

Formation of reactive metabolites from a species-specific hepatotoxic pyrazolopyrimidine derivative, 5-*n*-butyl-7-(3,4,5-trimethoxybenzoylamino)pyrazolo[1,5-*a*]pyrimidine, in chimeric mice with humanized liver

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Purpose: 5-*n*-Butyl-7-(3,4,5-trimethoxybenzoylamino)pyrazolo[1,5-*a*]pyrimidine (OT-7100) is a pyrazolopyrimidine derivative with potential analgesic effects. In contrast to no toxicological potential to experimental animals, exclusively limited elevations in serum levels of aspartate- and alanine-aminotransferase were abnormally observed in a clinical study. The OT-7100 metabolites included hydrolysates of amide moiety (M-19 and M-5) and oxidative products of *n*-butyl group (M-2 and M-3). The primary metabolite M-5 is assumed to be further metabolized to M-22OH (a C-6-position hydroxyl metabolite) and M-23OH (a C-3-position hydroxyl metabolite). Differences in the metabolic function of human and rat cytochrome P450 1A2 would be responsible for the human-specific metabolic activation of primary metabolite M-5 of the parent compound OT-7100. The purpose of this study was to clarify possible mechanism of metabolic activation of OT-7100 through M-5 formation associated with the hepatotoxicity in humans using chimeric mice with humanized liver.

Methods: The covalent binding of reactive metabolites of ¹⁴C-M-5 to liver microsomes from humans and rats was measured in the presence of an NADPH-generating system. In addition, the covalent binding to liver protein and the metabolite profiles in plasma and liver were investigated after intravenous administration of ¹⁴C-M-5 to rats and the chimeric mice transplanted with human or rat hepatocytes.

Results: Human and rat liver microsomes were capable of activating M-5 to covalently bound metabolite(s). The covalent binding to liver proteins was inhibited dose-dependently by reduced glutathione, L-cysteine or *N*-acetylcysteine. After administration of ¹⁴C-M-5 to the chimeric mice, the covalent binding in liver was formed. However, there were few differences in amount and ratio of covalent binding between the chimeric mice transplanted with human or rat hepatocytes. The M-23OH metabolized from M-5 was formed in the liver of the chimeric mice with humanized liver similarly as in an *in vitro* study used human liver microsomes. In the chimeric mice transplanted with rat hepatocytes, M-23OH and M-22OH would be further metabolized in the liver, and the metabolite profiles in the plasma of the chimeric mice with rat hepatocytes were similar to those in the plasma in rat *in vivo* study.

Conclusions: The covalent bound metabolite produced by activating M-23OH was observed in an *in vitro* metabolism study and also an *in vivo* administration study. The hepatotoxicity associated with OT-7100 is most likely related to the formation of a reactive metabolite from M-23OH. The present chimeric mice model with humanized liver is considered to be useful for estimating and predicting *in vivo* metabolism in humans.