Prediction of Drug-Induced Liver Injury Using Human Liver Microsomes

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Purpose
Drug-induced liver injury (DILI) has known as a major cause of both drug failures and drug withdrawal. Metabolic activation by drug metabolizing enzymes, especially cytochrome P450 (CYP), plays a critical role in DILI. In this study, an in vitro model using HepG2 cells in combination with pooled human liver microsomes (HLM) was developed for prediction of DILI.

Methods
Cytotoxicity induced by various chemicals such as cyclophosphamide (CPA), acetaminophen, diclofenac, leflunomide, nefazodone, bakuchiol, coumarin, and tolcapone was determined in HepG2 cells in the presence of HLM. To identify CYP isoforms involved in their metabolic activation/inactivation, 1-aminobenzotriazole (ABT), a potent non-specific CYP inhibitor and CYP isoform selective inhibitors were used. To determine effect of non-specific protein binding, chemical-induced cytotoxicity was measured in HepG2 cells cultured with HLM or heat-inactivated HLM. Moreover, metabolite identification of CPA was performed to compare metabolic profile in HepG2 cells cultured with or without HLM.

Results
Cytotoxicity of CPA, a model drug for bioactivation, was augmented in HepG2 cells cultured with HLM in an exposure time-, microsomal protein concentration- and NADPH-dependent manner. Experiments using pan or isoform-selective CYP inhibitors showed that CYP2B6 and CYP3A4 are responsible for bioactivation of CPA. In metabolite identification study employing LC-ESI-QTrap and LC-ESI-QTOF, dechloroethylcyclophosphamide, iminocyclophosphamide, 4-hydroxycyclophosphamide and phosphoramidemustard, a toxic metabolite of CPA, were detected in HepG2 cells cultured with HLM, but not without HLM. Acetaminophen- and diclofenac-induced cytotoxicity was also potentiated by HLM. 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-glucuronosyltransferases-dependent metabolism. Leflunomide-, nefazodone-, and bakuchiol-induced cytotoxicity was attenuated by HLM. The detoxification of leflunomide by HLM was attributed to CYP-dependent metabolism, especially CYP3A4. The protection against nefazodone cytotoxicity by HLM was mediated by both CYP-mediated metabolism and non-specific protein binding. Non-specific protein binding but not CYP-dependent metabolism played a critical role in the attenuation of bakuchiol cytotoxicity.

Conclusion
The present study suggests that HepG2 cells cultured with HLM can be a reliable model to predict DILI in the early drug discovery stage.