Regulated intracellular bilirubin levels in cellular protection against oxidative stress.

A’edah Abu-Bakar and Matti A. Lang, National Research Centre for Environmental Toxicology (Entox), University of Queensland, FHS, Queensland, Australia

The bile pigment bilirubin (BR) is formed in mammals via the catabolism of haem, a reaction catalysed by haem oxygenase. Haemoglobin released from senescent red blood cells and haem containing enzymes, such as cytochrome P450 (CYP), are the major source of haem for bilirubin synthesis. Bilirubin is cytotoxic at concentrations above 20 µM but an effective antioxidant at concentrations range of ~ 0.01 to 10 µM. Therefore, BR may serve to protect cells against oxidative free radicals (ROS) only when its intracellular level is strictly regulated during oxidative stress conditions.

Normally, BR is conjugated in the liver with glucuronic acid to promote its elimination in the bile, and the UDP-glucuronosyltransferase 1A1 (UGT1A1) is solely responsible for its conjugation in the liver. When BR glucuronidation capacity is reduced, as in neonatal jaundice and in hereditary forms of congenital jaundice, cytochrome P450 (CYP) driven BR oxidation has been suggested to contribute significantly to the maintenance of BR homeostasis.

Our recent work indicated that the mouse CYP2A5 and its human form CYP2A6 metabolise BR to mainly biliverdin and smaller dipyrrroles (Figure 1). Importantly, CYP2A5-driven BR oxidation is heightened during elevated BR levels caused by metal-induced oxidative stress. Bilirubin at high concentration is also found to be an inducer of the CYP2A5 protein. Importantly, concurrent induction of the enzyme that produces BR, haem oxygenase, and oxidizes BR, CYP2A5, is associated with reduced occurrence of hepatic lipid peroxidation during metal-induce oxidative stress (Figure 2). Under this condition some of the bilirubin oxidative metabolites were detected in the urine. The significance of hepatic BR homeostasis regulation during oxidative stress conditions is discussed.

![Figure 1: HPLC/ESI-MS profile of bilirubin incubated with rCYP2A5/6. (A) Incubation at 0 h. (B) After 1 h incubation in the presence of rCYP2A5/6. (C) After 1 h incubation in the presence of wildtype microsomes (control). The various ions detected are indicated in the order of their elution time: ions m/z 301 (2.6 – 3.2 min); ions m/z 333 (3.4 – 3.6 min); ions m/z 315 (5.6 – 5.7 min); ions BV m/z 583 (5.8 – 6.0); and ions BR m/z 585 (9.3- 10.3).](image-url)
Figure 2: Time-course effects of arsenite treatment on hepatic levels of haem oxygenase-1 (HMOX1), cytochrome P450 2A5 (CYP2A5), total bilirubin (BR), and malondialdehyde (MDA), a measure of lipid peroxidation. Male DBA/2J mice were given a single i.p. dose of 40 μmol sodium arsenite / kg bw. The livers were excised at indicated time after treatment. *Means difference to control group $p < 0.05$.