MOUSE MODEL OF DICLOFENAC-INDUCED LIVER INJURY: ROLES OF CYTOCHROME P450 AND HEME OXYGENASE-1
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Diclofenac induces idiosyncratic liver injury. It has been proposed that covalent binding of reactive metabolites from hepatic cytochrome P450 (P450)-mediated oxidation and/or acyl glucuronidation to critical protein targets are involved in the toxicity. We have observed metabolism-dependent covalent binding of [14C]diclofenac to liver microsomes in rats and mice, and reduced glutathione (GSH) greatly diminished the binding only in mice. It is possible that the reactive metabolites bind to the P450 enzyme activating diclofenac in rats, but to other proteins associated with the toxicity in mice. In the present study, we developed a mouse model of diclofenac-induced liver injury and examine the role of P450 and heme oxygenase-1 (HO-1). Overnight-fasted male CD-1 mice were treated intraperitoneally with diclofenac (150-300 mg/kg) and liver injury was assessed 3-24 hr after the treatment by alanine aminotransferase (ALT) leakage. Mice given diclofenac exhibited a minor but a significant increase in serum ALT, revealing an induction of hepatotoxicity. Administration of SKF-525A or ketoconazole prior to diclofenac was ineffective in reducing toxicity. On the other hand, HO-1 was highly induced with the development of hepatotoxicity, and pretreatment of mice with hemin (50 µmol/kg), an inducer of HO-1, effectively prevented the diclofenac-induced liver injury. In conclusion, there is no evidence for P450-dependent toxicity in the present acute model, whereas inducible heme oxygenase may play a protective role against the exaggeration of hepatotoxicity.

DIFFERENT SUSCEPTIBILITY OF C57BL/6 AND BALB/C MICE TO ACETAMINOPHEN-INDUCED LIVER INJURY
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Besides metabolic activation and covalent binding of acetaminophen (APAP), subsequent events, such as inflammation and apoptosis, called stage II are suggested to be critical for the pathogenesis in APAP hepatotoxicity. It is proposed that one possible mechanism underlying the toxicity is disturbing balance of cytokines, which can be classified into Th1 and Th2 types. In the present study, we investigated APAP-induced liver injury in two mouse strains, C57BL/6 and BALB/c, which develop predominantly Th1 and Th2 responses, respectively. Male C57BL/6 and BALB/c mice were given intraperitoneally APAP (150mg/kg or 250mg/kg) and were killed 8 and 24 hr after the treatment. More severe liver injury assessed by serum alanine aminotransferase leakage was observed in C57BL/6 mice than BALB/c mice after APAP treatment. Basal contents and activities of CYP2E1, CYP1A2 and CYP3A11, major P450 enzymes responsible for APAP activation, were identical in both the mouse strains. There was no strain difference in consumption of hepatic glutathione during in vitro or in vivo metabolism of APAP, suggesting that metabolic activation/detoxification of APAP or subsequent APAP-protein adduct formation dose not account for different susceptibility of the two strains. On the other hand, APAP-induced increase in hepatic TNF-α expression was more prominent in C57BL/6 mice than BALB/c mice. It is therefore likely that APAP-induced liver injury is associated with Th1-dominant response in Th1/Th2 balance, and TNF-α may play a pathological role in the toxicity.