Abstract

For a long time, bilirubin (BR) has been considered a waste molecule with potential toxic effects especially on the central nervous system. Later, it was found that BR exhibited cytoprotective effects and mildly elevated BR levels showed antioxidant, anti-inflammatory and immunomodulatory properties, however, exact mechanisms of the anti-inflammatory actions of BR have not been fully understood yet. The main aim of this study was to assess the protective effects of BR using experimental *in vivo* and *in vitro* models in relation to inflammation and oxidative stress. Partial goal was to establish validated analytical method for determination of BR and lumirubin.

Gunn and heterozygous rats were treated with lipopolysaccharide (LPS, 6 mg/kg, IP) or vehicle (saline). After 12 hours, blood and organs were collected for analyses of inflammatory and hepatic injury markers. Primary rat hepatocytes were treated with BR and TNF-α, HepG2 and SH-SY5Y cell lines were treated with BR and chenodeoxycholic acid. LPS-treated Gunn rats had a significantly decreased inflammatory response and hepatic injury compared to LPS-treated normobilirubinemic controls. We found different profile of leukocytes subsets and decreased systemic mRNA expressions and concentrations of IL-6, TNF-α, IL-1β and IL-10 in Gunn rats. Hepatic mRNA expression of LPS-binding protein was upregulated in Gunn rats before and after LPS treatment. In addition, activities of AST and ALT, markers of hepatocellular damage, were lower in Gunn rats as compared to LPS-treated controls. The exposure of primary hepatocytes to TNF-α resulted in the activation of the NF-κB pathway and phosphorylation of its p65 subunit, however, the degree of p65 phosphorylation was significantly decreased by BR. BR also reduced chenodeoxycholic acid-induced oxidative stress *in vitro*. A LC-MS/MS method for the simultaneous determination of lumirubin and BR was linear up to 400 μmol/L for BR and to 100 μmol/L for lumirubin with submicromolar limits of detection, with validity parameters relevant for use in clinical chemistry.

In conclusion, our results indicate that hyperbilirubinemia in Gunn rats is associated with an attenuated systemic inflammatory response and decreased liver damage upon exposure to LPS. Simultaneously, we confirmed the role of BR in protecting cells against oxidative stress induced by chenodeoxycholic acid. A LC-MS/MS method for the simultaneous determination of BR and lumirubin was established and validated.

Key words: bilirubin, hyperbilirubinemia, inflammation, LPS, NF-κB, oxidative stress, LC-MS/MS