hyperbilirubinaemia.

Most studies investigating the hepatic effects of ethanol were focused mainly on hepatocytes. However exciting evidence has now emerged implicating an indirect role of Kupffer cells in the toxic hepatic effects of ethanol. An elevated level of Kupffer-cell-secreted mediators was observed after ethanol treatment.

Kupffer cells produce several intercellular mediators, namely tumor necrosis factor-α (TNFα), ROS, eicosanoids, nitrogenmonoxide, carbonmonoxide, cytokines. Enteral ethanol feeding enhances the level of gut-derived bacterial endotoxins, which, after entering the bloodstream activate Kupffer cells to release prostaglandins other
eicosanoids, cytokines and ROS. Gadolinium-chloride (GdCl$_3$) is a well-known tool to examine the role of Kupffer cells. It is toxic for tissue macrophages, causes a decrease in Kupffer cell number by 80%. With GdCl$_3$ treatment the inductive effect of ethanol on CYP2E1 is absent. Therefore, it was suggested that Kupffer cells participate in ethanol-induced alterations of bilirubin glucuronidation.

In our in vitro experiments the effect of diabetes mellitus, fasting and chronic alcohol consumption were investigated on bilirubin UDP-glucuronosyltransferase (UGT1A1).

**Methods**

BB/Wor rat strain was used as diabetes mellitus type I model. In a series of experiments rats were starved for four days. Ketosis was modeled using rats treated with acetone added to drinking water (1%, v/v). Ethanol (3 g/kg of body wt. once a day) was administered by gastric intubation for two weeks. A group of ethanol treated rats was treated with gadolinium chloride (GdCl$_3$), for the inactivation of the Kupffer cells.

Rat liver microsomes (permeabilized and native) were prepared to measure pnp-UGT and bilirubin-UGT activities. Concentrations of glucose, acetone and conjugated bilirubin were determined in the sera of rats. The changes in the UGT1A1 level were investigated by Western blotting, using a specific rat UGT1A1 antibody. Total RNA were isolated from rat livers, and using the cDNA of UGT1A1, as a probe Northern-blot was performed for the analysis of UGT1A1 mRNA.

**Results**

In diabetic, fasted and acetone treated rats higher conjugated serum bilirubin concentrations were observed, whereas total bilirubin concentrations remained unaltered. $p$-Nitrophenol glucuronidation was not increased significantly in either impermeabilized or permeabilized diabetic microsomes compared to the controls, while
bilirubin-UGT activities were increased up to 6-fold in diabetic microsomes compared to the controls. UGT1A1 was increased in diabetes measured in rat liver microsomes by Western blot. In contrast to previous findings – in streptozotocin diabetic rats –, the latency of both phenol UGT and UGT1A1 was not influenced in spontaneously developed type I diabetes mellitus. Northern-blot analysis showed that significantly higher levels of UGT1A1 mRNA are present in the liver of diabetic rats compared to the control group.

Bilirubin glucuronidation was enhanced by 3.5-fold in microsomes prepared from acetonaemic rats, and by 6.6-fold in microsomes originated from fasted rats. The p-nitrophenol glucuronidation was not altered in these experimental groups. Western blot analysis indicated an increase in the amount of UGT1A1 isoforms both in acetone-treated or starved rats. The UGT1A1 mRNA levels were not changed in livers from acetone-treated rats, while an increase was shown in livers from starved rats.

Higher conjugated bilirubin concentrations in the serum, and an elevated bilirubin-UGT activity in the microsomes were observed after ethanol treatment.

Bilirubin-UGT activities were increased, while p-nitrophenol glucuronidation was not increased in intact or permeabilised microsomes prepared from ethanol treated animals. The chronic ethanol treatment increased the UGT1A1 expression by about 3-fold compared to the controls. Higher amount of UGT1A1 mRNA was detected by Northern hybridization in the liver of ethanol-treated rats compared to the controls.

Bilirubin-UGT the results showed that bilirubin-UGT activities were significantly higher in ethanol treated rats compared to those treated with GdCl₃ and ethanol simultaneously. The conjugated serum bilirubin level was also significantly higher in the ethanol treated rats than in the ethanol plus GdCl₃ treated animals. Simultaneous GdCl₃ administration prevented the increasing effect of ethanol on UGT1A1 expression measured by Western blot and on UGT1A1 mRNA level measured by Northern-blot.

Our results indicate that the UGT1A1 is under transcriptional control, for the induction of UGT1A1 by ethanol active Kupffer cells are required.
Main observations:

1. In liver microsomes prepared from diabetes mellitus type I rats, starved acetone treated rats or after chronic ethanol treatment
   a) elevated bilirubin-glucuronosyl-transferase activity
   b) elevated levels of UGT1A1 detected by Western-blot were shown.
2. In diabetes, starvation and upon chronic ethanol treatment the induction occurred at transcriptional level as increased UGT1A1 protein and UGT1A1 mRNA (detected by Northern-blot) were demonstrated. However, in acetone treated rats the UGT1A1 mRNA level was not changed in liver suggesting that in diabetes mellitus and starvation the UGT1A1 inducer is not the acetone.
3. UGT1A1 induction by chronic ethanol administration could be prevented by addition of Kupffer cell depleting gadolinium chloride to rats. Therefore, it is supposed that extrahepatocellular mechanisms are involved in the induction of UGT1A1.
Main observations in my thesis were published in the following papers:


Other papers:


