**ABSTRACT**

**Purpose:** To investigate the transport characteristics of several efflux transporters endogenously expressed in Caco-2 cells. **Methods:** The bidirectional permeability of digoxin, estrone-3-sulfate (E3S), and sulfasalazine were examined in Caco-2 and MDR-MDCK cells in the absence and presence of efflux transporter inhibitors: CsA (Pgp), FTC (BCRP) and MK571 (MRP). **Results:** Digoxin, E3S, and sulfasalazine exhibited different transport characteristics in Caco-2 and MDR-DMCK cells. Digoxin showed significant efflux ratio (ER) in both Caco-2 (ER = 17) and MDR-DMCK (ER = 120); whereas E3S and sulfasalazine only showed significant efflux in Caco-2 (ER = 15 and 88, respectively) but not in MDR-DMCK cells (ER = 1.1 and 1.3, respectively). The results demonstrated that E3S and sulfasalazine are subject to efflux mechanisms different from Pgp in Caco-2 cells. CsA at 10 μM showed complete inhibition of Dig efflux, and partial inhibition on E3S, but no effect on sulfasalazine. FTC and MK571 also resulted in different inhibitory effects on the efflux of the three compounds. **Conclusions:** The present study shows evidence for the functional expression of multiple efflux transporter systems in Caco-2 cells. The use of Caco-2 cells together with selected inhibitors of efflux transporters permit to obtain mechanistic information on drug-drug interaction with drug transporters under relevant physiological conditions.

**INTRODUCTION**

Caco-2 cells exhibit morphological characteristics of small intestinal cells (e.g., tight intercellular junctions and microvilli) and express P-glycoprotein (Pgp), multidrug-resistance associated proteins (MRPs), and breast-cancer resistance protein (BCRP). More importantly, in vitro permeability coefficient values obtained in the Caco-2 cell model generally exhibit a good correlation with in vivo fraction absorbed; thus Caco-2 cells have been widely used in pharmacological industry for drug screening purposes (1). Pgp is the best-understood efflux transporter. It is generally accepted that inhibition of this transporter (as a substrate, inhibitor, or inducer) can result in drug-drug interactions that could affect the pharmacokinetics and pharmacodynamics of the co-administered drugs. Therefore, the P-gp interaction potential of drug candidates needs to be evaluated as part of the drug development program. Observations indicate that some compounds undergo higher transport in the basolateral-to-apical direction (B-to-A) than in the apical-to-basolateral direction (A-to-B) in Caco-2 cells, whereas the same compounds did not exhibit such an asymmetric transport in MDR-MDCK cells. These results suggest that nortriptyline mediated transport was involved in transport of these compounds (2).

In the present study, three test compounds with different transport characteristics were selected: (1) digoxin; (2) estrone-3-sulfate (E3S); (3) sulfasalazine. We measured the bidirectional permeabilities of three selected test compounds in Caco-2 and MDR-MDCK cells in the absence and presence of cyclosporin A (CsA), furmitremorgine C (FTC) and MK571, which are inhibitors of Pgp, BCRP and MRP.

**METHODS**

**Cell Culture.** Caco-2 were obtained from American Type Culture Collection (Manassas, VA). MDR-MDCK cells were obtained from NIH (Bethesda, MD). All cells were maintained in high glucose (4.5 g/L) DMEM supplemented with 10% FBS, 1% NEAA, 1% L-glutamine, penicillin (100 U/mL), streptomycin (100 μg/mL) at 37°C in a humidified incubator with 5% CO₂. The culture media used for MDR-MDCK cells contained 80 mg/mL, colchicines (maintaining selective pressure).

**Transport Studies.** All cells were seeded at a density of 60,000 cells/cm² onto collagen-coated, microporous, polycarbonate membranes in 12-well Transwell® plates. The medium was changed every other day for certain period of time: three weeks for Caco-2 cells and six days for MDR-MDCK cells. The permeability assay buffer was Hanks’ Balanced Salt Solution containing 10 mM HEPES and 15 mM glucose at pH 7.4 (HBSSg buffer). The test compounds were prepared in HBSSg buffer to a final concentration of 10 μM each for digoxin and sulfasalazine, 10 μM estrone-3-sulfate (E3S), 10 μM furmitremorgine C (FTC), and 30 μM MK571 for 30 minutes and the same concentration of inhibitors was present in the assay buffer throughout the experiments. The transport experiment was conducted either in the apical to basolateral (A-to-B) direction or in the basolateral to apical (B-to-A) direction. At pre-selected time points, a 200-μL aliquot was taken from the insert and replaced with 200 μL of fresh buffer with or without inhibitor addition. All samples, except for [3H]-E3S, from the inserts and the wells were collected into 96 deep-well plate.

**LC/MS/MS Conditions.** All mass spectrometry was performed on a PE Sciex API300 triple quadrupole mass spectrometer in the multiple reaction monitoring mode using a turbo ionspray quadrupole mass spectrometer in the multiple reaction monitoring mode using a turbo ionspray quadrupole mass spectrometer. The Q1/Q3 settings are as follows: +798.6/391.5 for digoxin, and +398.9/381.1 for estrone-3-sulfate.

**RESULTS**

- In the absence of inhibitors, digoxin showed an efflux ratio of 10 in Caco-2 cells (Figure 1A). The presence of CsA completely abolished the polarized transport of digoxin, i.e., the efflux ratio was ~1. Similar results were obtained for the treatment with MK571. FTC also increased the absorptive transport (p<0.03) and decreased the secretory transport (p<0.01), but did not reduce the efflux ratio to ~1.

- In the absence of inhibitors, E3S showed an efflux ratio of 15 in Caco-2 cells (Figure 1B). CsA did not completely abolish the polarized transport of E3S. Both FTC and MK571 blocked the polarized transport of E3S, and the decrease in the secretory transport was very significant. Among all tested inhibitors, only FTC increased the absorptive transport significantly.

- Sulfasalazine exhibited a secretory transport 90 times greater than the absorptive transport in Caco-2 cells (Figure 1C). The presence of CsA did not affect either the absorptive or the secretory transport of sulfasalazine. FTC alone increased the absorptive transport and decreased the secretory transport of sulfasalazine. MK571 alone showed the inhibitory effect on the bidirectional transport of sulfasalazine in Caco-2 cells. Combination of FTC and MK571 completely eliminated the secretory transport of sulfasalazine.

**CONCLUSION**

- The current study provided functional evidence of existence of multiple efflux systems in Caco-2 cells.

- The presence of uptake systems in Caco-2 cells makes it a more relevant system to study human intestinal absorption.

- Together with different chemical inhibitors and other genetically modified cell lines, bidirectional permeability results obtained in Caco-2 cells may provide valuable mechanistic information on intestinal drug transporters.

**References**