Interactions of Azo Dyes Commonly Used in Oral Drug Products with the Organic Anion Transporting Polypeptide 2B1 (OATP2B1) and Human Gut Bacteria

Ling Zou, PhD
Postdoctoral Scholar
University of California, San Francisco
Disclaimer

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Azo dyes are Found in Foods and Drug Products: FD&C Red No. 40

- Used in 35 drugs with active ingredients such as phenytoin sodium and valproic acid (CERSI Excipient Browser¹)

- Commonly used in food industry.

- Estimated daily intake per person is 25 mg², which equals to 220 µM in intestine.

¹. http://excipients.ucsf.bkslab.org/
Complex Interplay Among Azo Dyes, Transporters and Gut Bacteria

Some azo dyes commonly added in food and drug products appear to be inhibitors of intestinal influx transporter, OATP2B1, in vitro.

Azo dyes can be metabolized by human gut bacteria, ex vivo.

Do azo dye metabolites inhibit OATP2B1?

What is the rate and extent of metabolism of azo dyes by human gut bacteria?
Azo Dyes are Potent Inhibitors of OATP2B1

Ki = 2.59 µM
Ki = 68.4 µM
Ki = 11.3 µM
Ki = 58.1 µM

DBF: 4′,5′-Dibromofluorescein
HEK FlpIn cells

FD&C Red No. 40
FD&C Yellow No. 6
D&C Red No. 6
D&C Red No. 33

Screening concentration: 2 µM; Uptake time: 3 min
Metabolites of Azo Dyes are Weak Inhibitors of OATP2B1

FD&C Red No.40
$K_i = 2.59 \, \mu M$

Metabolite 1
$K_i > 50 \, \mu M$

Metabolite 2
$K_i > 200 \, \mu M$

<table>
<thead>
<tr>
<th>Excipients with Azo Group</th>
<th>$K_i$ (µM)</th>
<th>Reduced Metabolites</th>
<th>$K_i$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD&amp;C Red No. 40</td>
<td>2.59</td>
<td>1-amino-2-methoxy-5-methylbenzene-4-sulfonic acid</td>
<td>&gt; 50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-amino-6-hydroxy-2-naphthalenesulphonic acid</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>FD&amp;C Yellow No. 6</td>
<td>68.4</td>
<td>Sulfanilic acid</td>
<td>&gt; 200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-amino-6-hydroxy-2-naphthalenesulphonic acid</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>D&amp;C Red No. 6</td>
<td>11.3</td>
<td>4-amino-3-hydroxy-[2]naphthoic acid</td>
<td>&gt; 200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-Aminotoluene-3-sulfonic acid</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>D&amp;C Red No. 33</td>
<td>58.1</td>
<td>3,5-diamino-4-hydroxy-naphthalene-2,7-disulfonic acid</td>
<td>&gt; 50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aniline</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>
Azo dyes are metabolized by bacteria from human stool samples

**Agar plate**

D&C Red No. 33  
D&C Red No. 6  
FD&C Red No. 40  
FD&C Yellow No. 6

**Culture medium**

% Metabolism

D&C Red No. 33  
D&C Red No. 6  
FD&C Red No. 40  
FD&C Yellow No. 6
Variable Azo Dye Metabolism Mediated by 24 Bacteria Strains Isolated from Human Stool Samples

<table>
<thead>
<tr>
<th>Isolate</th>
<th>E1.1. Bifidobacterium longum subsp. longum</th>
<th>D1.2_Clostridium pseudobutyricum</th>
<th>A1.2_Faecalibacterium plaeformium</th>
<th>A1.3_Eubacterioides uniformis</th>
<th>A2.2_Bacteroides fragilis</th>
<th>A3.2_Bacteroides uniformis</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2.1. Bifidobacterium longum subsp. longum</td>
<td>E2.2. Bifidobacterium bifidum</td>
<td>D2.1_Clostridium butyricum</td>
<td>C1.1_Eubacterium cylindroides</td>
<td>C2.2_Clostridium butyricum</td>
<td>C3.2_Bifidobacterium adolescentis</td>
<td></td>
</tr>
<tr>
<td>D1.1_Collinsella aerocolonies</td>
<td>D2.2_Ruminococcus gnavus</td>
<td>D3.1_Bifidobacterium longum</td>
<td>C1.1_Eubacterium cylindroides</td>
<td>C2.2_Clostridium butyricum</td>
<td>C3.1_Giapia massiliensis</td>
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</tr>
<tr>
<td>E1.1. Faecalibacterium prausnitzii</td>
<td>E2.3_Eisenbergiella massiliensis</td>
<td>E2.4_Bifidobacterium longum subsp. longum</td>
<td>E1.1. Bifidobacterium longum subsp. longum</td>
<td>E3.2_Bifidobacterium subtilis</td>
<td>E3.3_Dorea longicatena</td>
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</tr>
<tr>
<td>F1.2_Coprococcus sp</td>
<td>G1.3_Collinsella aerocolonies</td>
<td>H1.2_Erysipelotrichaceae</td>
<td>H3.3_Ruminococcus obeum</td>
<td>H3.3_Ruminococcus obeum</td>
<td>H3.3_Ruminococcus obeum</td>
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</tbody>
</table>

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<thead>
<tr>
<th>Azo dye</th>
<th>E1.1. Bifidobacterium longum subsp. longum</th>
<th>D1.2_Clostridium pseudobutyricum</th>
<th>A1.2_Faecalibacterium plaeformium</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD.C.Red.No.40</td>
<td>FD.C.Yellow.No.6</td>
<td>D.C.Red.No.6</td>
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<tr>
<td>D.C.Red.No.33</td>
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</tbody>
</table>
Azo Dyes Inhibit OATP2B1 Transport Activity and Gut Bacteria May Modulate These Effects Through Azo Reduction

1. Azo dyes commonly added in food and drug products are inhibitors of intestinal influx transporter, OATP2B1.
2. Azo dye metabolites are much less potent inhibitors of OATP2B1 compared with azo dyes.
3. Bacteria isolated from human stool samples show different capabilities in metabolizing azo dyes, e.g., *F. pleomorphus* avidly reduces four azo dyes tested vs. *B. bifidum* has poor reductive capability.
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