

Determination of Cytochrome P450 Profile in Supersomes™, by Inhibition in Human Liver Microsomes, or Correlation Analysis using Human Liver Microsomes

1. Objective:

The determination of cytochrome P450 profile in liver microsomes or Supersomes™ is designed to provide information about the involvement of the different human CYP isoenzymes in the metabolic fate of a test compound. This information helps identifying possible problematic drug-drug interactions occurring in patients with multiple medications.

2. Introduction

Liver microsomes are subcellular fractions (mainly endoplasmatic reticulum) containing many drug-metabolizing enzymes, e.g. cytochrome P450s (CYPs), flavin-monooxygenases, carboxylesterases, and epoxide hydrolase. Therefore they are widely used as an *in vitro* model system in order to investigate the metabolic fate of xenobiotics. The most prominent group of drug metabolizing enzymes is the super family of cytochrome P450s (CYPs) These haem-containing enzymes play a key role in the metabolism (mainly oxidation) of a variety of chemically diverse compounds including food compounds, pharmaceutical agents, carcinogens, and environmental pollutants.

Human liver microsomes contain the following CYP isoenzymes involved in drug metabolism: CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4. Of these isoenzymes CYP3A4 plays a major role in metabolism of xenobiotics as it is the most abundant CYP in human liver (approx. 28 %) and it is involved in metabolism of more than 50 % of all pharmaceuticals applied in present-day medication.

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CYP-specific chemical inhibitors or specific inhibitory antibodies (multiple concentrations) are employed to block or decrease enzyme activities of a single CYP isoform. By comparing the metabolism rates (loss of parent compound or metabolite formation) of inhibitor treated human liver microsomes with that of untreated control human liver microsomes the role of each single CYP isoenzyme can be determined.

A different human liver microsomes based approach is correlating the enzyme activities of known CYP isoform specific marker reactions/substrates to metabolism rates (loss of parent compound or metabolite formation) of the test compound using several single donor microsomes instead of the usually employed pooled microsomes. If the test compound demonstrates the same metabolism pattern as a specific marker reaction, e.g. 7-ethoxyresorufin O-deethylation for CYP1A2, then an involvement of the respective CYP isoform in the metabolism of the test compound is very likely.

SupersomesTM (human recombinant enzymes) are microsomes prepared from insect cells infected by baculovirus and containing cDNA of a single human CYP isoenzyme. Therefore SupersomesTM have the advantage of unique specification by expressing enzyme activity of one single CYP isoform.

3. Short summary: Cytochrome P450 Profile Procedure

a) CYP Specific Chemical or Antibody Inhibition in Human Liver Microsomes

- Preparation of buffers and stock solutions of test compound (usually in DMSO or water)
- Preincubation of human liver microsomes with reference inhibitor or inhibitory antibodies for 5 to 10 minutes.
- Incubation of reaction mix including human liver microsomes, reference inhibitor, test compound, and NADPH for a single time points, e.g. 60 minutes.
- End of reactions by addition of stop reagent, sample preparation

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- Determination of metabolite formation and loss of parent compound (compared to zero time point control and/or no NADPH-control) using LC-MS methods.
- Results: Metabolism rates (loss of parent compound or metabolite formation) and comparison of inhibitor treated samples with untreated samples (= 100 % enzyme activity).

b) Correlation Analysis

- Preparation of buffers and stock solutions of test compound (usually in DMSO or water)
- Incubation of reaction mix including human liver microsomes (single donor), test compound or a marker substrate, and NADPH for a single time points, e.g. 60 minutes.
- End of reactions by addition of stop reagent, sample preparation
- Determination of metabolite formation and loss of parent compound (compared to zero time point control and/or no NADPH-control) using LC-MS methods and determination of marker reaction activities.
- Results: Metabolism rates (loss of parent compound or metabolite formation), marker reaction activities, and correlation analysis of these two activities.

c) Supersomes™

- Preparation of buffers and stock solutions of test compound (usually in DMSO or water)
- Incubation of reaction mix including Supersomes™ (human recombinant enzymes), test compound, and NADPH for a single time points, e.g. 60 minutes.
- End of reactions by addition of stop reagent, sample preparation

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- Determination of metabolite formation and loss of parent compound (compared to no NADPH-control) using LC-MS methods.
- Results: Metabolism rates (loss of parent compound or metabolite formation).

4. Marker Reactions

The following marker reactions, marker substrates, and reference inhibitors can be employed in the course of cytochrome P450 experiments:

CYP	Marker Substrate	Marker Reaction	Reference Inhibitor
1A2	7-ethoxyresorufin	7-ethoxyresorufin O-deethylation	furafylline
2A6	coumarin	coumarin 7 - hydroxylation	8-methoxypsoralene
2B6	S-mephenytoin	S-mephenytoin N-demethylation	triethylenethiophosphoramidate
2C8	paclitaxel	paclitaxel 6a-hydroxylation	ketoconazole
2C9	diclofenac	diclofenac 4'-hydroxylation	sulfaphenazole
2C19	S-mephenytoin	S-mephenytoin 4'-hydroxylation	omeprazole
2D6	bufuralol	bufuralol 1'-hydroxylation	quinidine
2E1	chlorzoxazone	chlorzoxazone 6-hydroxylation	diethyldithiocarbamic acid
3A4	testosterone	testosterone 6 β -hydroxylation	ketoconazole, troleandomycin

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