Microsomal Protein Binding of Drugs

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Overview

• What is microsomal protein binding (MPB)?
• Why is measuring MPB important?
• Methods to measure MPB
• MPB study design
• Data example
What are microsomes?

• Microsome is a type of hepatic in vitro test system used to evaluate the drug interaction potential of an investigational drug.

• Microsomes are the test system in drug inhibition and drug metabolism studies.

• Formed from the endoplasmic reticulum when cells are disrupted and the homogenate is subjected to differential centrifugation.

• Contain CYP and UGT enzymes amongst others.

• Drug that is sequestered in microsomes in vitro is unavailable for direct interaction with metabolizing enzymes.
Preparation of microsomes from hepatocytes

Differential centrifugation
Overview

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Why measure microsomal protein binding?

1. 2020 FDA Guidance

Microsomal protein concentrations are usually less than 1 mg/mL. The sponsor should correct for nonspecific binding during the incubation if this binding is expected to influence the analysis of kinetic data. Nonspecific binding can be measured experimentally (e.g., using equilibrium dialysis or ultrafiltration) (Hallifax and Houston 2006) or predicted using in silico methods. It is recommended to experimentally determine nonspecific binding for highly lipophilic drugs (Gertz, Kilford, et al. 2008).

As per the FDA Guidance, measurement of MPB is required for Drug Inhibition studies, but not for Drug Metabolism studies

2. Better understanding of relationship between in vitro metabolism and in vivo pharmacokinetics
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Methods to determine MPB

Principle:
Physical separation and measurement of the unbound and bound drug to calculate their relative fractions.
Equilibrium Dialysis

Initial State → Equilibrium

Control

Drug

Test Sample

+ Microsomal protein
Rapid Equilibrium Dialysis (RED) Device

- Base plate has a 96-well footprint
- Holds 48 RED devices
- Dialysis membrane (MWCO 8K, 12K)
- Multiple sampling

Drawbacks:
- Non specific binding to membrane - underestimate fraction unbound
- Protein leakage - overestimate fraction unbound
- Volume shifts - dilute protein concentration.
Ultrafiltration

Separation of the protein and bound drug from free drug in solution occurs using a suitable membrane which retains the proteins and is assisted by positive pressure or centrifugation (2000 g).

**Advantage:**
Fast process

**Drawbacks:**
- Non specific binding to membrane—underestimate fraction unbound
- Molecular sieving (water from plasma passes through the membrane faster)—underestimate $F_u$
Ultracentrifugation

Spike test article in buffer containing microsomes
Centrifuge ~ 500,000 g for 5-6 hours at 37° C
Aliquot of supernatant
Determine levels by LC/MS

Advantage:
No non-specific binding

Disadvantages:
• Long process
• Cosedimentation of free and bound drug
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Study Design

Microsomal protein binding studies are offered as a stand-alone study or may be part of a drug inhibition study.

Selection of drug concentrations:

- Stand-alone: Client request
- Within drug inhibition protocol: Microsomal protein binding will be assessed at the IC$_{50}$ concentration with same human liver microsomal protein concentration as the IC$_{50}$ assay.

Impact of the microsomal binding will be discussed in the report.
Study Design

**Preliminary Assays**
- Stability (optional)
- Non-specific binding
- Time to reach equilibrium

**Microsomal protein binding Assay**
- Fraction unbound

**Human liver microsomes**
- Incubation time: 4h, 6h, 24h
- 1 Concentration

**CYP/UGT buffer**
- Incubation time: 4h, 6h, 24h
- 1 Concentration
Decision tree

Preliminary Assays

Stability ≥ 75%
Recovery ≥ 50%

- MPB assay with incubation for time required to attain equilibrium

Stability < 75%
Recovery < 50%

- Alternative method (cannot proceed with equilibrium dialysis i.e. RED device)
### Data example

<table>
<thead>
<tr>
<th>1 μM paroxetine with buffer Run</th>
<th>Buffer</th>
<th>Run</th>
<th>Fu</th>
<th>Mean Fu ± RSD (inter-day)</th>
<th>Binding (%)</th>
<th>Mean % bound ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0.1 mg/mL Human liver microsomes</strong>&lt;br&gt;CYP 1</td>
<td>1</td>
<td>0.476</td>
<td>0.456 ± 3.9</td>
<td>52.4</td>
<td>54.4 ± 3.3</td>
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<td>3</td>
<td>0.442</td>
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<tr>
<td><strong>1 mg/mL Human liver microsomes</strong>&lt;br&gt;CYP 1</td>
<td>1</td>
<td>0.066</td>
<td>0.0657 ± 5.85</td>
<td>93.4</td>
<td>93.4 ± 0.4</td>
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<td>0.069</td>
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<td>0.062</td>
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<td>93.8</td>
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<tr>
<td><strong>0.1 mg/mL Human liver microsomes</strong>&lt;br&gt;UGT 1</td>
<td>1</td>
<td>0.623</td>
<td>0.631 ± 4.5</td>
<td>37.7</td>
<td>36.9 ± 7.7</td>
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<tr>
<td><strong>1 mg/mL Human liver microsomes</strong>&lt;br&gt;UGT 1</td>
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<td>0.115</td>
<td>0.118 ± 2.2</td>
<td>88.5</td>
<td>88.2 ± 0.3</td>
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</table>
Summary of variables in microsomal protein binding studies

- **Protein:** Human liver microsomes
- **Microsomal concentrations:** 0.1 or 1 mg/mL
- **Buffer:** CYP or UGT
- **Positive control:** 1 µM paroxetine
For questions or further resources

Thank You!