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EXAMINATION OF PERMEABILITY AND DRUG EFFLUX TRANSPORTER LIABILITIES OF A SERIES OF OVERACTIVE BLADDER AGENTS IN A CELL CULTURE MODEL OF THE BLOOD-BRAIN BARRIER

Hypothesis / aims of study
The passage of drugs from the blood to the brain is restricted by the brain microvessel endothelial cells that form the blood-brain barrier (BBB). Drug efflux transport proteins such as P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP) expressed in the brain microvessel endothelial cells limit the passage of compounds into the central nervous system (CNS). The aim of the present study was to evaluate the permeability, and drug efflux transporter interactions of a series of over-active bladder (OAB) agents in a cell culture model of the BBB. The hypothesis of the studies was that interactions of OAB agents with drug efflux transporters expressed in the brain endothelial cells limit the BBB permeability of these agents.

Study design, materials and methods
The drug efflux transporter activity and permeability of a series of six OAB agents, darifenacin (DAR), solifenacin (SOL), tolteradine (TOL), fesoteradine (FES), trospium (TRO) and oxybutynin (OXY) was examined. Drug efflux transporter interactions were assessed using membrane ATPase, cell accumulation and cell permeability studies. Transporter dependent hydrolysis of ATP was examined in cell membranes containing P-gp, MRP or BCRP under basal conditions and following exposure to various concentrations (0-100 □M) of OAB agents. Primary cultured bovine brain microvessel endothelial cells (BMEC) were used to assess BBB drug efflux transporter interactions and permeability. Cellular accumulation of rhodamine 123 (R123), a P-gp fluorescent probe, and 2, 7-bis-(carboxy-ethyl)-5(6)-carboxyfluorescein (BCECF), a mixed P-gp/MRP fluorescent probe, were examined in BMEC monolayers under control conditions and following treatment with various OAB agents (0-100 □M). Permeability of the OAB agents were examined in BMEC monolayers in both the apical to basolateral (absorptive) and basolateral to apical (efflux) directions. Those OAB agents displaying higher efflux permeability compared to absorptive permeability, as determined by efflux permeability ratios of 2 or greater, were further assessed with specific inhibitors of P-gp, BCRP, and MRP transporters. Permeability was determined over a 60 minute period and concentrations of OAB agents in the donor and receiver compartments were measured using LC/MS/MS.

Results
Of the six OAB agents examined, only DAR produced concentration dependent increases in P-gp ATPase activity (EC50 = 1.6 □M). In the MRP ATPase assay, only OXY displayed activity (EC50 = 0.1 □M). None of the OAB agents examined had any activity in the BCRP ATPase assay. In BMEC monolayers, both DAR and OXY caused significant increases in BCECF accumulation, with EC50 concentrations of 1.0 nM and 0.1 nM, respectively. In contrast, R123 retention in BMEC monolayers was only enhanced by DAR. The rank order absorptive permeability of the OAB agents in BMEC monolayers was TRO = FES > DAR > SOL > TOL = OXY. A significantly greater efflux permeability was observed with DAR and TRO in BMEC monolayers (efflux permeability ratios of 3 and 6, respectively), and this was inhibited by the P-gp inhibitor, GF120918.

Interpretation of results
DAR clearly and consistently displayed P-gp activity. Bidirectional permeability studies in BMEC indicated DAR and TRO both have significant P-gp transporter liabilities in the BBB. While OXY displayed MRP activity in multiple assays, the high passive diffusion of OXY appears to be sufficient to overcome any drug efflux activity at the BBB. The remaining OAB agents, FES, SOL, and TOL showed no drug efflux transporter activity in any of the assays.

Concluding message
The P-gp transporter liabilities of DAR and TRO contribute to the reduced BBB permeability of these OAB agents. The P-gp transporter liabilities of DAR and TRO at the BBB is likely to result in clinically significant reductions in the brain accumulation and CNS side effects for these particular OAB agents.

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