Determination of Enzyme Kinetic Parameters in Human Liver Microsomes o Supersomes™

1. Objective:

The determination of enzyme kinetic parameters in human 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.

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.

Supersomes™ (human recombinant enzymes) are microsomes prepared from insect cells infected by baculovirus and containing cDNA of a single human CYP

isoenzyme. Therefore Supersomes™ have the advantage of unique specification by expressing enzyme activity of one single CYP isoform.

The two most important enzyme kinetic parameters are:

- K_m Michaelis-Menten constant (concentration at which 50 % of the maximal velocity is observed)
- V_{max} maximum reaction velocity of enzyme

3. Short summary: Enzyme Kinetic Parameters Procedure

a) Human Liver Microsomes

- Preparation of buffers and stock solutions of test compound (usually in DMSO or water)
- Incubation of reaction mix including human liver microsomes, test compound (multiple concentrations, usually 8 to 10), 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.
- Results: Determination of turnover rates and calculation of enzyme kinetic parameters (K_m, V_{max}).

b) 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 (multiple concentrations, usually 8 to 10), 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.
- Results: Determination of turnover rates and calculation of enzyme kinetic parameters (K_m, V_{max}).

4. Marker Reactions

The following marker reactions and marker substrates can be employed as quality criteria of the metabolic capability of the microsomes or the Supersomes™:

CYP	Marker Substrate	Marker Reaction
1A2	7-ethoxyresorufin	7-ethoxyresorufin O-deethylation
2A6	coumarin	coumarin 7 - hydroxylation
2B6	S-mephenytoin	S-mephenytoin N-demethylation
2C8	paclitaxel	paclitaxel 6a-hydroxylation
2C9	diclofenac	diclofenac 4'-hydroxylation
2C19	S-mephenytoin	S-mephenytoin 4'-hydroxylation
2D6	bufuralol	bufuralol 1'-hydroxylation
2E1	chlorzoxazone	chlorzoxazone 6-hydroxylation
3A4	testosterone	testosterone 6ß-hydroxylation