Tariquidar increases in-vitro transport of Etoposide across a P-glycoprotein expressing monolayer— a model for blood brain barrier disruption

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No relevant financial disclosures or conflicts of interest.
The blood brain barrier (BBB) is a unique entity within the central nervous system (CNS) that consists of both passive and active mechanisms to regulate access to the brain.

- Multiple surface proteins expressed by vascular endothelium contribute to the integrity and polarity of the BBB.
- P-glycoprotein (P-gp)— a 170-kD transmembrane protein of the ABC transporter family is encoded by the ABCB1 (or MDR1) gene and is localized on the apical membrane of vascular endothelial cells.
- Facilitates transport of substrates of a wide specificity including cytotoxic compounds by efflux into luminal spaces such as the ventricular system for elimination.
- Also implicated in the development of a multidrug-resistance phenotype in many tumors refractory to conventional chemotherapy.
Tariquidar exhibits high affinity for P-gp and various other multidrug transporters such as BCRP.

• Tariquidar, developed from rational drug design, is an anthranilamide derivative which binds P–gp non–competitively at nanomolar concentrations.

• Prior in vivo studies in humans have shown that Tariquidar has been able to inhibit P-gp at the BBB, with an increased cerebral uptake of a known P-gp substrate, 11C-N-desmethyloperamide, by PET imaging.

• However, the utility of Tariquidar has not been studied in a neuro-oncologic model and the utility of this drug as an adjuvant to conventional chemotherapy warrants investigation.

• We used an in-vitro model of the BBB endothelium to screen for chemotherapy drug candidates who’s transport across a P-gp expressing monolayer can be increased by addition of Tariquidar.
We used an in-vitro model of the BBB endothelium to screen for chemotherapy drug candidates whose transport across a P-gp expressing monolayer can be increased by addition of Tariquidar.

- A monolayer of LLC-PK1 cells expressing MDR1 on a pcDNA vector was seeded on transwell plates and used as an in-vitro model for P-gp activity.
- Various drugs (vincristine, paclitaxel, irinotecan, etoposide, and temozolomide) were screened using a transwell assay to determine if Tariquidar (100 nM) can inhibit P-gp.
- Differential etoposide concentrations in triplicate transwell chambers measured by GC-MS were significant (p<0.05) between LLC-PK1/MDR1+ and LLC-PK1/MDR1–, and LLC-PK1/MDR1+ treated with Tariquidar.
- No significant difference in differential drug concentrations were observed in vector cells and LLC-PK1/MDR1+ treated with Tariquidar.
## Differential Drug Transport Augmented by Tariquidar

<table>
<thead>
<tr>
<th></th>
<th>A. LLC-PK1 pcDNA MDR1−) Negative Control</th>
<th>B. LLC-PK1 pcDNA MDR1+ Positive Control</th>
<th>C. LLC-PK1 pcDNA MDR1+ (+100 nM Tariquidar)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Loperamide (Positive Control)</strong></td>
<td><strong>Top:Bottom Drug Concentration</strong></td>
<td>1.75 ± 0.78</td>
<td>43.66 ± 6.21</td>
<td>1.76 ± 0.40</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>0.01 (vs B)</td>
<td>(0.01 vs C)</td>
<td>0.96 (vs A)</td>
</tr>
<tr>
<td><strong>Temozolomide</strong></td>
<td><strong>Top:Bottom Drug Concentration</strong></td>
<td>18.14 ± 3.64</td>
<td>16.20 ± 6.63</td>
<td>16.98 ± 4.85</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>0.77 (vs B)</td>
<td>0.91 (vs C)</td>
<td>0.39 (vs A)</td>
</tr>
<tr>
<td><strong>Irinotecan</strong></td>
<td><strong>Top:Bottom Drug Concentration</strong></td>
<td>45.39 ± 16.81</td>
<td>325.56 ± 26.22</td>
<td>205.97 ± 53.03</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>0.0005 (vs B)</td>
<td>0.0185 (vs C)</td>
<td>0.0196 (vs A)</td>
</tr>
<tr>
<td><strong>Etoposide</strong></td>
<td><strong>Top:Bottom Drug Concentration</strong></td>
<td>19.35 ± 12.23</td>
<td>224.97 ± 55.97</td>
<td>55.25 ± 3.47</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>0.03 (vs B)</td>
<td>0.03 (vs C)</td>
<td>0.06 (vs A)</td>
</tr>
</tbody>
</table>

Loperamide is a P-gp substrate, which can be reversed by Tariquidar.

TMZ is not a P-gp substrate, *in-vitro*.

Irinotecan is a P-gp substrate, which can be slightly reversed by Tariquidar.

Etoposide is a P-gp substrate, which can be reversed by Tariquidar.
• A primary glioma cell line (D54) derived from surgical resection was obtained to determine if glioma cells express P-gp.
• Expression was determined by western blot using the C219 antibody and cytotoxicity assays determined if Tariquidar can increase sensitivity of tumor cells to chemotherapy.
• Because our in-vitro transwell assay revealed that Tariquidar increases transport of Etoposide the most, treatment of D54 primary glioma cells expressing MDR1 with Tariquidar increased etoposide toxicity by approximately 1.94-fold (p<0.05).
Our data suggests that Tariquidar, a third generation P-gp inhibitor can increase the transport of Etoposide across a polarized epithelium expressing MDR1.

- We have begun to explore the role of Tariquidar in the cerebral uptake of P-gp substrates in an in-vitro mouse glioma model.
- Rhodamine-123 (Rh-123) is a known P-gp substrate and a fluorescent dye. Using a cranial window approach, live-cerebral imaging with florescence microscopy can measure in real-time the cerebral uptake and clearance of Rh-123 with and without Tariquidar administration.
- This work can give insights into the dynamics of how Tariquidar and its substrates interacts with the BBB in real time, and is currently undergoing.