Various members of the ABC transporter family (MDR1, BCRP, and MRP2) play a significant role in drug resistance and drug transport. These transporters are involved in the cellular uptake and efflux of compounds, which can affect the pharmacokinetics and pharmacodynamics of drugs. Caco-2 cells are widely used as an in vitro model to study the active transport of compounds across the intestinal barrier, and to evaluate the effect of transporters on the absorption of drugs.

**METHODS:**
Caco-2 cells were maintained in a humidified incubator at 37°C and 5% CO2 and were harvested for assay ready 24-well plates. The endogenous levels of MDR1, BCRP, and MRP2 in the Caco-2 wt cells were sufficient to transport substrates. All three transporters in the C2BBe1 cell line could be inhibited with standard concentrations of inhibitors.

**RESULTS:**
The endogenous transporter expression levels of MDR1, BCRP, and MRP2 in the Caco-2 wt cells were 0.48, 0.91, and 0.83 fmol/µg of plasma membrane protein, respectively. All three substrates exhibited B to A / A to B ratios >14 in the Caco-2 wt cells. In the MDR1 KO cells the ratios for the substrates markedly decreased (≤1.3), suggesting BCRP (topotecan) or MRP2 (vinblastine) involvement. The lack of inhibitor specificity for some transporters can further convolute assay results.

**CONCLUSIONS:**
The endogenous levels of MDR1, BCRP, and MRP2 in the Caco-2 cells are sufficient to transport substrates. The function, protein levels, and the specific gene deletion of these transporters have been confirmed in both the KO and the double gene knockouts. This in vitro model allows for the study of transport compounds without the use of non-specific or multiple MDR1, BCRP, and MRP2 inhibitors.

**INTRODUCTION**
Various members of the ABC transporter family (MDR1, BCRP, and MRP2) play an important role in the absorption and distribution of xenobiotics. Caco-2 cells are widely used as a model to assess passive permeability and MDR1, BCRP, and MRP2 mediated active efflux of compounds. The expression of multiple transporters in Caco-2 makes it more difficult to clearly determine which transporter is involved. The lack of inhibitor specificity for some transporters can further confound assay results.

**Table 1**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Parent ion</th>
<th>Product ions</th>
<th>Product ion (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>1539.5</td>
<td>1251.3, 1330.9</td>
<td>1251.3 (blue)</td>
</tr>
<tr>
<td>BCRP</td>
<td>1619.5</td>
<td>1330.9, 1539.5</td>
<td>1330.9 (blue)</td>
</tr>
<tr>
<td>MRP2</td>
<td>1685.5</td>
<td>1406.5, 1619.5</td>
<td>1406.5 (blue)</td>
</tr>
</tbody>
</table>

**Figure 1**
Cell Culture for Assay Ready 24-well Plates

**Figure 2**
Preparation and Quantification of Transporters by LC-MS/MS

**Figure 3**
Quantitative Transporter Expression of MDR1, BCRP, and MRP2 in C2BBe1 and Knockout Cells

**Figure 4**
Inhibition of MDR1, BCRP, and MRP2 Efflux in C2BBe1 Cells

**References:**

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