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# INDUCTION OF HEMOXYGENASE 1 PREVENTS ACUTE HEPATIC CHOLESTASIS PRODUCED BY OXIDATIVE STRESS IN THE RAT

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**INTRODUCTION:** Cholestasis is defined as the reduction of bile flow, and the consequent accumulation of toxic compounds in liver and blood. Under oxidative stress (OS) conditions, reactive oxygen species (ROS) are generated due to mitochondrial damage. Bilirubin (BR) is an endogenous biliary pigment derived from heme metabolization by enzymes hemoxygenase 1 (HO1) and biliverdin reductase. In a previous study, we demonstrated that unconjugated BR exerts an important protective effect on biliary secretory failure induced by OS, even at physiological concentrations.

**AIM:** To study the effect of HO1 induction and the consequent increase in endogenous levels of BR on the hepatocellular redox status and the function of two key hepatocanalicular transporters, Bsep and Mrp2.

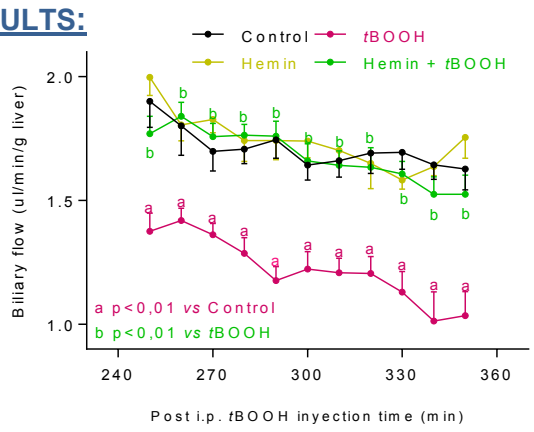
**HYPOTHESIS**: Hepatic diseases bearing an oxidative background would have a more severe outcome in terms of hepatobiliary function in the absence of BR, and the modulation of endogenous BR levels would have a beneficial effect on the course of oxidative cholestatic pathologies.

**MATERIALS AND METHODS**: Wistar rats (♂) were divided into 4 groups: **Control**: treated with DMSO (vehicle); **tert-butyl hydroperoxide** (**tBOOH**): treated with 440 µmol/kg p.c. tBOOH (prooxidant agent), i.p.; **Hemin**: treated with 20 mg/kg p.c. hemin (HO1 inductor), i.p.; and **Hemin + tBuOOH**: treated with 20 mg/kg p.c. hemin i.p. + 440 µmol/kg p.c. tBOOH i.p. At 4 h post tBOOH treatment, bile flow was monitored and **bile samples** were collected every 10 minutes for 2 h. After euthanasia, **liver tissue samples** were collected and stored (-70°C).

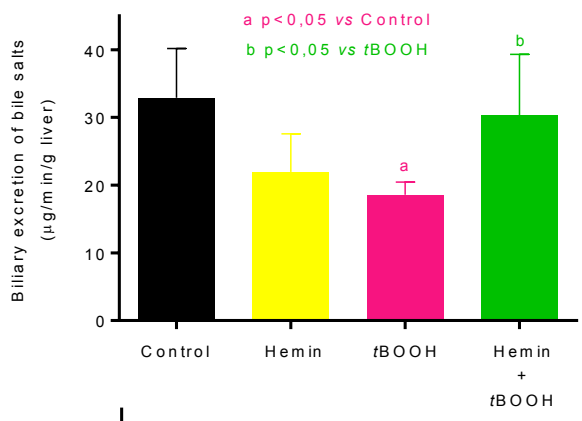
- **REDOX STATUS**
  - Lipid peroxidation & protein carbonyls
  - Oxidized gluthatione/Total glutathione ratio (GSSH/GSHt)
  - Catalase (CAT) & superoxide dismutase (SOD)
- **HEPATOCELLULAR SECRETORY FUNCTION**
  - Bile flow
  - Biliary excretion of bile salts (BS)
  - Biliary excretion of GSHt
  - Localization of Bsep & Mrp2

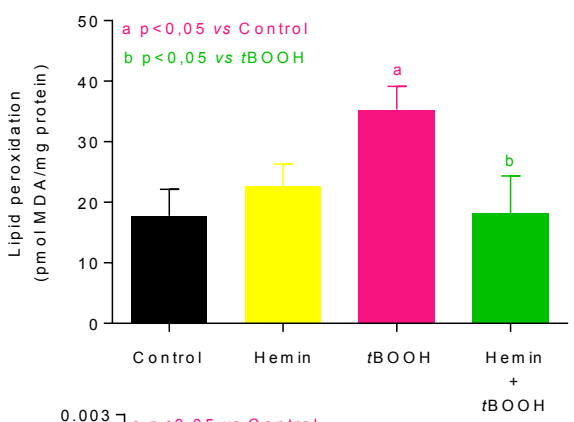
**Oxidative damage** to lipids and proteins was assessed in liver tissue by the reaction of the **Thiobarbituric Acid Reactive Substances (TBARS)** and the determination of **protein carbonyls** by the **Levine method**, respectively. Antioxidant defenses were evaluated in liver tissue through **CAT activity,** measured by spectrophotometrically monitoring the disappearance of $H_2O_2$ at 240 nm (**Beers and Sizer method**), and **SOD activity**, measured using a commercial kit (Randox, Crumlin, UK). In bile samples, **GSHt and GSSG** were measured by the **Griffith method** (modified by Tietze), as a sensible marker of OS.
**Biliary secretory** function was evaluated through the determination of biliary excretion of **bile salts** (Bsep substrates), by the **Talalay method** (modified by Berthelot) and biliary excretion of GSHt (Mrp2 substrate), as described above. **Localization of hepatocanalicular transporters** was studied in liver tissue samples through fluorescence confocal microscopy.

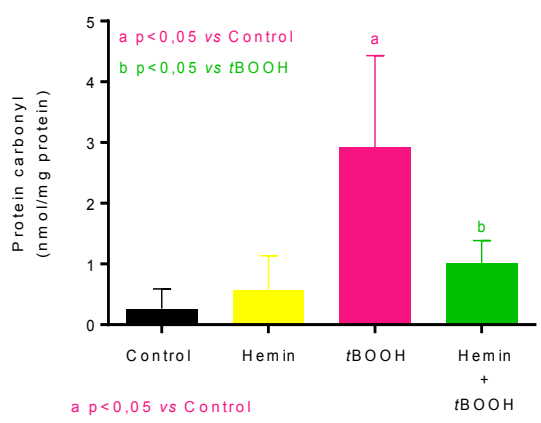
## RESULTS:

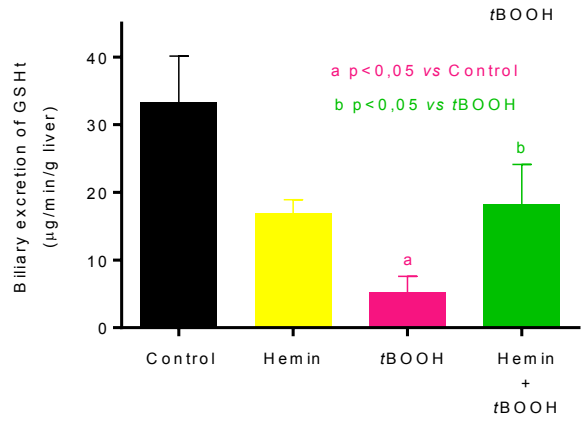


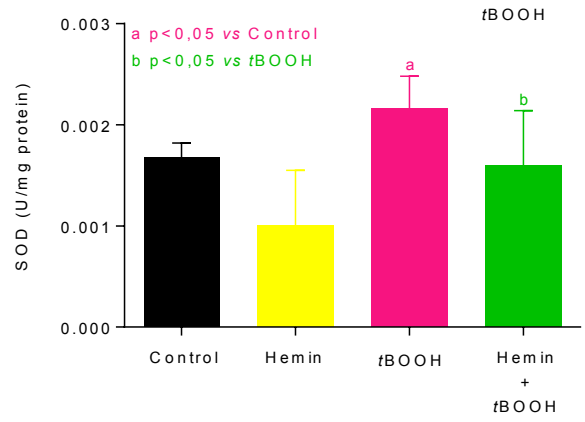

**HO1 INDUCTION PREVENTS OS-INDUCED BILIARY SECRETORY FAILURE**: tBOOH caused a drop of bile flow, whereas pretreatment with hemin (Hemin + tBOOH group) was able to prevent this drop throughout the study period (4 to 6 h post injection of tBOOH).

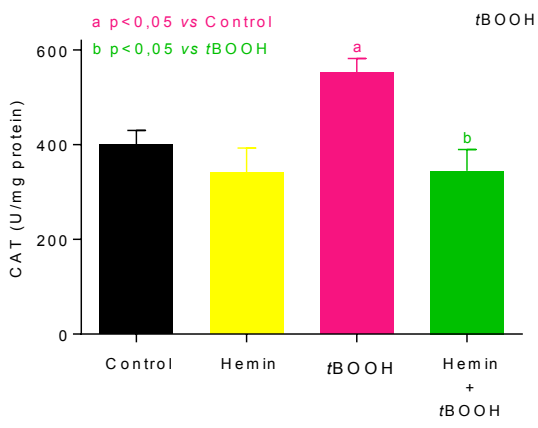


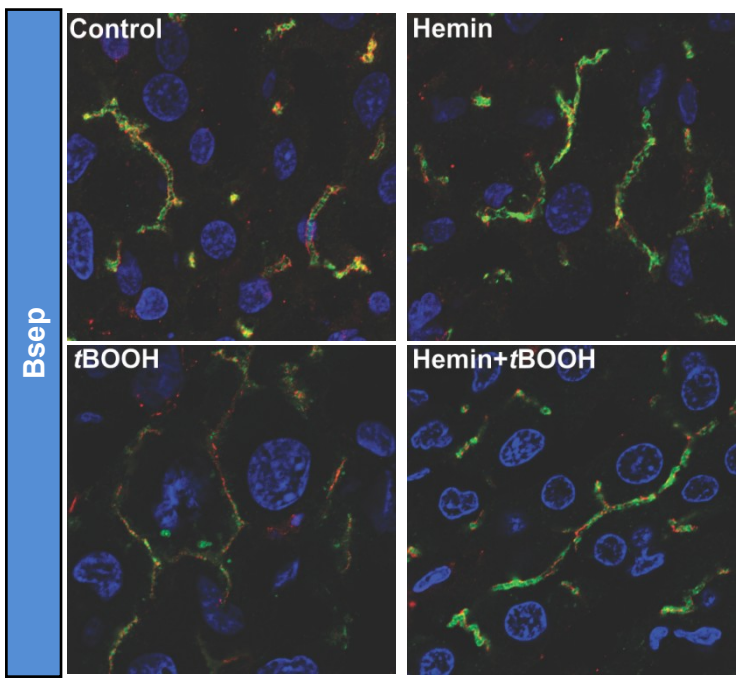



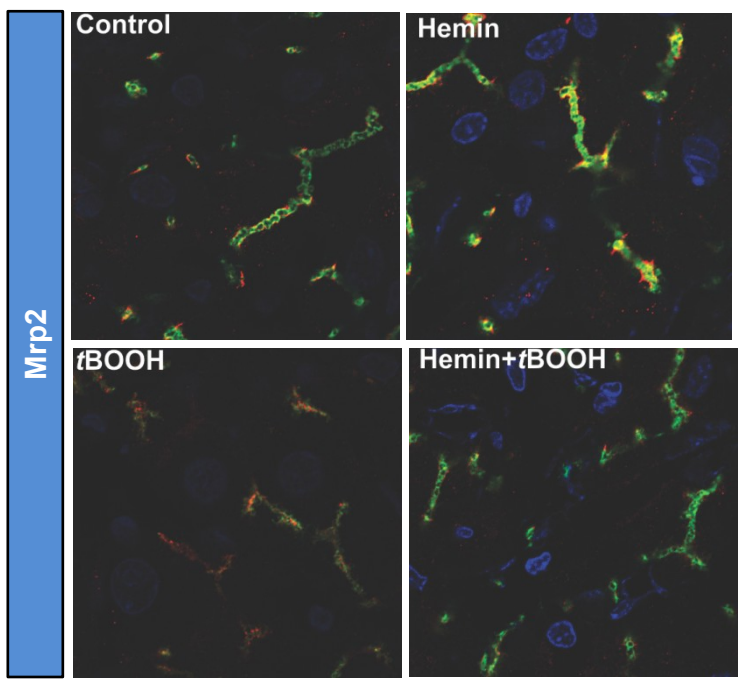




**HO1 INDUCTION PREVENTS OXIDATIVE INJURY PRODUCED BY tBOOH** : tBOOH caused an increase in CAT & SOD activities; also in lipid peroxidation and protein carbonyl levels, as well as in GSSG/GSHt ratio, compared to Control, whereas pretreatment with hemin (Hemin + tBOOH group) prevented these increases.




**LOCALIZATION OF HEPATOCANALICULAR TRANSPORTERS**:
In the Control group, Mrp2 and Bsep were confined to the canalicular space, while in the tBOOH group a relocation from the canalicular space to the pericanalicular region was observed, thus suggesting endocytic internalization of these transporters. Besides, pretreatment with hemin prevented this phenomenon, as can be inferred from the distribution of Mrp2 and Bsep similar to Control in the Hemin + tBOOH group.

**CONCLUSION**: Induction of HO1 and consequent elevation of BR levels protect the liver from oxidative injury, thus contributing to limit the progression of cholestatic liver diseases that concurrs with OS.