In Vitro Evaluation of Excipients as Inhibitors of Human Intestinal P-glycoprotein

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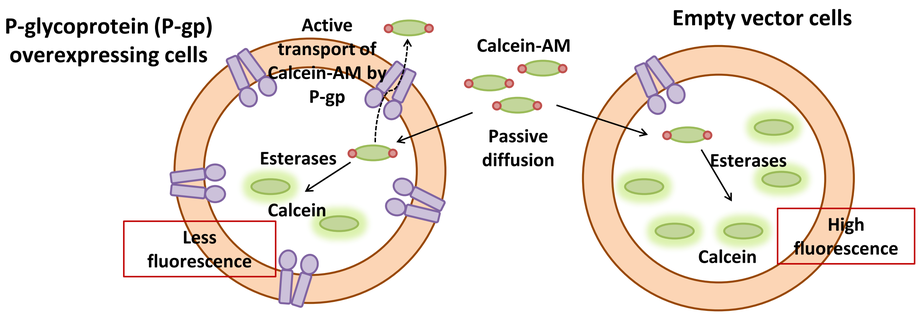
P-glycoprotein (P-gp) is one of the major barriers to oral bioavailability and contributes to the interindividual variability observed in intestinal absorption of P-gp substrates. A well characterized source of variable P-gp function in the intestine is inhibition by co-administered drugs. However, the potential interplay between intestinal P-gp and pharmaceutical excipients used in orally administered drug products has not been systematically studied and is the focus of this investigation. A fluorescence assay using calcein AM (5 μM), which served as a model substrate for P-gp, was used to assess inhibition of P-gp transport function by 130 excipients in HEK293 cells stably expressing human P-gp or transfected with an empty vector (control). Specific inhibition of P-gp-mediated calcein AM efflux by excipients was expressed as a ratio of inhibition observed in P-gp overexpressing cells over control cells. An inhibition screen was performed at an excipient concentration of 200 μM where possible, or at 50 μM where excipient solubility was limiting. Six potential inhibitors (inhibition ratio >1.3) were identified, including five dyes (D&C Red #6, D&C Brown#1, Naphthol blue black, Acid blue 9, Light green CF yellowish) and one suspending agent (Butylparaben). None of these potential inhibitors achieved >30% inhibition of calcein-AM efflux at concentrations up to 300 μM. Intestinal concentrations of these potential inhibitors estimated from the maximum amount of each excipient allowed per unit dose (http://excipients.ucsf.bkslab.org/) ranged from 0.50-2.3 μM, with the exception of light green CF yellowish (214 μM). While these in vitro findings indicate minimal inhibition of human P-gp at intestinal excipient concentrations typically expected from marketed drugs, further studies are needed to assess the impact of these excipients on the oral bioavailability of P-gp substrates.

Calcein-AM Assay for Measuring P-glycoprotein Function

Figure 1: Cell based Calcein-AM assay



Protocol

Seed cells at density of 0.8*10^6
↓
Wash 3X with PBS
↓
Add reagents: Calcein-AM, inhibitor in PBS
↓
Incubate at 37°C for 1 hr
↓
Wash 3X with PBS
↓
Measure fluorescence 492nm/535nm

Data Analysis

$$\text{P-gp+I/P-gp-I ratio} = \frac{\text{Fluorescence in P-gp cell line with potential inhibitor}}{\text{Fluorescence in P-gp cell line without potential inhibitor}}$$

$$\text{EV+I/EV-I ratio} = \frac{\text{Fluorescence in Empty Vector cell line with potential inhibitor}}{\text{Fluorescence in Empty Vector cell line without potential inhibitor}}$$

$$\text{(P-gp+/-)/(EV+/-) ratio} = \frac{\text{EV+I/EV-I ratio}}{\text{P-gp+I/P-gp-I ratio}}$$

Figure 2: Optimization of Calcein-AM assay with respect to concentration of Calcein-AM [A,B] and time [C,D]

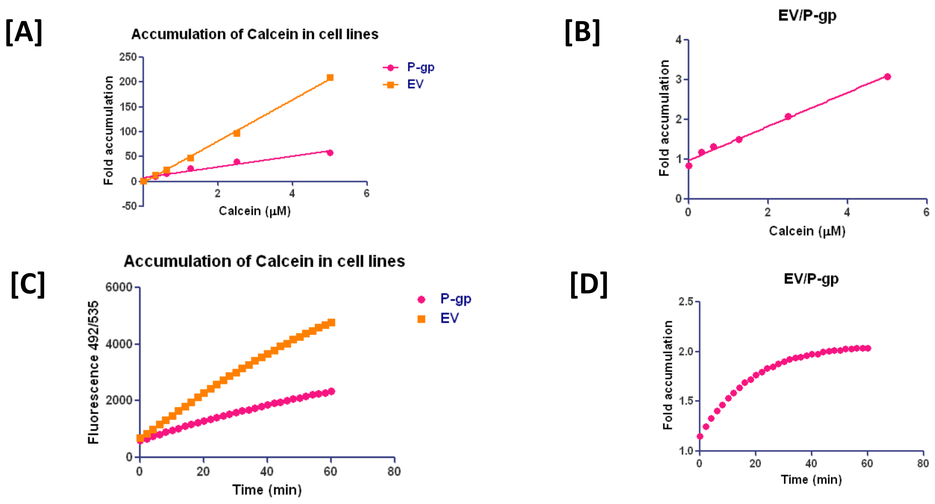
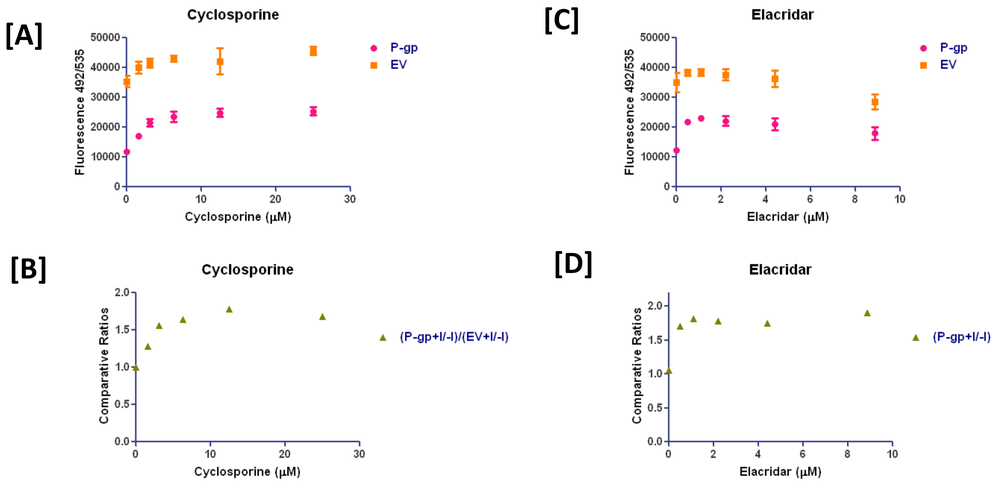


Figure 3: Calcein-AM assay is sensitive to known inhibitors



Screening of Oral Excipients for Inhibition of P-glycoprotein

Figure 4: Screening of oral excipients for inhibition of P-gp using Calcein-AM assay

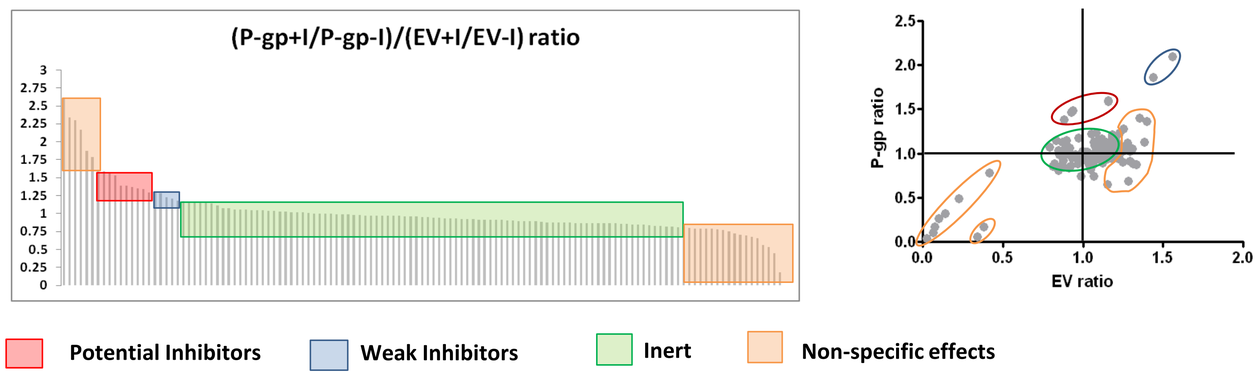


Figure 5: Excipients that inhibit Calcein-AM transport by P-gp cannot be classified based on [A] molecular weight, [B] LogP, [C] polar surface area, [D] number of hydrogen bond acceptors or [E] number of hydrogen bond donors

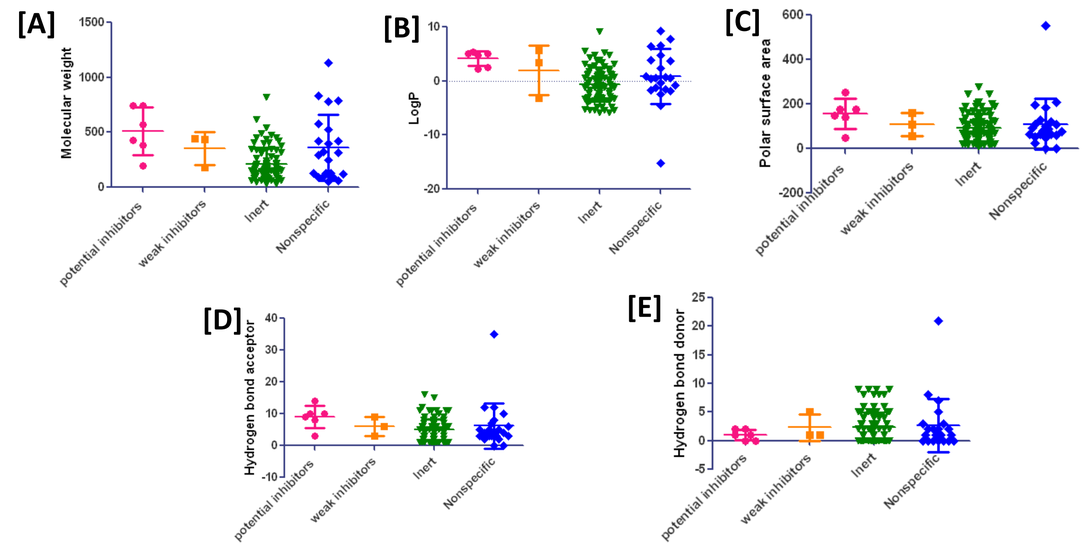


Figure 6: None of the potential inhibitors showed significant concentration-dependent inhibition of P-gp at concentrations up to 300 μM

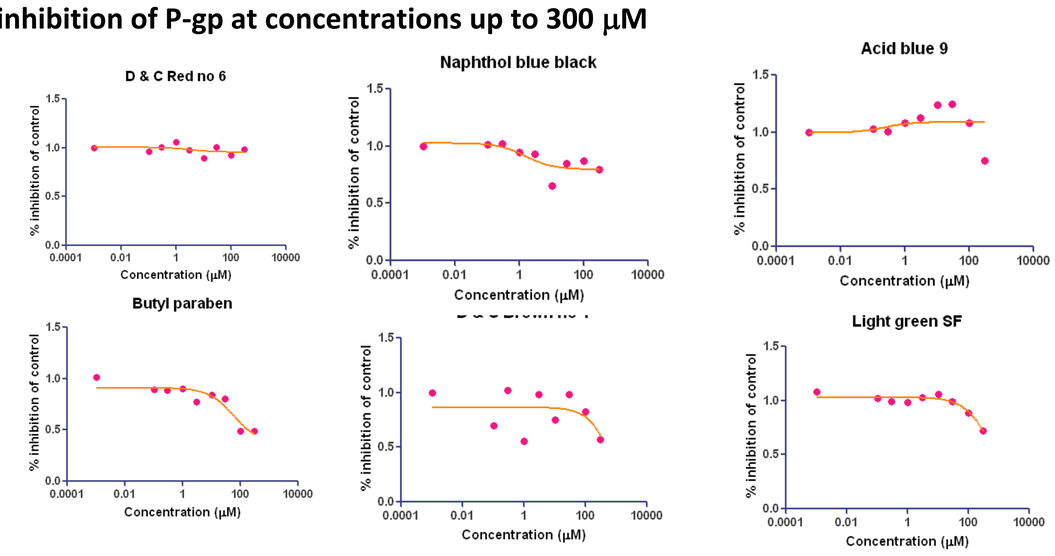


Table 1: Estimate of intestinal concentrations of potential inhibitors

Inactive ingredient	Route	Dosage form	Maximum potency per unit dose (mg)	Molecular weight (g/mole)	Intestinal concentration (mg/ml)	Intestinal Concentration (μM)
D&C Red no 6	Oral	Tablet	0.2	430	0.008	1.86
Acid blue 9 ammonium	Oral	Tablet	0.45	782	0.0018	2.30
Naphthol blue black	Oral	Tablet	0.08	616	0.00032	0.52
Light green CF yellowish	Oral	Tablet	40	749	0.16	213.62
Bytylparaben	Oral	Tablet	0.002	194	0.000008	0.04

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Conclusions and Future Directions:

- Oral excipients at concentrations recommended by the FDA are not significant inhibitors of P-gp.
- These results are being confirmed using digoxin flux in a MDCK-MDR cell line.