01B09-3

FUROSEMIDE HEPATOTOXICITY IN MICE: COMPARISON OF THE ROLE OF REACTIVE METABOLITES WITH ACETAMINOPHEN

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Acetaminophen (APAP) is widely used to induce hepatotoxicity in experimental animals, while other models of hepatotoxicity than APAP are needed to differentiate general events in drug-induced liver injury from specific events for APAP. In the present study, we developed hepatotoxicity by treatment of mice with furosemide (FS), and compared some events accompanied by the toxic responses to those with APAP. Overnight-fasted male CD-1 mice were given FS or APAP intraperitoneally. Hepatotoxicity was assessed by serum leakage of alanine aminotransferase (ALT). Glutathione (GSH) concentrations were determined by the dithionitrobenzoic acid-glutathione disulfide reductase recycling assay. Liver cytokine mRNA expression was assayed by real-time RT-PCR. Significant increase in serum ALT was observed after the administration of FS at a dose of 0.9 mmol/kg, which was slightly less than hepatotoxic dose of APAP. Diethylthiocarbamate and ketoconazole protected mice against FS-induced liver injury as well as those by APAP, suggesting that hepatotoxicity requires metabolic activation, mainly by CYP2E1 and CYP3A. Early depletion of hepatic GSH, which has been observed prior to initiation of hepatotoxicity by APAP, was not found in FS-treated mice, suggesting a difference in the chemical nature of reactive metabolites. Despite the lack of early GSH depletion, N-acetylcysteine, which alleviated the APAP-induced liver injury, also partially alleviated the FS hepatotoxicity. In both mouse models, liver expressions of TNF-α and IL-6 were induced during the development of toxicity. In conclusion, FS could be an alternative to APAP for developing metabolism-dependent hepatotoxicity, whereas pathophysiological roles of the reactive metabolites may be different between APAP and FS-induced toxicities.

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PHARMACOKINETICS OF BOSENTAN IN RATS WITH BILE DUCT LIGATION-INDUCED LIVER DYSFUNCTION

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Bosentan, an endothelin receptor antagonist, is metabolized by hepatic CYP proteins; however, OATP proteins are also involved in hepatic uptake of the drug. The purpose of this study was to evaluate mechanisms of the pharmacokinetic variability of bosentan using rats with 7-day bile duct ligation (BDL). Bosentan was administered intravenously at the constant rate (I) of 24, 40 or 60 μg/min/kg. Blood concentration of bosentan at steady-state (Cₐ) was measured by HPLC, and apparent clearance (CL_app) of the drug was estimated as I/Cₐ. The CL_app values in normal rats were 29.3 and 18.7 mL/min/kg at 24 and 60 μg/min/kg, respectively. The blood bosentan concentration in BDL rats was much higher than that in normal rats, and the CL_app values in BDL rats were 3.65 and 3.05 mL/min/kg at 24 and 60 μg/min/kg, respectively. BDL reduced hepatic mRNA expressions of CYP3A1 and CYP3A2 by 18.3% and 67.0%, respectively. On the other hand, hepatic mRNA expressions of Oatp1a1, Oatp1a4, and Oatp2b1 in BDL rats were decreased by 54.0%, 20.8%, and 64.0%, respectively. In addition, BDL caused 120-fold increases of plasma concentration of total bile acids. When taurocholic acid was administered concomitantly at the rate of 10 mg/min/kg, CL_app of bosentan (at I = 40 μg/min/kg) in normal rats was decreased by 81.1%. These results suggested that the decreased bosentan clearance in BDL rats was caused not only by reduced expression of the drug metabolizing enzyme, but also by impaired liver uptake of the drug.