System-Dependent Inhibition of BCRP Transport by High- and Low-Permeable Compounds in Bi-Directional MDCKII Cells and Membrane Vesicles

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Introduction

Breast cancer resistance protein (BCRP, *ABCG2*) is an efflux transporter that blocks absorption at the apical membrane of enterocytes in the intestine, blood-testis barrier, blood–brain barrier, mammary gland and other cells. At the apical membranes of hepatocytes and renal proximal tubule cells, BCRP enhances excretion and elimination of xenobiotics.[1] Inhibition of BCRP by drugs or xenobiotics has the potential to cause pharmacokinetic drug-drug interactions whereby increasing the exposure of co-administered compounds typically excreted from cells by BCRP. For example, inhibition of BCRP in the intestine upon oral administration of GF120918 caused a 2.4-fold increase in the AUC of topotecan, a BCRP substrate.[2]

Therefore, the recent FDA and EMA Guidance recommends evaluation of BCRP inhibition by new drug candidates,[3] which is typically conducted in one of two test systems: 1) Bi-directional cell-based transport assays (e.g. MDCKII-BCRP cells) or 2) BCRP-expressing inverted membrane vesicles. In the case of cell-based assays, BCRP function is evaluated by measuring active efflux across the cell monolayer. Inasmuch as the substrate binding site(s) is intracellular or in the cell leaflet, the compound has to cross lipid membranes to interact with the transporter. Conversely, BCRP function in inverted membrane vesicles is evaluated by monitoring the uptake of a probe substrate into the vesicles. Compounds do not have to cross a lipid membrane inasmuch as the substrate binding domain is located on the outside of the vesicle and is exposed to free drug available in the incubation medium. Because of this difference in substrate or inhibitor exposure to the substrate binding domain of BCRP, compounds with various intrinsic permeabilities may cause test system-dependent inhibition of BCRP in vitro.

In this study, we evaluated the system-dependent effects of both a high- and low-permeability compound on BCRP-mediated efflux, namely Ko143 and sulfasalazine, in MDCKII-BCRP cells and BCRP-expressing membrane vesicles. Ko143 is a potent and specific inhibitor of BCRP with moderate permeability (predicted $logD_{7.4}$ >2 and total polar surface area (TPSA) of ~98 Å^2) as referenced in **Table 1**. Sulfasalazine, an anti-inflammatory agent, was reported to cause potent inhibition of BCRP transport (IC_{50} ~ 1 µM) in BCRP-expressing oocytes.[4] However, sulfasalazine, with a predicted $logD_{7.4}$ ~ 0 and TPSA of >140 Å^2 (**Table 1**), is poorly absorbed with limited permeability across physiological membranes. We hypothesize that, due to limited membrane permeability, sulfasalazine would cause significantly less inhibition of BCRP transport in cell-based assays.

Materials and Methods

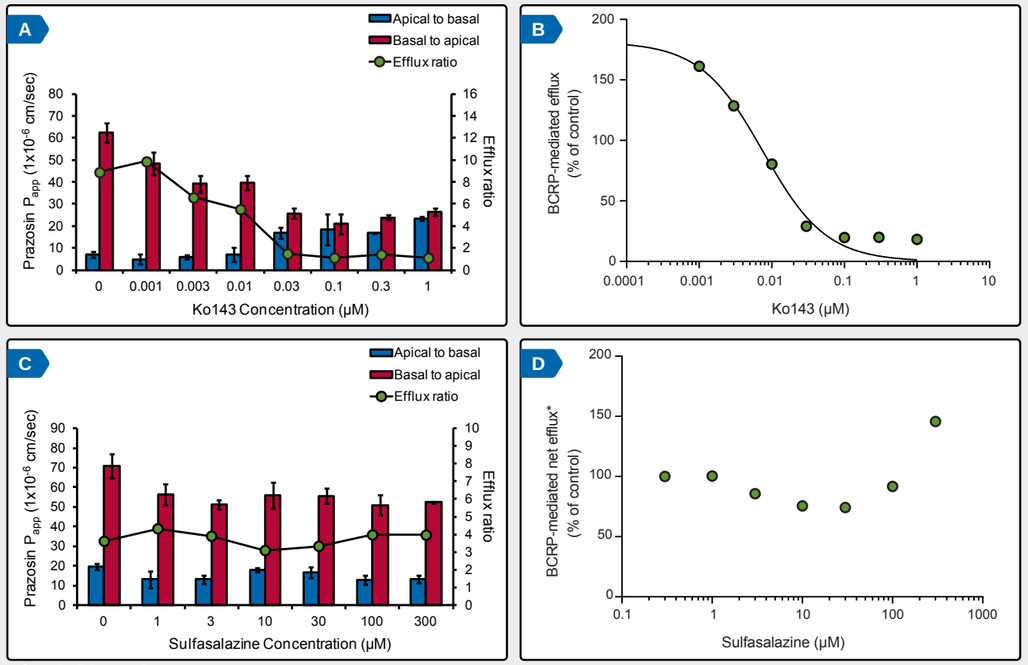
MDCKII-BCRP cells were obtained from The Netherlands Cancer Institute. BCRP-expressing inverted membrane vesicles were obtained from Solvo Biotechnologies (Budaors, Hungary). Sulfasalazine, Ko143 and Prazosin were obtained from Sigma (St. Louis, MO, USA). [^{3}H]Estrone 3- sulfate (E3S) was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA, USA).

The bidirectional permeability of prazosin across MDCKII-BCRP and control cells was measured in the presence of Ko143 (0.001 - 1 µM) and sulfasalazine (1 - 300 µM) in triplicate in transwell plates (Corning Life Sciences, Lowell, MA, USA). Culture media was removed and incubation media (HBSS supplemented with 25 mM HEPES and 25 mM glucose) was added to the cells at 37 ± 2 °C. TEER values were recorded approximately ten min after the incubation media was added and then the cells were warmed to 37 ± 2 °C for 30 – 60 minutes. After the preincubation, incubation media with prazosin (1 µM final concentration) containing the solvent control or inhibitor were added to the donor or receiver chambers. Aliquots (20 µL) were taken from the donor chambers at zero minutes and the end of the incubation and from the receiver chambers (100 µL) at the end of the incubation (120 min). Samples containing prazosin were mixed with internal standard and analyzed by LC-MS/MS. P_{app}, efflux ratios and net efflux ratios (efflux ratio on transporter expressing cells ÷ efflux ratio on control cells) were determined for prazosin.

BCRP activity was evaluated in inverted membrane vesicles according to the manufacturer's protocol. Briefly, the accumulation of E3S in BCRP expressing vesicles was measured in the presence of Ko143 (0.001 - 1 µM) and sulfasalazine (0.01 – 100 µM) with Mg-ATP or Mg-AMP in duplicate (n = 2). The membrane vesicles were diluted in incubation media and added to the incubation plate (39 µL). The inhibitor or solvent control was added to the membrane vesicles at 1% of final reaction volume and preincubated (37 ± 1 ºC) for 15 minutes. The reaction was initiated by the addition of 10.5 µL of incubation media containing the substrate and Mg-ATP (4 mM) or Mg-AMP (4 mM) and incubated for one minute. Reactions were stopped by the addition of ice-cold washing mix (typically 200 µL) and transferred to a filter plate and filtered. The filter plate was washed five times with 200 µL of ice-cold washing-mix. After the filter plate was dried, the amount of substrate inside the filtered vesicles was determined by adding an aliquot of scintillation fluid to the plate and counting with a liquid scintillation counter.

Tables and Figures

Figure 1. Inhibition of Prazosin efflux across BCRP-expressing MDCKII cells in the presence of Ko143 and Sulfasalazine



*Control cell data not shown

Figure 2. Inhibition of Estrone 3-sulfate uptake into BCRP-expressing membrane vesicles in the presence of Ko143 and Sulfasalazine

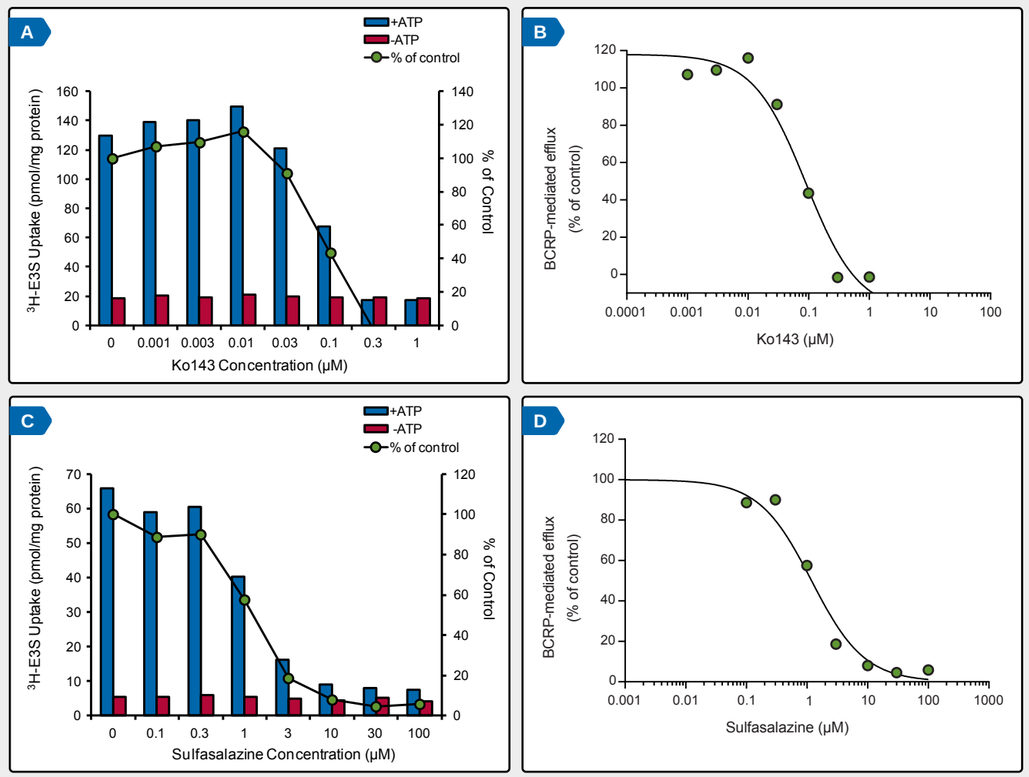
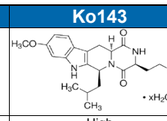
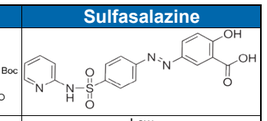


Table 1. Summary of physiochemical properties and inhibition parameters of Ko143 and Sulfasalazine in in vitro test systems

Compound Properties	Ko143	Sulfasalazine
Chemical structure¥		
Permeability	High	Low
$logD_{7.4}$†	>2	~0
Total Polar Surface Area†	~98 Å^2	>140 Å^2
IC_{50} (MDCKII cells)	0.012 ± 0.003 µM	>300 µM
IC_{50} (membrane vesicles)	0.089 ± 0.012 µM	1.2 ± 0.1 µM

¥: Chemical structures were referenced from the vendor (Sigma, St. Louis, MO, USA).
†: Reported values were referenced from ChemSpider database.

Results

- **Figure 1 (A and B)** illustrates the inhibition of prazosin efflux across BCRP-expressing MDCKII cells in the presence of Ko143. The highly permeable compound, Ko143, inhibited BCRP-mediated efflux in BCRP-transfected MDCKII cells with an IC_{50} value of 0.012 µM **(Table 1)**.
- **Figure 1 (C and D)** illustrates the inhibition of prazosin efflux across BCRP-expressing MDCKII cells in the presence of sulfasalazine. In this case, the poorly permeable compound did not inhibit BCRP-mediated prazosin efflux in BCRP-transfected MDCKII cells (tested up to 300 µM).
- **Figure 2 (A and B)** illustrates the inhibition of E3S uptake into BCRP-expressing membrane vesicles in the presence of Ko143. The highly permeable compound, Ko143, inhibited BCRP-mediated uptake of E3S into BCRP-expressing membrane vesicles with an IC_{50} value of 0.09 µM **(Table 1)**. These results were similar to those reported above for inhibition of BCRP in MDCKII cells.
- **Figure 2 (C and D)** illustrates the inhibition of E3S uptake into BCRP-expressing membrane vesicles in the presence of sulfasalazine. The poorly permeable compound, sulfasalazine, inhibited BCRP-mediated uptake of E3S into BCRP-expressing membrane vesicles with an IC_{50} value of 1.2 µM **(Table 1)**. These results were markedly different than those reported above for inhibition of BCRP in MDKCII cells.
- Sulfasalazine caused potent inhibition of BCRP in membrane vesicles (IC_{50} 1.2 µM) but little to no inhibition of BCRP in MDCKII cells. Thus, sulfasalazine was identified as a system-dependent inhibitor of BCRP.

Conclusions

- The highly permeable compound, Ko143, inhibited BCRP-mediated efflux in BCRP transfected MDCKII cells and membrane vesicles with similar potency.
- Sulfasalazine was identified as a system-dependent inhibitor of BCRP inasmuch as it did not inhibit BCRP-mediated prazosin efflux in BCRP-transfected MDCKII cells but caused potent inhibition of BCRP in membrane vesicles.
- Compounds with high or low permeability may exhibit system-dependent transporter inhibition with common in vitro test systems.
- Therefore, it is imperative to consider the expression system and the physiochemical properties of the compound when evaluating the inhibition of efflux transporters.

References

1. Konig J et al. (2013) *Pharmacol Rev.* **65**: 944-966.
2. Zhang L et al. (2011) *Clin Pharmacol Ther.* **89**: 481-484.
3. [EMA] European Medicines Agency. EMA/CHMP/EWP/125211/2012—Guideline on the Investigation of Drug Interactions, Committee for Human Medicinal Products (CHMP), London, England.
4. Elsby R et al. (2011) *Eur J Pharm Sci.* **43**: 41-49.