Current Approaches and Applications of Phenotyping Methods for Drug Metabolizing Enzymes and Transporters

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Definition

Phenotyping is quantifying the *in vivo* activity of an enzyme or a transporter (E/T) mediating a *rate-limiting process* in pharmacokinetics.

(probably to be added: at a *specific site of expression*)
Dextromethorphan AUC as a Metric of Hepatic CYP2D6 Activity

(marginally simplified model of human body)
E/T Activity vs. PK Parameters

**Reasonable unit for activity:**
- amount of metabolite formed per unit of time per amount of enzyme at a given substrate concentration

**Examples:**
- nmol
- minute
- nmol of enzyme, g of liver, cm of jejunum, entire liver…
- Km, infinity (=Vmax), XX µmol/L

**What we optimally get is clearance:**
- volume of plasma cleared per unit of time per amount of enzyme at changing substrate concentrations (which are unknown at the binding site)

**Examples:**
- mL
- minute
- entire person
- ?

What we optimally get is clearance:
Current Approaches

Standard approach:
1. systemic (mostly oral) administration of a specific substrate of the E/T (often as a cocktail),
2. blood/plasma sampling for a **complete PK profile**, 
3. calculation of a metric reflecting systemic (or organ-specific?) E/T **activity** **and** at the same time **substrate exposure** (AUC in most cases).

Alternative substrates: endogenous/dietary compounds

Alternative sampling: less data points, different matrices including DBS

Alternative metrics: systemic clearance, partial clearance, intestinal extraction, renal clearance, renal secretion, metabolic ratios (in plasma, saliva, urine), single point concentrations…
Number of DDI Studies in clinicaltrials.gov
Received in the Last 2 Years

<table>
<thead>
<tr>
<th>Search terms: drug interaction + …</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>(none)</td>
<td>1137</td>
</tr>
<tr>
<td>Cocktail</td>
<td>18</td>
</tr>
</tbody>
</table>

### Individual CYP probes

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number (CYP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midazolam</td>
<td>39 (CYP3A4/5)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>19 (CYP1A2)</td>
</tr>
<tr>
<td>Tizanidine</td>
<td>0 (CYP1A2)</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>10 (CYP2D6)</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>5 (CYP2D6)</td>
</tr>
<tr>
<td>Desipramine</td>
<td>0 (CYP2D6)</td>
</tr>
</tbody>
</table>

### Individual Transporter probes

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number (Transporter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin</td>
<td>13 (P-gp)</td>
</tr>
<tr>
<td>Dabigatran etexilate</td>
<td>3 (intestinal P-gp)</td>
</tr>
<tr>
<td>Fexofenadin</td>
<td>2 (P-gp)</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>12 (OATP1B1&amp;3)</td>
</tr>
<tr>
<td>Pitavastatin</td>
<td>2 (OATP1B1)</td>
</tr>
<tr>
<td>Metformin</td>
<td>11 (OCT2, MATE1, MATE2-K)</td>
</tr>
</tbody>
</table>
Applications / Objectives of Phenotyping

- (Improve the understanding of pharmacokinetic processes)
- (Quantify the absolute activity of an E/T in an individual to personalize dosing)
- Assess the effect of potential covariates on E/T activity (mainly DDIs) to predict the effect on pharmacokinetics of E/T substrates
CYP3A and CYP1A2 Phenotyping to Individualize Erlotinib Treatment in NSCLC?

Day 1 (oral drugs):
- erlotinib 150 mg
- midazolam 2 mg
- caffeine 100 mg
- 6 samples until 6 hours postdose

For erlotinib and metabolite, 4 additional trough values during 10 weeks

→ ”phenotyping not suitable to substitute therapeutic drug monitoring”

Parra-Guillen ZP et al., Basic Clin Pharmacol Toxicol. 2017 Apr 26. [Epub ahead of print]
Applications / Objectives of Phenotyping

- (Improve the understanding of pharmacokinetic processes)
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Frequent Study Design in DDI Studies

perpetrator drug at highest chronic dose until steady state is reached

about 24 healthy volunteers

Average bioequivalence approach to estimate effect

Weak effect: 1.25-fold to 2-fold
Moderate effect: 2-fold to 5-fold
Strong effect: more than 5-fold
400-fold Mean AUC Range of Midazolam 15 mg Single Oral Dose

- ▲ 4 days 200 mg itraconazol o.d. (-2 hours to midazolam)
- △ 4 days after end of itraconazol
- ○ no co-medication
- □ 4 days after rifampicin
- ■ 5 days 600 mg rifampicin o.d. (-13 hours to midazolam)

DDI studies designed to see maximal effect size

### Some Established and/or Reasonable Phenotyping Methods

<table>
<thead>
<tr>
<th>Enzyme/transporter</th>
<th>target site of expression</th>
<th>Substrate</th>
<th>Dose</th>
<th>Route of administration</th>
<th>metric</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>liver</td>
<td>caffeine</td>
<td>50 - 150 mg</td>
<td>oral</td>
<td>AUC (F=1)</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>liver</td>
<td>bupropion</td>
<td>20 mg</td>
<td>oral</td>
<td>AUC (F&lt;0.9)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>liver</td>
<td>tolbutamide</td>
<td>125-500 mg</td>
<td>oral</td>
<td>AUC (F?)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>liver</td>
<td>flurbiprofen</td>
<td>10 mg</td>
<td>oral</td>
<td>AUC (F=0.8)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>liver</td>
<td>omeprazole</td>
<td>10 - 20 mg</td>
<td>oral</td>
<td>AUC (F=0.4)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>liver</td>
<td>dextromethorphan</td>
<td>7 – 22 mg</td>
<td>oral</td>
<td>AUC (F = 0.8 in PMs, 0.01-0.2 in EMs?)*</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>liver</td>
<td>midazolam</td>
<td>3 µg – 7.5 mg</td>
<td>i.v.</td>
<td>AUC</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>gut</td>
<td>midazolam</td>
<td>3 µg – 7.5 mg</td>
<td>oral (plus i.v.)</td>
<td>intestinal extraction</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>gut + liver</td>
<td>midazolam</td>
<td>3 µg – 7.5 mg</td>
<td>oral</td>
<td>AUC (F=0.3)</td>
</tr>
<tr>
<td>P-gp</td>
<td>kidney</td>
<td>digoxin</td>
<td>0.25-0.5 mg</td>
<td>oral</td>
<td>renal secretion</td>
</tr>
<tr>
<td>P-gp</td>
<td>gut + liver + kidney</td>
<td>digoxin</td>
<td>0.25-0.5 mg</td>
<td>oral</td>
<td>AUC (F= 0.7)</td>
</tr>
<tr>
<td>P-gp</td>
<td>gut + liver + kidney</td>
<td>fexofenadin</td>
<td>0.1-125 mg</td>
<td>oral</td>
<td>AUC (F = 0.3)</td>
</tr>
<tr>
<td>OATP1B1&amp;3</td>
<td>liver</td>
<td>rosuvastatin</td>
<td>25 µg-10 mg</td>
<td>oral</td>
<td>AUC (F=0.2)</td>
</tr>
<tr>
<td>OCT2, MATE1, MATE2-K</td>
<td>kidney</td>
<td>metformin</td>
<td>500 mg</td>
<td>oral</td>
<td>AUC (F=0.55)</td>
</tr>
</tbody>
</table>

„Oral Clearance“ – a Confusing Misnomer

Example:

(text) The main CYP enzyme and its contribution to the clearance of each model substrate is listed in Table 1.

Table 1: Cytochrome P450 (CYP) model substrates and the fraction of the dose metabolized by the enzyme that they mark (fm)

<table>
<thead>
<tr>
<th>CYP</th>
<th>Substrate</th>
<th>fm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Phenacetin</td>
<td>0.71</td>
</tr>
<tr>
<td>2B6</td>
<td>Bupropion</td>
<td>0.60</td>
</tr>
<tr>
<td>2C9</td>
<td>Tolbutamide</td>
<td>1.00</td>
</tr>
<tr>
<td>2C19</td>
<td>Omeprazole</td>
<td>0.76</td>
</tr>
<tr>
<td>2D6</td>
<td>Desipramine</td>
<td>0.91</td>
</tr>
<tr>
<td>3A4/5</td>
<td>Midazolam</td>
<td>0.87/0.1</td>
</tr>
<tr>
<td>3A4/5</td>
<td>Alprazolam</td>
<td>0.95/0.05</td>
</tr>
</tbody>
</table>

Barter ZE et al., Clinical Pharmacokinetics 2013, 52: 1085–1100
The Average Human Small Intestine and Hepatic CYP Pies (enzymes quantified by LC-MS/MS)

Regulatory Phenotyping Approach for DDIs

- Sensitive probe drugs should be used\(^a, b\)
- Key metric: AUC after oral administration\(^a, b\)
- Clearance through a specific pathway recommended if no specific substrate available\(^a\)
- If renal secretion is affected, renal clearance should be considered (e.g., digoxin for P-gp)\(^a\)

but…

Regulatory Phenotyping Approach for DDIs

- Sensitive probe drugs may not be representative for all respective drugs depending on E/T and contribution of first pass metabolism
- AUC depends on bioavailability (F); for many phenotyping drugs, F is low, highly variable or even unknown
- AUC reflects exposure but often is not very helpful for mechanistic considerations
- For transporter substrates, AUC typically reflects activity of multiple transporters at multiple sites
- Renal clearance consists of glomerular filtration plus renal secretion – it appears that using renal secretion would be more informative to assess transporter activity
Phenotyping Cocktails

Combined administration of phenotyping drugs with no (?) mutual interaction at low doses

Emphasis on CYPs (well established, more mechanism-related):
- Cologne: caffeine + tolbutamide + omeprazole + dextromethorphan + midazolam + digoxin (all orally) + midazolam (i.v.) (see poster Gazzaz et al. on ethanol effect)
- Basel: caffeine + efavirenz + losartan + omeprazole + metoprolol + midazolam (all orally)
- Geneva: caffeine + bupropion + flurbiprofen + omeprazole + dextromethorphan + midazolam + fexofenadine (all orally)
- CIME, Pittsburgh, Karolinska...

Emphasis on transporters (exploratory, more exposure-related):
- (Boehringer): digoxin + furosemide + metformin + rosuvastatin.
- (Merck): midazolam + dabigatran etexilate + pitavastatin + rosvastatin + atorvastatin (all orally)
- (Cologne): pitavastatin + metformin + adefovir dipivoxil + sitagliptin + digoxin (all orally) (see poster Stoffel et al. for potential mutual interactions)
In summary, the (cocktail) phenotyping approach is an established procedure only in the area of DDIs, but with a number of limitations to be addressed, including:

- 1: Lack of important PK information for many probe substrates
- 2: (extent of) intestinal metabolism
- 3: extent of hepatic first pass metabolism
- 4: „bottleneck“ property / specificity in all situations
- 5: temporal variability of covariates (mainly co-medication)
- 6: focused on exposure instead of E/T activity, thus results have limited predictive precision for other substrates