SUMMARY

The liver is a unique organ with a number of vital functions. Pivotal one is its participation on bile formation and secretion, import, detoxification and excretion of endogenous substances and xenobiotics.

Bile formation is essential for both absorption of lipids in intestine and excretion of various endogenous compounds and xenobiotics (e.g. bile acids, bilirubin, cholesterol, phospholipids and drugs). This function is markedly impaired during extrahepatic and intrahepatic cholestasis with partial or complete stoppage of bile flow. Consequently, hepatic and further systemic accumulation of toxic biliary constituents, such as bile acids and bilirubin, occurs. In an effort to compensate this situation, spontaneous anti-cholestatic mechanisms are activated, which provide alternative excretory routes for toxic accumulating compounds (e.g. renal elimination of bile acids and xenobiotics into urine). These mechanisms include changes in the expression, localization and function of respective transporters in liver and kidneys. Another mechanism with a significant impact on bile formation and transport of compounds between bile and blood is blood-biliary barrier formed by connection of hepatocytes by „tight-junctions“ and „gap-junctions“. While „gap-junctions“ exchange substances among cells, „tight-junctions“ represent the real blood-biliary barrier and are, together with transporters, necessary for the bile formation. „Tight-junctions“ regulate transport of water, solutes and ions through the paracellular spaces of hepatocytes.

In this work both mechanisms responsible for bile formation and secretion – transporters and blood-biliary barrier – were studied. Furthermore, acute and chronic cholestasis induced changes in transporter expression and integrity of blood-biliary barrier were evaluated. In addition, changes in pharmacokinetics of drugs, acting as substrates of selected transporters, were examined, too. In the first study, we focused on the evaluation of biliary and renal excretion of rhodamine 123, a substrate of P-glycoprotein (P-gp), during acute and chronic extrahepatic cholestasis in rats induced by 1 and 7 days lasting bile duct obstruction. Recently, many studies have confirmed increased expression of P-gp at both mRNA and protein level during obstructive cholestasis. P-gp is the only efflux transporter expressed at the canalicular membrane of hepatocytes with increased expression during cholestasis. One of the explanations is a decline in its substrate concentration in hepatocytes due to down-regulation of basolateral uptake transporters – “Organic cation transporter 1“ (Oct1) and “Organic anion transporting polypeptide 1a4“ (Oatp1a4). This fact could partially explain the disagreement between increased expression

of P-gp protein and unchanged or even decreased biliary excretion of rhodamine123.
Comparison of rhodamine 123 renal and hepatic excretion showed that this compound is eliminated mostly by the kidney with only approximately 23% of the eliminated amount being excreted into bile. Decreased bile production and biliary excretion of rhodamine 123 during acute cholestasis and preserved bile excretion during chronic cholestasis are in disagreement with increased P-gp expression in livers. Physicochemical properties of the drug could partly explain this fact since rhodamine 123 is a fluorescent dye which exhibits a positive charge. Despite the assumption that it enters the cells by passive diffusion, partial water solubility at physiological pH eventuated the potential contribution of active transport at the basolateral membrane of hepatocytes via Oct1 transporter. Other explanation could be increased accumulation of endogenous and exogenous substances such as bilirubin and bile acids, which cross the basolateral membrane of hepatocytes mostly by passive diffusion and consequently activate nuclear receptors that regulate P-gp expression (CAR, PXR). Rhodamine 123 is actively excreted into urine via P-gp and P-gp expression changes are accompanied with P-gp-mediated renal tubular secretory clearance changes. During acute cholestasis, the net tubular secretion clearance and expression of P-gp protein were preserved, which complies with the reported nephroprotective effect of cytokines released from the liver. Decreased renal excretion and renal clearance of rhodamine 123 during chronic cholestasis may be explained by down-regulation of P-gp expression. In this study, the employed model of extrahepatic cholestasis with reconstituted bile flow offers possibility to investigate functional consequences of cholestasis for the pharmacokinetics and pharmacodynamics of endogenous substances and xenobiotics.

In the second study, we investigated changes in permeability of blood-biliary barrier using rhamnose/meliobiose test during acute obstructive cholestasis induced by 1 day lasting bile duct obstruction. Biliary excretion of both sugars was increased during the first 60 min (from total 240 min) after i.v. application of the compounds. Regarding cumulative biliary excretion, the melibiose/rhamnose ratio was increased throughout the whole experiment. Biliary excretion of both sugars was negligible in comparison with their renal elimination. When administered rhamnose intravenously to mammals, it is eliminated primarily by the kidneys with 65% and 75% contribution in rats and humans, respectively. In comparison to rhamnose, melibiose is not supposed to be metabolised. In this study, total excretion of rhamnose and melibiose was 62% and 71%, respectively, with highly prevailing urinary excretion. Renal clearance was not

influenced by cholestasis. Plasma to bile concentration ratios of rhamnose suggested that passive diffusion of this sugar through hepatocytes occurs similarly to that of enterocytes and that rhamnose quickly equilibrates between plasma and new formed bile. In conclusion, the used dual-sugar permeability test is convenient to describe the alteration of the blood-biliary barrier during acute cholestasis in rats. This test demonstrated that the blood-biliary barrier becomes leaky during acute cholestasis and this impairment could be measured during the first 60 min after administration of both sugars.

The third study was focused to description of changes in the pharmacokinetics of methotrexate (MTX) using the *in vivo* model of extrahepatic (bile duct obstruction for 1 and 7 days) and intrahepatic cholestasis (lipopolysaccharide administration, LPS) in rats. Simultaneously, we analyzed changes in the expression of main transporters responsible for MTX transport at both mRNA and protein level. Moreover, kinetics of conjugated bilirubin, an endogenous substrate of Mrp2, was evaluated to qualify the severity of cholestasis. In humans, 10–30% of applied dose of MTX is excreted by bile. Nevertheless, the contribution of hepatic transporters seems to be higher because the ratio of MTX biliary/renal excretion in humans is 0.94. This indicates extensive entero-hepatic cycling and active reabsorption of the drug from gastrointestinal tract. Some kinetic studies demonstrated that biliary excretion of drugs is impaired during extrahepatic cholestasis. This was confirmed in this study as well, when biliary excretion and biliary clearance of MTX was decreased and led to significant reduction of its systemic clearance. In comparison to obstructive cholestasis, reduction of MTX biliary clearance was less intensive during intrahepatic cholestasis. Expression changes of MTX transporters in livers partly corresponded to alterations of MTX biliary excretion. MTX transporters include (1) for uptake into hepatocytes – “Organic anion transporting polypeptide 1a1, 1a4 and 1b2“ (Oatp1a1, Oatp1a4 and Oatp1b2); (2) for efflux into bile – “Multidrug resistance-associated protein 2“ (Mrp2) and “Breast cancer resistance protein“ (Bcrp); (3) for efflux from hepatocytes into plasma – “Multidrug resistance-associated protein 3 and 4“ (Mrp3 and Mrp4). Protein expression of transporters corresponds with that of mRNA suggesting transcriptional regulation of the expression during extrahepatic cholestasis. Administration of LPS produced changes in hepatic transporter expression mostly at the mRNA level. Only Oatp1b2 and Mrp3 were influenced at the protein level. Although we observed a rise in renal excretion of MTX, renal clearance was only slightly increased. This proves that increased expression of efflux

transporters such as Mrp2 in kidneys cannot compensate systemic accumulation of anionic compounds during obstructive cholestatis. On the other hand, induction of Mrp2 may protect the kidney proximal tubular cells from the accumulation of MTX during acute cholestasis and thus contribute to nephroprotective effect. The increased expression of Mrp3 may make for this effect. Intrahepatic cholestasis led to down-regulation of Mrp2 and Mrp3 transporters in kidney proximal tubular cells. In addition, glomerular filtration was reduced in LPS-induced cholestasis and the reduction of MTX renal clearance may be attributable not only to altered transporter expression but especially to reduced kidney perfusion. This effect could be caused by increased cytokine production. Changes in MTX pharmacokinetics and respective transporter expression alterations suggest important differences between the two widely used cholestatic models.