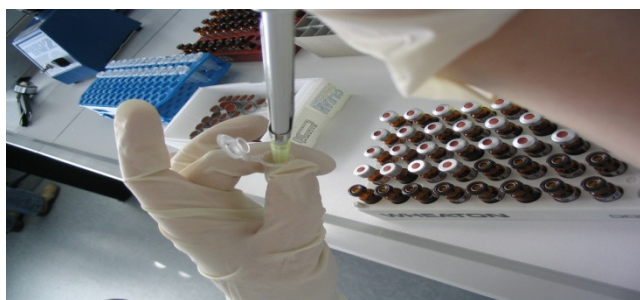


Cytochrome P450 Inhibition in Liver Microsomes

Purpose

The simultaneous administration of two different drugs can result in unwanted and potentially harmful drug-drug interactions. In most cases this effect is a result of drug-induced inhibition or induction of cytochrome P450 (CYP) enzymes. Since inhibition of CYP enzymes can have significant impact on the safety profile of co-administered drugs, the determination of the inhibitory potency of a new drug is important for assessing its potential to induce biologically significant drug-drug interactions in humans at therapeutic doses.



Assay protocol

Following a P450 inhibitor screening using recombinant CYP enzymes, for a more detailed and validated investigation on CYP inhibition, individual CYP isoforms are tested for their capability to convert specific probe substrates (outlined in **Table 1**) in presence or absence of the putative inhibitor.

As test system, human liver microsomes (HLM) are used. Specific metabolites are detected and quantified by LC/MS-MS. Compounds are tested at a single concentration (typically 10 μ M) or at multiple concentrations for determination of IC_{50} values.

Table 1: Probe substrates for specific CYP isoforms (further isoforms available upon request)

| isoform | enzyme reaction |
|---------|-------------------------------|
| CYP1A2 | phenacetin-O-deethylation |
| CYP2C9 | diclofenac-4'-hydroxylation |
| CYP2C19 | S-mephenytoin-4-hydroxylation |
| CYP2D6 | bufuralolol-1-hydroxylation |
| CYP3A4 | midazolam-1-hydroxylation |

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Results

In **Figure 1**, representative inhibition curves for CYP isoforms CYP1A2 (●), CYP2C9 (▼), CYP2C19 (■), CYP2D6 (◆), and CYP3A4 (▲) are plotted, using furaphylline, sulfaphenazole, tranlycypromine, quinidine and ketoconazole as prototypic inhibitors, respectively.

CYP1A2 was inhibited by furaphylline with an IC_{50} of 496 ± 29 nM, and sulfaphenazole resulted in an IC_{50} value of 277 ± 14 nM. For tranlycypromine, an IC_{50} of 9.4 ± 1.9 μ M was measured for CYP2C19 inhibition, whereas quinidine was a potent inhibitor of CYP2D6, leading to an IC_{50} of 74.2 ± 14.6 nM. For the CYP3A4 inhibitor ketoconazole an IC_{50} value of 21.7 ± 4 nM was determined.

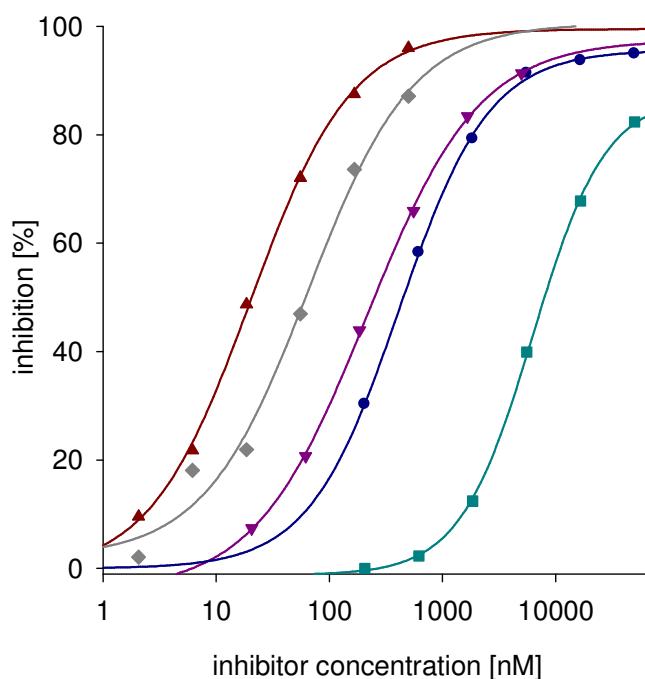


Figure 1: CYP inhibition in human liver microsomes

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