Structural Basis of Species Differences between Rat and Human CYP1A1s in Metabolism of Polychlorinated Biphenyls

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14 Feb. 2014
Cytochrome P450 (CYP) monooxygenases

- Monooxygenase reaction
- Endogenous and exogenous compounds as a substrate
- Broad and overlapping substrate specificity

Mammalian liver microsome

P450 reductase

\[ \text{RH} + \text{O}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{NADP}^+ \]

Critical roles in drug metabolism in mammals
Dioxin-like toxicity of PCBs

Persistent organic pollutants (POPs)
- Persistency in the environment
- Wide distribution
- Bioaccumulation through the food chain

Adverse effects to wildlife and human health by dioxin-like toxicity such as
- Carcinogenicity
- Hepatotoxicity
- Teratogenicity
- Mutagenicity

<table>
<thead>
<tr>
<th>Congener</th>
<th>TEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,3',4,4'-TeCB (#77)</td>
<td>0.0001</td>
</tr>
<tr>
<td>3,4,4',5-TeCB (#81)</td>
<td>0.0003</td>
</tr>
<tr>
<td>3,3',4,4',5-PeCB (#126)</td>
<td>0.1</td>
</tr>
<tr>
<td>3,3',4,4',5'-HxCB (#169)</td>
<td>0.03</td>
</tr>
<tr>
<td>2',3,4,4',5-PeCB (#123)</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3',4,4',5-PeCB (#118)</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4'-PeCB (#105)</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,4,4',5-PeCB (#114)</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3',4,4',5,5'-HxCB (#167)</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4',5-HxCB (#156)</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4',5'-HxCB (#157)</td>
<td>0.00003</td>
</tr>
<tr>
<td>2,3,3',4,4',5,5'-HpCB (#189)</td>
<td>0.00003</td>
</tr>
</tbody>
</table>

TEF; Toxic equivalency factor
WHO, 2005
Metabolism of PCBs in microsomal fractions of rat and human CYP1A1s

Reaction condition

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomal fraction containing rat or human CYP1A1</td>
<td>40 pmol</td>
</tr>
<tr>
<td>NADPH</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>G6P</td>
<td>5 mM</td>
</tr>
<tr>
<td>G6PDH</td>
<td>1 U</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>3.3 mM</td>
</tr>
<tr>
<td>Potassium phosphate buffer</td>
<td>100 mM</td>
</tr>
<tr>
<td>CB126, CB77</td>
<td>~2 ppm</td>
</tr>
<tr>
<td>37°C, 2-hour incubation</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

1. Addition of NADPH to start reaction
2. A reaction mixture without NADPH is used as a control.
3. Addition of $^{13}$C-OH-PCBs after stopping a reaction
4. Extraction of metabolites with hexane
5. Dryness and methylation
6. Quantification and identification of metabolites with high resolution GC/MS
Metabolism of CB126 by rat and human CYP1A1s

Tetrachlorobiphenyl

Pentachlorobiphenyl

Signal intensity ($\times 10^4$) vs. Retention time (min)

Rat CYP1A1 + NADPH

Rat CYP1A1 - NADPH

Human CYP1A1 + NADPH

Human CYP1A1 - NADPH

Standard

S1

S2

4'-OH-CB79

5-OH-CB66

4'-OH-CB127

4

1.6

0

3.2

31

32

33

Cl

Cl

Cl

Cl

Cl

Cl

OH

OH

Cl

Cl

Cl

Cl

Cl

Cl

Cl

Cl

OH

OH

4'

5

- OH

- OH

Metabolism of CB126 by rat and human CYP1A1s
F316 is conflicted with Y263, and then flipped to the side of substrate binding pocket. It results in a small volume of substrate binding pocket in rat CYP1A1.
Structures of amino acid residues consisting of a substrate binding pocket in mammalian CYP1A1s

<table>
<thead>
<tr>
<th>Number (rat)</th>
<th>Human</th>
<th>Rat</th>
<th>Dog</th>
<th>Golden hamster</th>
<th>Guinea pig</th>
<th>Monkey</th>
<th>Mouse</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A120</td>
<td>Ser</td>
<td>Ala</td>
<td>Thr</td>
<td>Thr</td>
<td>Ser</td>
<td>Ser</td>
<td>Thr</td>
<td>Thr</td>
</tr>
<tr>
<td>Y263</td>
<td>Tyr</td>
<td>Tyr</td>
<td>Tyr</td>
<td>Tyr</td>
<td>Ser</td>
<td>His</td>
<td>Tyr</td>
<td>Tyr</td>
</tr>
<tr>
<td>F316</td>
<td>Leu</td>
<td>Phe</td>
<td>Leu</td>
<td>Leu</td>
<td>Leu</td>
<td>Leu</td>
<td>Leu</td>
<td>Leu</td>
</tr>
</tbody>
</table>

The Y263 causing the conflict is well conserved. Both residues A120 and F316, seen in rat CYP1A1, are rare in other well-studied mammalian CYP1A1s.

These findings suggest that the cavity shape and catalytic activity of rat CYP1A1 may be unique among experimental animals.
Docking model of CB126 with rat and human CYP1A1s

Orientation

Rat CYP1A1
3 orientations

Human CYP1A1
5 orientations

CB126 is more stable in the cavity of rat CYP1A1 than that of human CYP1A1.

Distance from the iron of the heme (Fe-C4 ≤ 5 Å)

Rat CYP1A1
3 conformations

Human CYP1A1
0 conformations

CB126 is more accessible to the heme of rat CYP1A1 than that of human CYP1A1.
Species differences on metabolism of PCBs between human and rat CYP1A1s

The rat is not always a suitable animal for estimating effects in humans, such as dioxin toxicity and endocrine disruption, if they are metabolized by CYP1A1.