Summary

The study of the potential importance of pravastatin in the prevention of the cholestatic liver impairment.

Obstructive cholestasis is a clinical syndrome accompanying numerous liver diseases. Early diagnosis and appropriate treatment of obstructive jaundice is very important because untreated condition leads to irreparable changes in the liver. This gives rise to liver fibrosis, which later passes into biliary cirrhosis with all its consequences (portal hypertension, esophageal varices, liver failure). The causal therapy such as surgical removal of the obstruction is quite often impossible. Therefore it is necessary to search for pharmacotherapeutic approaches that can positively modulate the developing disease.

In the present thesis, the effect of pravastatin on the liver damage during chronic obstructive cholestasis was investigated in rats. Pravastatin, belonging to a group of widely used inhibitors of HMG-CoA reductase, possesses not only lipid-lowering action but also anti-inflammatory and antioxidant effects, so called pleiotropic effects. The decisive fact for choosing pravastatin as a suitable substance for our study was that pravastatin has already shown positive effects in several patients with cholestasis and its hydrophilic nature does not require metabolism in impaired liver and allows compensatory excretion in the kidney.

Detailed elucidation of pravastatin effect on development of liver injury during bile duct obstruction (BDO) in rats was performed using complex biochemical and histological examinations together with the evaluation of changes in enzyme and transport mechanisms for bile acids (BA), bilirubin, cholesterol, and iron.

Biochemical and histological findings in untreated BDO animals were in accordance with the specifications reported in the literature and thus confirmed reliability of the model. Obstructive cholestasis was the cause of hyperbilirubinemia, hypercholesterolemia, and elevated plasma concentration of BA. Histological analysis revealed bile duct proliferation and early fibrotic remodeling of liver tissue. Administration of pravastatin to BDO animals induced significant changes, which, however, differed accordingly to the dose applied (1 (P1) or 5 (P5) mg/kg/day). While in BDO-P1 group both decrease in plasma concentrations of BA, bilirubin, and cholesterol and attenuation of proliferative and fibrotic changes were observed, in the group with higher dose of pravastatin an increased mortality and worsening of biochemical and histological parameters were noted when compared with BDO animals.

Changes in the expression of several enzymes and transporters were induced during biliary obstruction in BDO group, which is supposed to preclude accumulation of potentially toxic substances (especially BA and bilirubin) in the liver. Administration of pravastatin in the lower dose caused a decrease in plasma concentrations of BA and bilirubin in BDO rats. This effect originated from a decreased synthesis of these substances due to downregulation of responsible enzymes Cyp7a1 and Cyp8b1 (BA) and hemeoxygenase-1 (bilirubin). Conversely, further deterioration of liver excretory function occurs in BDO-P5 rats as a consequence of downregulation of canalicular transporters Bsep and Mrp2.

Hypercholesterolemia is a common symptom of obstructive cholestasis. Our study brings new findings about underlying mechanisms. Increased cholesterol levels in plasma and liver of BDO animals were related to the increase in liver content of HMG-CoA reductaseand decrease in efflux of cholesterol due to downregulation of Abca1 and Abcg5/g8 transporters. Administration of pravastatin in lower dose caused a decrease in liver cholesterol content through reduction of its synthesis (HMG-CoA reductase), uptake (LDL receptor), esterification and deposition (Acat2), and increased efflux (Abca1).

Oxidative stress plays an important role in liver injury during obstructive cholestasis. Iron is one of the potential sources of free oxygen radicals whose role has not been yet described during chronic extrahepatic cholestasis in rats. Positive effect of pravastatin during biliary obstruction consisted in increased iron storage in a metabolically inactive form of ferritin that is not a source of free oxygen radicals. Increased expression of transferrin receptors responsible for iron uptake into hepatocytes along with increased ferritin formation and increased expression of efflux transporter ferroportin 1 can explain the rise of liver iron content that was significantly decreased in BDO group. Furthermore, administration of lower dose of pravastatin restored expression of hepcidin, a key regulator of iron metabolism, whose expression was suppressed during obstructive cholestasis. Increased content of pSTAT3 in this group suggests reduction of bile acidinhibitory effect on regulatory pathway IL6 – gp130 – pSTAT3 – hepcidin.

The acquired data enabled to summarize the potentially beneficial effect of pravastatin on the liver damage induced by obstructive cholestasis. The choice of proper drug dose seems to be entirely fundamental. The effect itself includes a number of regulatory and executive mechanisms and is not associated with hypocholesterolemic effect of the drug. So we can classify this effect as so-called pleiotropic effect of the statins.