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

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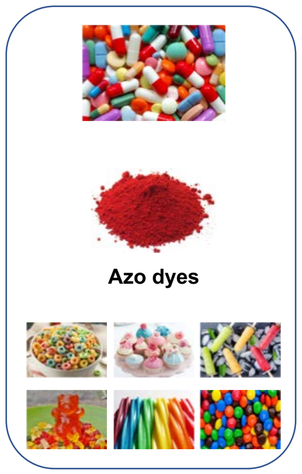
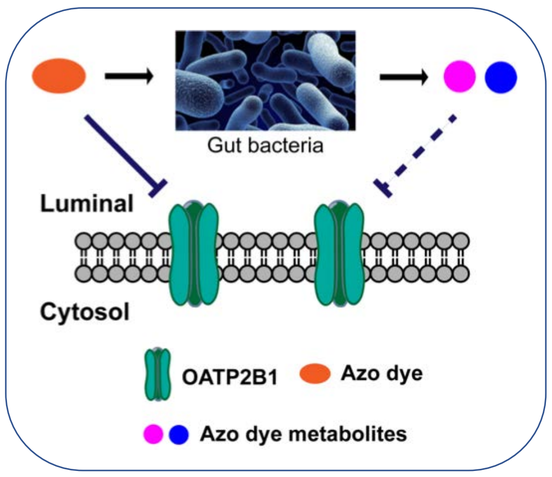
Introduction

1. Azo dyes are commonly added to food and drug products for improved esthetics.
2. The amount of azo dyes certified by the FDA increased 5-fold from 1955 to 2010 and intake may vary dramatically among individuals.
3. Azo dyes are metabolized by human gut bacteria.
4. OATP2B1, the intestinal influx transporter, is abundantly expressed along the GI tract and is important for the absorption of many drugs.
5. OATP2B1 can mediate drug-drug and drug-food interactions.
6. The complex interactions of azo dyes with OATP2B1 and gut bacteria are largely unknown.





Goal

Investigate the effects of azo dyes as inhibitors of the intestinal transporter, OATP2B1 and determine whether gut bacteria modulate these effects.

Methods

- A fluorescent assay using 4',5'-dibromofluorescein (DBF) as substrate was developed to assess inhibition of OATP2B1 transport activity by six orally used azo dyes and their metabolites in stably transfected HEK cells.
- Gut bacteria obtained from human stool samples were screened for azo dye metabolism (negative control: 100 μM dyes without metabolism) and individual bacterial species capable of reducing the dyes were identified by 16S rRNA gene sequencing.

Results

Figure 1. Establishment of screening assay and the identification of azo dyes as inhibitors of OATP2B1

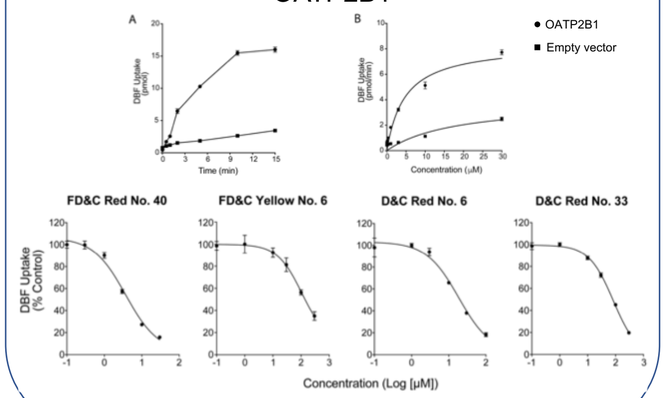


Table1. Azo dyes are more potent inhibitors of OATP2B1 than their metabolites.

Excipients with Azo Group	K_i (μM)	Reduced Metabolites	K_i (μM)
FD&C Red No. 40	2.59	1-amino-2-methoxy-5-methylbenzene-4-sulfonic acid	> 50
		5-amino-6-hydroxy-2-naphthalenesulphonic acid	> 200
FD&C Yellow No. 6	68.4	Sulfanilic acid	> 200
		5-amino-6-hydroxy-2-naphthalenesulphonic acid	> 200
D&C Red No. 6	11.3	4-amino-3-hydroxy-[2]naphthoic acid	> 200
		4-Aminotoluene-3-sulfonic acid	> 200
D&C Red No. 33	58.1	3,5-diamino-4-hydroxy-naphthalene-2,7-disulfonic acid	> 50
		Aniline	> 200

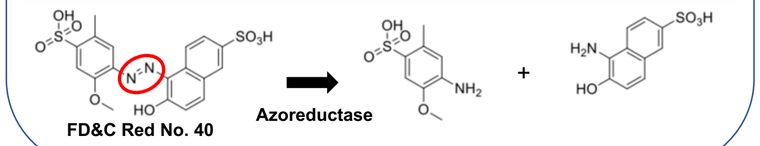


Figure 2. Azo dyes are metabolized by bacteria from human stool samples.

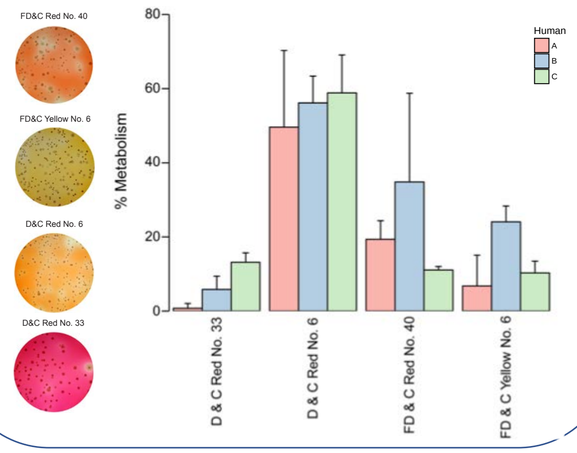
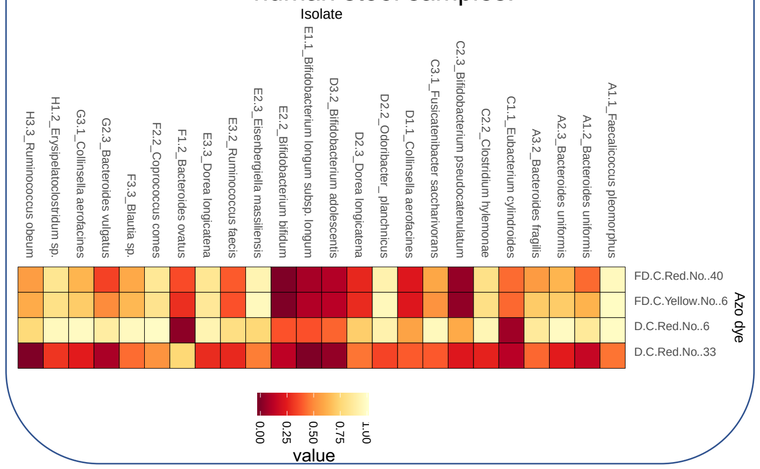


Figure 3. Differences in capabilities of azo dye metabolism among 24 bacteria strains isolated from human stool samples.



Conclusion

This study is the first to show that azo dyes have inhibitory effect on OATP2B1 in vitro, and that gut bacteria may alleviate the transporter inhibition through azo reduction. Future studies will focus on ascertaining the effect of these dyes on the oral absorption of OATP2B1 substrate drugs *in vivo.*

Acknowledgement

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