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Transport deficient (TR$^-$) hyperbilirubinemic rats are resistant to acetaminophen hepatotoxicity

Vanessa M. Silva [a,1], Michael S. Thibodeau [a,2], Chuan Chen [b], José E. Manautou [a,*]

[a] *Toxicology Program, Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, 69 North Eagleville Road Unit 3092, Storrs, CT 06269-3092, USA*
[b] *Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS, USA*

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Abstract

The biliary excretion of acetaminophen (APAP) is reduced in transport deficient (TR$^-$) hyperbilirubinemic rats lacking the multidrug resistance-associated protein 2 (Mrp2). This mutant strain of Wistar rats has impaired biliary excretion of organic anions and increased hepatic glutathione. The rational for this study was to determine if there is an altered risk for liver damage by APAP in the absence of Mrp2. Therefore, the susceptibility of TR$^-$ rats to APAP hepatotoxicity was investigated. Male Wistar and TR$^-$ rats were fasted overnight before APAP treatment (1 g/kg). Hepatotoxicity was assessed 24 h later by plasma sorbitol dehydrogenase activity and histopathology. In other studies, TR$^-$ rats received buthionine sulfoximine before APAP to reduce hepatic glutathione to values similar to those in Wistar rats. mRNA expression of APAP metabolizing enzymes was also measured in naïve animals. Wistar rats treated with APAP showed significant elevations in plasma sorbitol dehydrogenase activity, while no increases in enzyme activity were observed in TR$^-$ rats. Histopathology was in agreement. Hepatic non-protein sulfhydryls were significantly lower in Wistar rats receiving APAP than in TR$^-$ rats. TR$^-$ rats treated with buthionine sulfoximine and APAP showed dramatic increases in hepatotoxicity. TR$^-$ rats had increased mRNA expression of several APAP metabolizing enzymes. Mrp2 expression not only is important in biliary excretion, but also influences the toxic potential of reactive intermediates by controlling intrahepatic GSH and possibly drug metabolism.

Keywords: Acetaminophen; Mrp2; TR$^-$ rat; Liver; Biliary; Hepatotoxicity

1. Introduction

Acetaminophen (APAP) and its conjugated metabolites can be found in urine and bile of mice and rats. A considerable amount of APAP is excreted in bile, mainly as the glutathione (APAP-GSH) and glucuronide (APAP-GLUC) conjugates [1,2]. Transport mechanisms for the hepatobiliary excretion of APAP and its metabolites are not completely understood. Recent studies indicate that the multidrug resistance-associated proteins 2 and 3 (Mrp2 and 3, ABCC2 and ABCC3, respectively) are involved in this process. The biliary excretion of APAP-GLUC and APAP-sulfate was reduced in isolated perfused livers from transport deficient (TR$^-$) rats [3]. This mutant strain of Wistar rats lacks expression of functional Mrp2 [4,5].

Mrp2 is involved in the biliary excretion of amphiphilic organic anions including non-bile acid organic anions, glucuronide and glutathione conjugates [6–9]. Excretion of these compounds into bile is impaired in TR$^-$ rats [10,11]. Our laboratory demonstrated that TR$^-$ rats receiving APAP have decreased biliary excretion of APAP-GSH, APAP-GLUC and APAP-*N*-acetylcysteine [1]. Our studies

Abbreviations: TR$^-$, transport deficient hyperbilirubinemic rats; Mrp, multidrug resistance-associated protein; APAP, acetaminophen; APAP-GSH, acetaminophen glutathione; APAP-GLUC, acetaminophen glucuronide; ICG, indocyanine green; NAPQI, *N*-acetyl-*p*-benzoquinoneimine; SDH, sorbitol dehydrogenase; NPSH, non-protein sulfhydryls; BSO, buthionine sulfoximine; GSH, glutathione; PBS, phosphate buffered saline; γ-GCS, gamma-glutamylcysteine synthetase; UGT, UDP-glucuronosyltransferase; CYP450, cytochrome P450

* Corresponding author. Tel.: +1 860 486 3852; fax: +1 860 486 5792.
E-mail address: jose.manautou@uconn.edu (J.E. Manautou).
[1] Present address: Procter and Gamble Company, 6110 Center Hill Avenue 1B17, Cincinnati, OH 45249, USA.
[2] Present address: Boehringer Ingelheim Pharmaceuticals Inc., Toxicology and Safety Assessment, 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT 06877, USA.

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also showed that Mrp2 deficiency results in increased urinary excretion of APAP-GLUC in TR$^-$ rats. This is most likely due to compensatory up-regulation of the basolateral efflux transporter Mrp3 in TR$^-$ rats [12]. Studies in Mrp3$^{-/-}$ mice conclusively demonstrated that the sinusoidal efflux transport of APAP-GLUC is highly dependent on Mrp3 function [13].

A key question not previously addressed is whether there is an altered risk for hepatic damage by APAP in the absence of Mrp2. Since the organic anion indocyanine green (ICG) not only produces changes in the biliary disposition of APAP similar to those seen in TR$^-$ rats [14], but also has a protective effect against its hepatotoxicity [15], we decided to investigate the susceptibility of TR$^-$ rats to APAP toxicity.

Hepatic GSH is important in the detoxification of the reactive metabolite of APAP, *N*-acetyl-*p*-benzoquinoneimine (NAPQI). GSH is transported into bile by a common transport-mediated process with organic anions [16]. TR$^-$ rats have increased hepatic GSH levels, demonstrating that Mrp2 is important for GSH transport into bile [17]. Although the higher GSH content in liver should make these mutant rats more resistant to APAP toxicity, the dramatic changes in hepatobiliary disposition of APAP in these rats makes it difficult to anticipate their ultimate response to a toxic dose of APAP.

The results of the present studies show that TR$^-$ rats are highly resistant to APAP toxicity. To investigate the role of higher hepatic GSH in this resistance, hepatic GSH content in TR$^-$ rats was normalized to values in naïve Wistar rats with the use of the GSH-depleting agent buthionine sulfoximine (BSO) prior to APAP administration. Changes in gene expression of several APAP metabolizing enzymes between strains of rats were investigated also.

2. Materials and methods

2.1. Reagents

4-Acetamidophenol (APAP), buthionine sulfoximine, glutathione, trichloroacetic acid, EDTA, 5,5′-dithiobis(2-nitrobenzoic acid), trizma hydrochloride, trizma base and β-nicotinamine adenine dinucleotide (reduced form) were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were of reagent grade or better.

2.2. Animals

Male Wistar rats were obtained from Charles River Laboratories (Wilmington, MA) and TR$^-$ rats were bred in our animal facilities. The mutational status of our TR$^-$ rats was confirmed in previous studies [18]. Weight-matched adult Wistar and TR$^-$ rats were used. Animals had free access to tap water and standard laboratory rodent diet. Lights were maintained on a 12:12 h light/dark cycle, and the room temperature was maintained at 22 °C. Animal studies were approved by the University of Connecticut Institutional Animal and Care Use Committee (Protocol No. A05-010).

2.3. Treatment of animals

Animals were fasted overnight prior to treatment. To elicit APAP-induced liver injury, Wistar and TR$^-$ rats received 1 g/kg APAP in 0.2% Gum Arabic, intraperitoneally (i.p.). Controls received vehicle only. Liver injury was determined 24 h later by plasma sorbitol dehydrogenase (SDH) activity and histopathology. Hepatic GSH levels were determined by the non-protein sulfhydryls (NPSH) assay. This dose of APAP was selected from pilot studies where fasted Wistar rats received doses of 750, 1000 or 1250 mg/kg. The dose of 1 g/kg was selected because it produced significant, but no overt liver toxicity or lethality.

To investigate the role of GSH in the susceptibility of TR$^-$ rats to APAP liver injury, BSO was used to modulate hepatic GSH content. Wistar and TR$^-$ rats were dosed with 0.89 g/kg of buthionine sulfoximine (BSO) in phosphate buffered saline (PBS), i.p. [19,20]. NPSH content was determined before and 3 h after BSO treatment. The goal was to decrease hepatic GSH levels in TR$^-$ rats by BSO treatment to levels in untreated Wistar rats.

To determine whether a reduction in hepatic GSH concentration by BSO treatment would increase the susceptibility of TR$^-$ rats to APAP toxicity, mutant rats were fasted overnight and dosed with 0.89 g BSO/kg or PBS vehicle 3 h prior to challenge with APAP (1 g/kg). Animals were sacrificed at 24 h for assessment of hepatotoxicity and hepatic NPSH analysis.

2.4. Biochemical assays

Plasma sorbitol dehydrogenase (SDH) activity was used as an indicator of hepatic injury. Briefly, rats were anesthetized with a combination of 100 mg ketamine/kg and 10 mg xylazine/kg, i.p., and blood was withdrawn from the abdominal aorta. Blood was collected in heparinized tubes and plasma SDH activity was measured following the procedure of Gerlach and Hiby [21].

Hepatic NPSH content was measured as an indicator of reduced glutathione (GSH). Liver samples were processed as previously described for NPSH quantification by the colorimetric procedure of Ellman [22,23]. NPSH content was determined by comparison to a GSH standard curve.

2.5. Histopathology

A piece of the left lateral lobe of the liver was fixed in 10% formalin and processed for histopathological examination as previously described [24,25]. Liver sections were scored using a scale from 0 to 5 depending on the severity of centrilobular necrosis and degeneration. Histopathology scoring was as follows: no injury = grade 0;