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False Negatives May Occur in BCRP Substrate Assessments Depending on the In Vitro Assay System Used

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Abstract

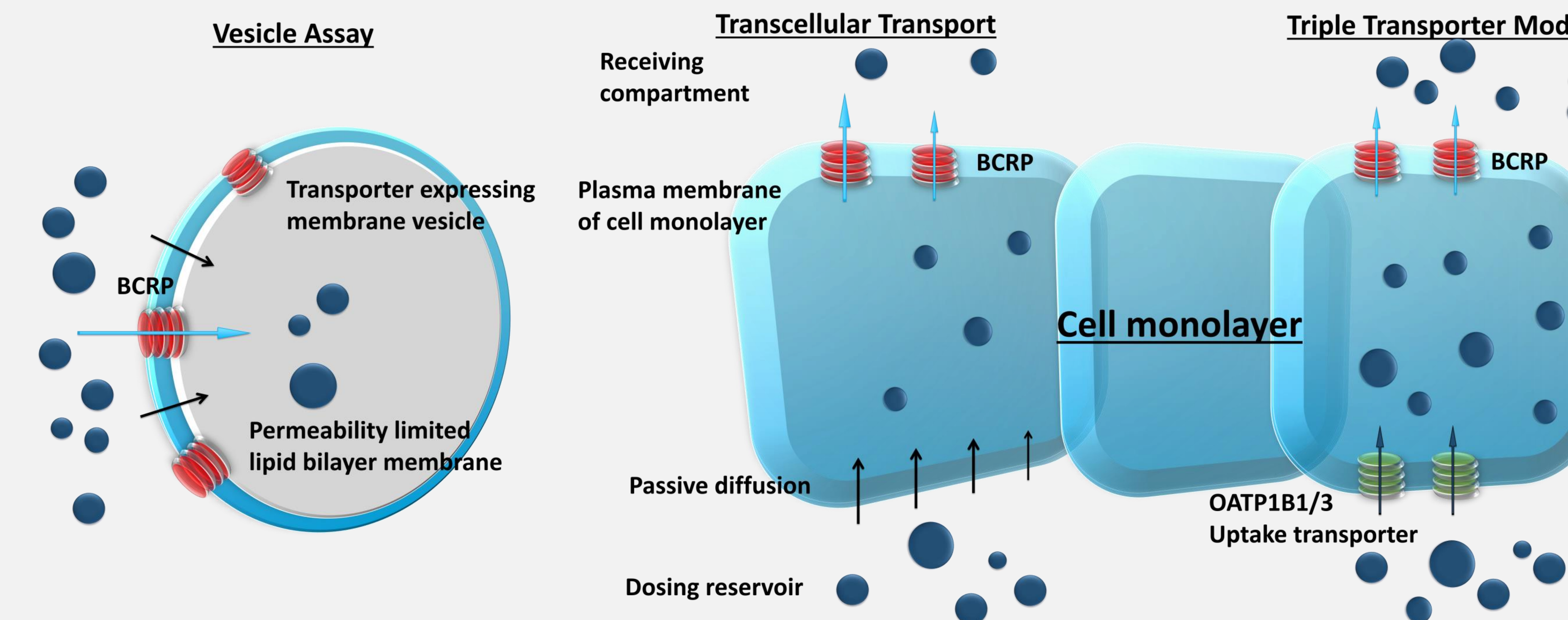
Purpose: BCRP (Breast Cancer Resistant Protein) is one of the major drug efflux transporters involved in drug disposition in the liver and at the blood brain barrier. Therefore, characterization of compounds as potential BCRP substrates or inhibitors is recommended by regulatory agencies for all clinical-stage drugs. Traditionally, BCRP activity has been measured using bidirectional transport assays in polarized cells overexpressing BCRP or in uptake assays with BCRP overexpressing membrane vesicles. However, false negatives were observed for some drugs tested in different systems. To elucidate the mechanism for such inconsistency, membrane vesicles and MDCK cells transfected with BCRP along with relevant uptake transporters were compared to evaluate BCRP-mediated drug disposition.

Methods: Radioisotope-labeled BCRP substrates were incubated with BCRP vesicles +/- ATP. MDCK monolayers cultured on transmembrane inserts were transiently-transfected with plasmids encoding BCRP alone, a combination of OATP1B1 and OATP1B3 transporters, or a combination of OATP1B1, OATP1B3, and BCRP. Uptake rates were measured for vesicle assays, and transcellular flux and intracellular accumulation were measured for the B->A transcellular assays.

Results: Some compounds (e.g., CCK-8) were substrates in vesicle assays, but did not appear to be substrates in cell-based assays transfected with only BCRP (cell transport rate <0.2 pmole/min/cm^2). Others (e.g., genistein) appeared to be substrates in cell-based assays, but not in vesicle assays due to high membrane permeability (~7 pmole/min/cm2). The most consistent results were obtained by comparing transport rates of cells with OATP1B1+OATP1B3 *vs.* OATP1B1+OATP1B3+BCRP, in which the true BCRP activity could be determined by introducing sufficient intracellular accumulation (>5 pmole/cm^2) to allow measurement of BCRP-dependent transcellular flux.

Conclusion: BCRP vesicle assays work well only for poorly permeable compounds, while cells transfected with only BCRP are most useful for highly permeable compounds, as these compounds do not depend on uptake transporters to gain entry to BCRP. Importantly, to avoid false negative results, BCRP-transfected cells should be co-transfected with basolateral uptake transporters, allowing drugs with low permeability to sufficiently accumulate in the intracellular space.

Introduction to screening BCRP substrate

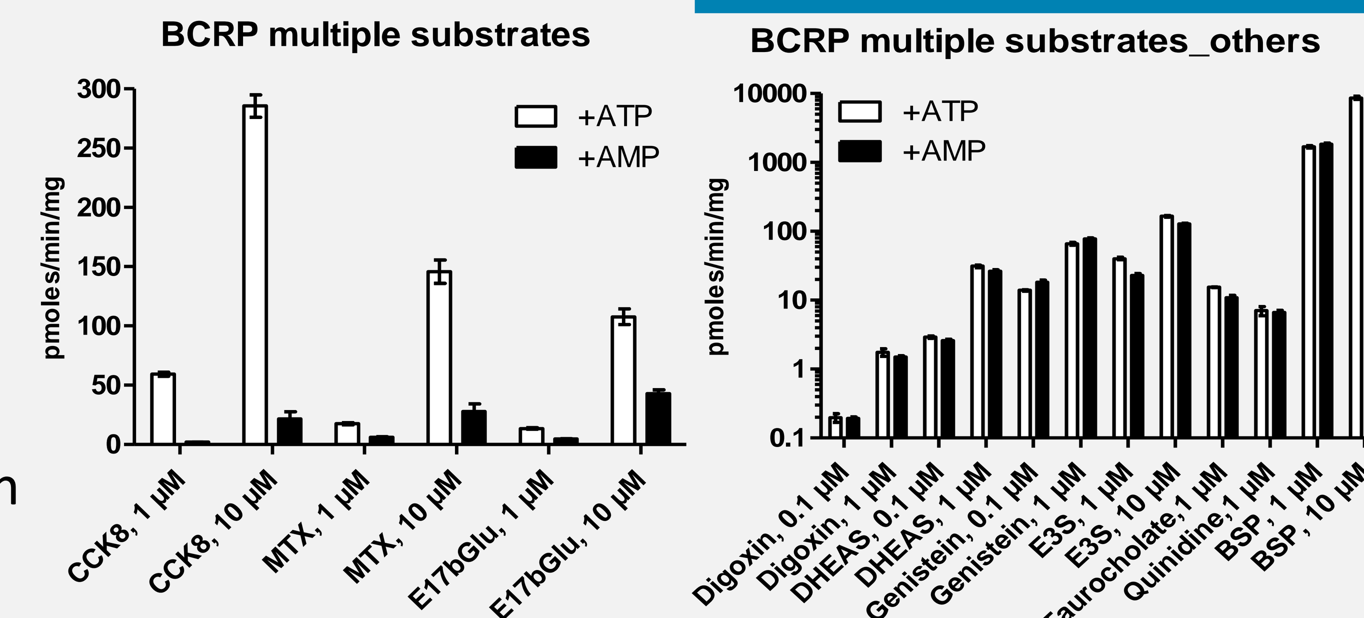


1) BCRP overexpressing vesicles are not suitable for screening highly permeable compounds
2) BCRP-only overexpressing cells may not be suitable for screening compounds with low permeability as they may not reach sufficient cellular concentration to allow BCRP efflux

Methods

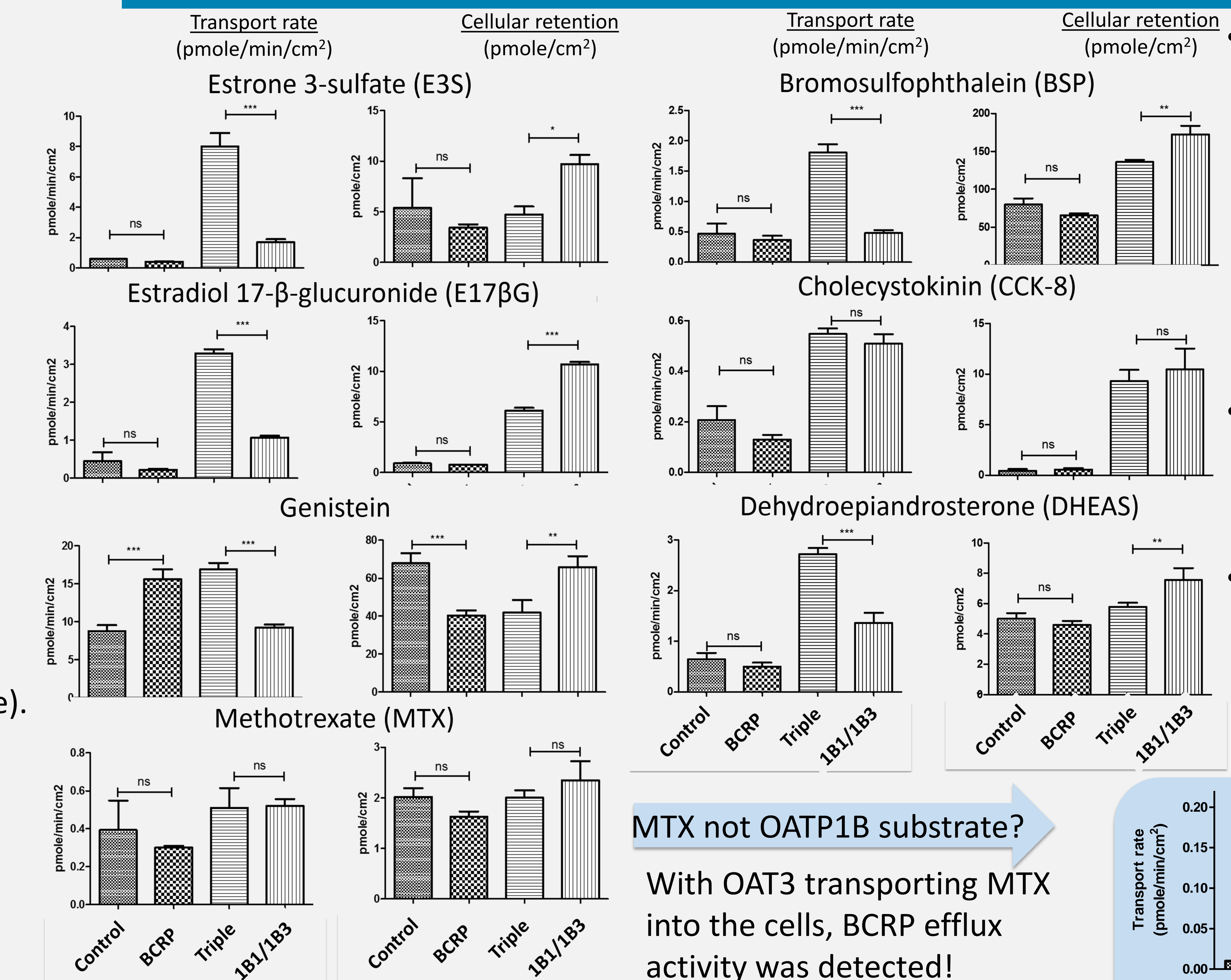
1. BCRP-overexpressed or control membrane vesicles were purchased (Genomembrane).
2. MDCK cells were seeded at 60K cells/well and cultured on PCF porous membrane inserts (0.4 µm pore size, Millipore) using standard culture conditions.
3. Cells were transiently transfected by proprietary OPTI-Expression^{TM} using cDNAs encoding BCRP alone, a combination of OATP1B1 and OATP1B3 transporters, or a combination of OATP1B1, OATP1B3, and BCRP (triple model).
4. Radioisotope-labeled BCRP substrates were applied to the basal side of the cell membrane for the cell based assays. For the vesicle assays, substrates were incubated in the presence of ATP or AMP.
5. Uptake rates were measured for vesicle assays, and transcellular flux and intracellular accumulation were measured for the B->A transcellular assays.

Result 1: Vesicle Assay



- Using membrane vesicle assays, CCK-8, MTX, E3S and E17βG were identified as BCRP substrates.
- Digoxin, DHEAS, genistein, taurocholate, quinidine, and BSP did not appear to be BCRP substrates.

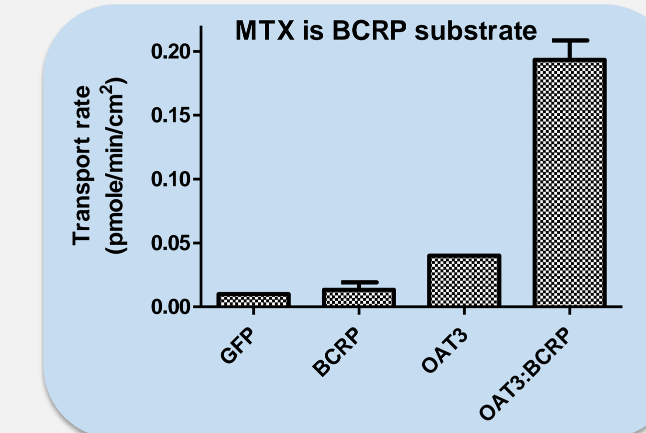
Result 2: Cell-based BCRP-only *vs.* Triple Transporter Model



- E3S, BSP, E17βG, and DHEAS did not appear to be BCRP substrates using the BCRP-only model, but were shown to be substrates in the triple model.
- CCK-8 and MTX did not appear to be substrates in either cell-based model.
- Genistein was shown to be a BCRP substrate in both cell-based models.

MTX not OATP1B substrate?

With OAT3 transporting MTX into the cells, BCRP efflux activity was detected!



Discussion

BCRP Substrate	Vesicle Assay	BCRP-only	Triple model
Genistein	False Negative	O	O
MTX	O	False Negative	With OAT3
E17bG	O	False Negative	O
DHEAS	False Negative	False Negative	O
E3S	False Negative	False Negative	O
BSP	False Negative	False Negative	O
CCK-8	O	False Negative	Not significant difference at given experiment
Accuracy	43%	14%	83%

1. The triple transporter (OATP1B1/1B3/BCRP) model was the most effective model for identifying BCRP substrates. This model identified BCRP substrates giving false negative results in vesicle assays and in BCRP-only cell-based assays.
2. CCK-8 was identified as an ATP-dependent BCRP substrate using the vesicle assay, but not with cell models, possibly due to subcellular sequestration or metabolism.
3. E17βG appeared negative in the BCRP-only model due to poor permeability, preventing uptake into the cytosolic space.
4. Importantly, as MTX shows, the correct combination of uptake transporter(s) is crucial for assessing efflux activity and avoiding false negatives.

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