INDUCTION OF HEMOXYGENASE 1 (HO1) PREVENTS ACUTE HEPATIC CHOLESTASIS PRODUCED BY OXIDATIVE STRESS (OS) IN THE RAT

Here, we studied the effect of HO1 induction and consequent increase in endogenous levels of bilirubin (BR) on OS-induced cholestasis. Wistar rats were treated with Hemin (H) and biliary concentrations of BR were determined, finding that it increased 6-8h post i. p. injection of 20 mg/kg H (12.6±2.5 vs 5.3±0.6 for vehicle, p<0.001; n=4). Oxidative cholestatic injury was induced by tert-butyl hydroperoxide (tBOOH, 440 μmol/kg, i.p.) and bile flow (μl/min/g liver) was monitored finding that it decreased 4-6h post treatment (p<0.05 vs control; n=6). Pretreatment with H completely prevented reduction of bile flow (1.65±0.04 and 1.30±0.03, respectively; p<0.01; n=6). Redox state was evaluated by measuring levels of lipid peroxidation (LP), oxidized glutathione/total glutathione ratio (GSSG/GSHt) and activity of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). We found that tBOOH caused an increase in LP (0.170±0.022 nmol MDA/mg protein vs C, 0.090±0.005 nmol MDA/mg protein p<0.05; n=6) while pretreatment with H prevented this increase (0.082±0.001 nmol MDA/mg protein 0.05 vs tBOOH; n=6). GSSG/GSHt ratio increased after treatment with tBOOH (0.40±0.09 vs C, 0.14±0.12, p<0.05; n=6) while pretreatment with H prevented this increase (0.20±0.01, p<0.05; n=6). CAT and SOD activities were increased in tBOOH group (p<0.05 vs C, n=3, for both enzymes) while pretreatment with H completely prevented these increases (p<0.05 vs tBOOH, n=3).

We also studied the function of two key hepatocanalicular transporters, Bsep and Mrp2, by determining biliary excretion of their specific substrates, bile salts (BS) and GSHt, respectively. Biliary excretion of both BS and GSHt decreased after treatment with tBOOH, and pretreatment with H prevented these decreases (p<0.05 vs tBOOH, n=4). We conclude that induction of HO1 and consequent elevation of BR protect the liver from oxidative injury and contribute to limit the progression of cholestatic liver diseases concurring with OS.

Keywords: Oxidative stress, cholestasis, HO1, bilirrubin.