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 andconsequent 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 (*t*BOOH,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 Hcompletely 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 *t*BOOH caused an increase in LP (0.170±0.022nmol MDA/mg proteín*vs* C, 0.090±0.005nmol MDA/mg protein $p<0.05$;n=6) while pretreatment with H prevented this increase (0.082±0.001nmol MDA/mg protein 0.05 *vst*BOOH; n=6). GSSG/GSHt ratio increased after treatment with *t*BOOH (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 *t*BOOH group ($p<0.05$ *vs* C, n=3, for both enzymes) while pre-treatment with H completely prevented these increases ($p<0.05$ *vst*BOOH,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 *t*BOOH, and pretreatment with H prevented these decreases ($p<0.05$ *vst*BOOH, 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.