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Unraveling CYP3A Gut vs Liver Drug Metabolism and P-glycoprotein Activity

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BACKGROUND

More than half of marketed drugs are metabolized by the cytochrome P450 3A (CYP3A) enzyme. In most cases, CYP3A bio-transformation of a drug results in a more hydrophilic and less biologically active compound, which can be readily excreted [1]. Since various drugs also induce or inhibit CYP3A activity, understanding how an investigational product interacts with CYP3A as well as with other drugs, is an important step in getting a drug to the market.

CYP3A substrates (e.g. midazolam), inducers (e.g. rifampin[1], phenytoin, carbamazepine) and inhibitors (e.g. itraconazole) are commonly utilized in clinical drug-drug interaction (DDI) studies during the development of new drugs [2]. While midazolam holds the advantage of being both a sensitive and selective substrate for CYP3A, the latter does not apply for clinical index inducers and inhibitors, which are known to affect multiple CYPs or drug transporters **(Table 1)**. This may pose a challenge when attempting to elucidate complex DDI results involving multiple metabolic pathways. Moreover, CYP3A is expressed in liver and intestinal tissue, both of which contribute to first-pass metabolism. Variable expression and activity of CYP3A4 across tissues, differential effects of distinct CYP3A inducers, and involvement of multiple transcription factors in CYP3A4 expression can add to this complexity.

Table 1. CYP3A Index Substrates, Inducers and Inhibitors for DDI Studies

CYP3A Index Characteristic	Drug	Induction Pathway	Effect on other CYPs/Transporters
Substrate	Midazolam	-	None
Strong Inhibitor	Clarithromycin	-	P-gp inhibitor
	Itraconazole	-	P-gp inhibitor
Moderate Inhibitor	Erythromycin	-	P-gp inhibitor
	Fluconazole	-	Strong CYP2C19, moderate CYP2C9 inhibitor
	Verapamil	-	P-gp inhibitor
Strong Inducer	Rifampin[1] (no longer available for HV DDI studies)	PXR	• Strong CYP2C19, moderate CYP 1A2, CYP2B6, CYP2C8, CYP2C9 inducer • P-gp clinical inducer • OATPB1/3 inhibitor (single dose)
	Phenytoin	CAR, minor PXR	• Moderate CYP1A2, CYP2C19 inducer • P-gp inducer
	Carbamazepine	CAR, minor PXR	• Strong CYP2B6, weak CYP2C9 inducer • P-gp clinical inducer
Moderate Inhibitor	Efavirenz	CAR, minor PXR	Moderate CYP2B6 and CYP2C19 inducer

Adapted from FDA DDI table [2]. ***CAR****, constitutive androstane receptor;* ***HV****, healthy volunteer;* ***OATPB1/3****, organic anion transporting polypeptides B1/3;* ***P-gp****, P-glycoprotein;* ***PXR****, pregnane X receptor.*

[1]Rifampin is currently not available due to nitrosamine impurity risk – see References [3, 4] for more details.

DISCERNING GUT VS LIVER CYP3A ACTIVITY

By abundance, CYP3A represents approximately 80% of all CYPs expressed in the gut [5]. While CYP3A expression in the intestine is estimated to be only a fraction of that found in the liver, gut metabolism plays an important role for various drugs. For example, ciclosporin, midazolam, tacrolimus, nifedipine, felodipine and verapamil all demonstrate significant intestinal first-pass metabolism [6] Grapefruit juice is considered a selective intestinal CYP3A inhibitor since its effect is restricted to the gut. For example, consumption of grapefruit juice with IV administered cyclosporine, felodipine, or saquinavir had no effect on PK profiles, however when these drugs were orally co-administered with grapefruit juice intake, a significant increase in drug exposure was observed [6]. Grapefruit juice can in fact increase drug exposure by up to 1400% [7]. This elevation in drug PK can lead to adverse effects and, thus, patients are instructed to avoid grapefruit and other citrus juices when taking oral drugs that are CYP3A substrates. Grapefruit juice is thought to reversibly inhibit intestinal CYP3A as well as even decrease CYP3A protein concentration upon prolonged intake. It also interacts and inhibits uptake transports such as OATPB [7]. Therefore, the consumption of grapefruit juice with co-administration of an investigational product can provide valuable information about intestinal metabolism. In addition, administration of IV and oral midazolam in a two-part study, both before and after dosing with a study drug as perpetrator, can help assess the relative contribution of gut vs. liver to CYP3A induction or inhibition. For example, if an effect is observed upon IV midazolam co-administration, but not with the oral formulation, then the effect would be established at the level of the liver.

CYP3A AND P-GP SYNERGISTIC EFFECTS IN THE INTESTINE

CYP3A expression is regulated by nuclear receptors PXR and CAR, and strong clinical CYP3A induction drugs (e.g. rifampin, phenytoin and carbamazepine) stimulate CYP3A via these pathways **(Table 1)**. P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), is an efflux transporter that influences the absorption, distribution and excretion of several compounds, while its expression is also regulated by PXR, especially in the intestine [8]. The transporter is expressed on the apical membranes of enterocytes in the intestine, hepatocytes and kidney cells as well as endothelial cells in the brain. P-gp acts to regulate systemic exposure of substrates, since co-administration of a drug that inhibits or induces P-gp may increase or decrease systemic exposure of a P-gp substrate, respectively. Known P-gp substrates include digoxin, fexofenadine, dabigatran etexilate and talinolo. Interestingly, the magnitude of reduction in substrate exposure upon P-gp induction tends to be less than that typically observed upon CYP3A induction. For example, when rifampin was co-administered with dabigatran etexilate, a 67% reduction in the substrate exposure was noted, yet when rifampin was co-administered with a sensitive CYP3A substrate like midazolam, 90-95% exposure reduction was observed [8]. Moreover, rifampin is the most potent P-gp inducer compared to other DDI drugs. Phenytoin or carbamazepine co-administration with P-gp substrates tends to lower substrate exposure by 12-42%, while rifampin generally reduces P-gp substrate exposure by 20-67% [8]. In a multi-part or cocktail DDI study, a P-gp specific substrate can assist in distinguishing CYP3A vs P-gp effects. For example, dabigatran etexilate is a prodrug that is rapidly converted to dabigatran, which is an intestinal P-gp substrate [8]. Thus, changes in dabigatran exposure can serve as an "intestinal P-gp monitor" when co-administered with the study drug. Digoxin (P-gp substrate) and quinidine (P-gp inhibitor) can also be leveraged to explore P-gp function in combination with CYP3A probes and perpetrators in a cocktail or multi-part DDI study.

CONCLUSION

Intestinal CYP3A metabolism can play a critical role in drug absorption and distribution. Grapefruit juice consumption is a simple tool to distinguish a gut vs. liver effect. In addition, CYP3A vs P-gp effects can be dissected by leveraging multi-part or cocktail DDI studies to discern the contribution of each pathway. When first-pass metabolism leads to sub-optimal systemic drug concentrations, re-formulation may be needed to achieve appropriate concentrations. Here we described several approaches to elucidate the role of CYP3A intestinal drug metabolism that can be evaluated early in drug development to optimize program planning.

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