ABSTRACT
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Title of diploma thesis: Alterations in gene expression of hepatobiliary transporters as potential mechanisms for drug-induced cholestasis by amoxicillin and clavulanic acid

The combination of amoxicillin and clavulanic acid (AMO/CLA) represents one of the most frequent causes of the idiosyncratic type of drug-induced liver injury (DILI) nowadays. Despite difficulties in diagnosis and causality assessment, the clinical features have already been reported and in most of the cases categorized as cholestatic damages. Number of descriptions of the molecular mechanisms of drug-induced cholestasis has been rising recently and the role of hepatobiliary transporters has turned out to be crucial in the pathogenesis. However, the mechanisms of AMO/CLA-induced DILI at the molecular level still remain indistinct. In order to investigate the hepatotoxic effects of AMO/CLA and AMO alone in vitro, HepG2 and human Upcyte hepatocytes were used as hepatocellular models. The mRNA levels of key bile acid (BA) transporters, enzymes and nuclear receptors (NRs) were measured by quantitative real-time polymerase chain reaction. The cytotoxic concentrations of AMO/CLA and AMO were excluded using cell viability tests. Moreover, the protein expression levels of key BA transporters were determined by Western blot analysis and image documentation of cultured cells was provided by digital microscopy. Results evidenced that AMO/CLA caused extensive alterations in gene expression levels of BA transporters, enzymes and NRs, whereas AMO affected tested genes minimally. Specifically, AMO/CLA was able to decrease the mRNA levels of the bile salt export pump (BSEP), which could represent the most significant pro-cholestatic mechanism discovered up to now. On the contrary, the gene and protein expression of a transporter mediating uptake of conjugated BAs, the Na⁺-taurocholate co-transporting polypeptide (NTCP), was reduced by AMO/CLA, which could denote a compensatory response to accumulated BAs. Nevertheless, it is anticipated that other additional factors could also be involved in the pathogenesis of cholestasis by AMO/CLA. Therefore, results allow concluding for the first time that AMO/CLA negatively influences the expression of key BA transporters, which could hamper normal bile flow and promote cholestatic DILI.