OS42-3 **Prediction of metabolism-dependent hepatotoxicity using human liver microsomes**

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Drug-induced liver injury (DILI) via metabolic activation by drug-metabolizing enzymes, especially cytochrome P450 (CYP), is a major cause of drug failure and drug withdrawal. In this study, an *in vitro* model using HepG2 cells in combination with human liver microsomes (HLM) was developed for prediction of DILI. The cytotoxicity of cyclophosphamide, a model drug for bioactivation, was augmented in HepG2 cells cultured with microsomes in a manner dependent on exposure time, microsomal protein concentration, and NADPH. Experiments using pan- or isoform-selective CYP inhibitors showed that CYP2B6 and CYP3A4 are responsible for bioactivation of cyclophosphamide. In a metabolite identification study employing LC-ESI-QTrap and LC-ESI-QTOF, cyclophosphamide metabolites including phosphoramide mustard, a toxic metabolite, were detected in HepG2 cells cultured with microsomes, but not without microsomes. The cytotoxic effects of acetaminophen and diclofenac were also potentiated by microsomes. The potentiation of acetaminophen cytotoxicity was dependent on CYP-dependent metabolism, and the augmentation of diclofenac cytotoxicity was not mediated by either CYP- or UDP-glucuronosyltransferase-dependent metabolism. The cytotoxic effects of leflunomide, nefazodone, and bakuchiol were attenuated by microsomes. The detoxication of leflunomide by microsomes was attributed to mainly CYP3A4-dependent metabolism. The protective effect of microsomes against nefazodone cytotoxicity was dependent on both CYP-mediated metabolism and nonspecific protein binding. Nonspecific protein binding but not CYP-dependent metabolism played a critical role in the attenuation of bakuchiol cytotoxicity. The present study suggests that HepG2 cells cultured with HLM can be a reliable model in which to predict DILI via bioactivation by drug metabolizing enzymes