

Cytochrome P450 Induction Studies in Human Hepatocytes

Purpose

The isoforms of the cytochrome P450 (CYP) family are the key enzymes of drug metabolism. Some of these enzymes, e. g. human CYP1A1, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1, or CYP3A4 are inducible. Induction of a CYP isoenzyme is one of the major reasons for drug-drug interactions, if the metabolism of one drug is increased by co-administration of another. To investigate the CYP induction potential of a drug candidate, primary cultured human hepatocytes (plateable cryopreserved or fresh) are exposed to the test compound over a three-day period and subsequently, enzyme activity of CYP isoforms is monitored.

Assay protocol

Cryopreserved human hepatocytes are plated on 24-well plates. After reattachment, cells were induced with either chemical inducers or test compounds at different concentrations, respectively, for 72 hours. Following the induction period, the hepatocytes were incubated with CYP-specific substrates outlined in Table 1. The samples were analyzed by LC/MS-MS and the formation of specific products catalyzed by the respective CYP isoenzymes was monitored.

Table 1: Chemical inducers and probe substrates for CYP induction

Isoenzyme	Chemical inducer	Enzyme reaction
CYP1A2	β -naphthoflavone	7-ethoxyresorufin-O-deethylation
CYP2C9	rifampicine	diclofenac-4'-hydroxylation
CYP2C19	rifampicine	S-mephenytoin-4-hydroxylation
CYP3A4	rifampicine	testosterone-6 β -hydroxylation

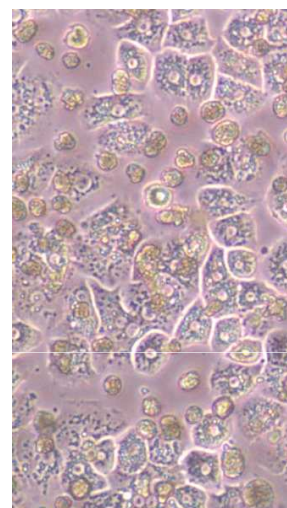


Figure 1:
Human hepatocytes

Results

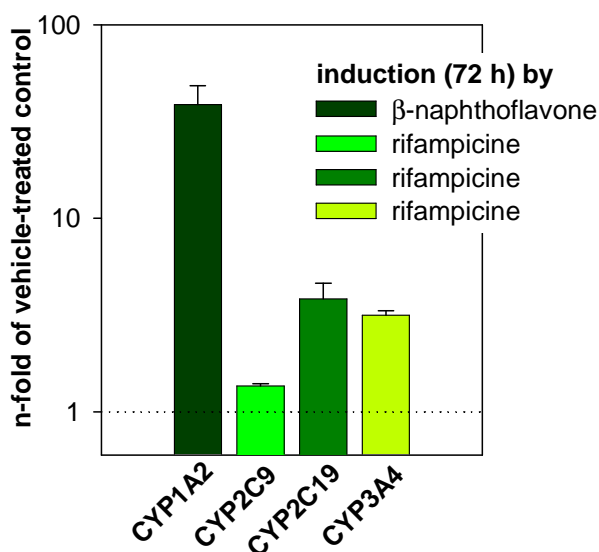


Figure 2: CYP induction in human hepatocytes

After induction with chemical inducers, enzyme activities of CYP1A2, CYP2C9, CYP2C19 and CYP3A4 in cryopreserved human hepatocytes were significantly increased in comparison to vehicle-treated controls.

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