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# Human liver *in vitro* models for evaluation of drug metabolism and disposition

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av

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# Human liver *in vitro* models for evaluation of drug metabolism and disposition

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The administrated dose of a drug is adjusted to give a therapeutic effect in patients without causing side-effects or toxicity. Cytochrome P450 (P450) and UDP-glucuronosyltransferase (UGT) enzymes, uptake and efflux transporters and nuclear receptors regulating these enzymes, expressed in the liver and in other tissues, are all important players in drug metabolism, disposition and elimination. Many drugs are substrates and/or inhibitors of the same enzymes and may cause drug-drug interactions (DDIs) in a patient that takes several drugs at the same time, which can result in loss of therapeutic effect, side-effects or toxicity. The detection of major metabolites, reactive metabolites, metabolizing enzymes and transporter proteins for all new drug candidates is of high importance during preclinical evaluations. Reliable *in vitro* test systems of the human liver are essential for a complete and accurate preclinical evaluation of a new drug candidate. Primary human hepatocytes lose their hepatic functions within a few hours or days when maintained in suspension or cultured in two-dimensions (2D).

In this work, important hepatic functions were investigated in the human hepatoma cell line, HepaRG, and fresh human hepatocytes in suspension and in a dynamic three-dimensional (3D) bioreactor system. Fresh human hepatocytes cultured in 3D retained P450, UGT and OATP1B1 uptake activities for at least one week. Further, all major *in vivo* metabolites of AZD6610 and diclofenac were detected in “fresh” human hepatocytes after 6 days culture in 3D. Three P450 enzymes, CYP2J2, CYP4A11 and CYP4F3B, which are normally not involved in the metabolism of drugs, were identified to take part in the hydroxylation of AZD6610. Furthermore, the UGT activity was higher and the P450 and OATP1B1 activities were lower in HepaRG cells compared to primary human hepatocytes, for the model substrates evaluated in this study. The HepaRG cells maintained P450 activities for several weeks and UGT activities for at least one week in the bioreactor culture. Moreover, effects of rifampicin and ketoconazole on P450 activities in HepaRG cells cultured in the bioreactor predicted well the effects observed *in vivo*. The primary human hepatocytes and HepaRG cells were polarized in the bioreactor and formed tissue-like structures, which resembled the human liver tissue. In addition, the detection of glucuronides in the bioreactor medium indicated an active efflux of conjugated metabolites from 7 days old primary human hepatocytes cultured in the bioreactor back to the circulating medium. Knockdown of drug transporters in Caco-2 cells using short hairpin RNA (shRNA) was shown to be a valuable tool to understand potential sites of transporter-mediated pharmacokinetic interactions and the involvement of hepatic transporters in drug disposition. This model clearly showed the involvement of P-gp but not of MRP2 in the efflux of ximelagatran, hydroxy-melagatran and melagatran. The liver bioreactor using either fresh human hepatocytes or HepaRG cells retained biotransformation and transporter capacities for at least one week. This is a compelling feature of the 3D model, which open up for long-term cultures required for detection of metabolites from slowly metabolized drugs as well as induction, DDI and toxicity investigations.

**Keywords:** 3D, drug, HepaRG, hepatocytes, liver, metabolism, P450, transporter, UGT