

The P450 Reaction Phenotyping:

- Profiling with Recombinant Human CYP's
- Knock-out Experiments with Human Liver Microsomes

Purpose

The cytochrome P450 phenotyping provides information about the involvement of the different human hepatic CYP450 isozymes in the metabolism of a test compound. This information is an important parameter of a new chemical entity (NCE) in the lead optimization process because it allows an early estimation of potential drug drug interactions (DDI) and potential pharmacokinetic variations by polymorphic enzymes. Using specific recombinantly expressed human CYP isozymes (from baculovirus transfected insect cells) allows the identification of the major drug metabolizing isozymes that biotransform the test item. In order to estimate the contribution of the particular major drug metabolizing isozymes to the total turnover of a test compound in liver, a selective "knock-out" of specific CYP isoenzymes present in human liver microsomes can be offered using specific diagnostic cytochrome P450 inhibitions.

Assay Protocol

Profiling: Incubation solutions are prepared containing NADPH, MgCl₂ and insect cell control Supersomes™ or Supersomes™ containing the human recombinant CYP isoenzyme 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 or 3A4 in phosphate buffer. The reaction is initiated by the addition of test compound after pre-incubation at 37°C. The incubation reaction is stopped by acetonitrile causing protein precipitation. The corresponding loss of parent compound is determined by LC-MS/MS in the supernatant.

Knock-out experiment: For each isoenzyme, buffered solutions are prepared containing NADPH, MgCl₂, human liver microsomes (HLM) and an isozyme-specific knock-out inhibitor. As a control the incubation is performed without knock-out inhibitor. The reaction is initiated by the addition of test compound after pre-incubation at 37°C. The reaction is stopped after incubation by acetonitrile causing protein precipitation. The corresponding loss of parent compound in presence of the different knock-out inhibitors is determined by LC-MS/MS in the supernatant and compared with the control.

Data analysis

The amount of test compound in the samples is expressed in percentage of remaining compound compared to the run performed with the control.



Table 1: Isozyme specific knock-out inhibitors in HLM

CYP Isoenzyme	Specific Inhibitor
CYP1A2	Furafylline
CYP2C9	Sulfaphenazole
CYP2C19	Tranylcypromine
CYP2D6	Quinidine
CYP3A4	Ketoconazole

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