Abstract

Introduction

Estrogen-induced cholestasis is a disease characterized by a failure of bile flow and bile production. It can develop in women after oral contraceptives use, hormone replacement therapy or during pregnancy. The estrogen metabolism is a complex process leading to formation of metabolites with different biological activities. It takes place primarily in the liver (Phase I and Phase II including hydroxylation, methylation, sulfation and glucuronidation). The enzymes from UDP-glucuronosyltransferases family , abbreviated UGT, are responsible for the glucuronidation of estrogens.

Aims

The objective of my work is to define estrogen metabolism and gene expression of UGT1A1, CYP1A2 and SULT1A1 and characterize cholestatic liver damage in the UGT1A1 deficient rat strain (Gunn rats) compared to rats with normal enzyme activity and try to define possible mechanisms responsible for the liver damage.

Methods

Adult female Gunn and corresponding heterozygous rats were treated with ethinylestradiol (EE, 5 mg/kg body weight SC) for 5 days, while control rats received propanediol (vehicle). Day six, the animals were sacrificed and plasma and liver tissue were collected for analysis. Markers of cholestasis and liver damage ALP, AST, ALT and bilirubin were determined using an automatic analyzer, total bile acids were measured spectrophotometrically. I isolated mRNA from the liver, transcribed it into cDNA and I performed a quantitative real-time PCR of key enzymes of estrogen metabolism by TaqMan analysis. I evaluated gene expressions of UGT1A1, CYP1A2, and SULT1A1.

Results

EE administration significantly increased cholestatic markers in Gunn rat strain (serum bile acids 10-fold, ALP 5.4-fold, $p \leq 0.05$) in comparison with heterozygotes. Expression of UGT1A1 was significantly increased in heterozygous rats (3-fold, $p = 0.02$) after application of EE as compared to

controls. Application of EE also led to more pronounced decrease in CYP1A1 (54%) in and SULT1A1 (84%) expressions in Gunn rats as compared to the corresponding heterozygous rat strain (27% and 70%,respectively,p≤0.05).

Conclusion

We found that UGT1A1 deficient rats developed significantly more severe cholestasis as compared to the corresponding heterozygotes. UGT1A1 defect leads to a different metabolism of estrogen, impaired hydroxylation, glucuronidation and sulfation, which may cause accumulation of toxic metabolites of estrogens and development of cholestasis.

Keywords: Gilbert syndrome, UGT1A1, Gunn, glucuronidation, UDP-glukuronosyltranferase, ethinylestradiol, cholestasis, CYP1A2, SULT1A1, estrogen metabolism, intrahepatic cholestasis of pregnancy