ABSTRACT

Evaluating the potential impact of p-glycoprotein (p-gp, MDR1) on drug absorption and disposition is an imperative task for preclinical stage drug development. In attempt to determine drug transport kinetic constants, cell-based in vitro system has been widely used. However, it was observed the apparent permeability (Papp) measured in cell monolayers cannot sufficiently describe the efflux activity of apically located p-gp activity, due to the dosing concentration not corresponding to the intracellular drug amount. Here, we demonstrate the measurement and the use of intracellular unbound concentration (Ccell,u) to describe intrinsic disposition kinetic parameters.

MDR1-MDCKII cell monolayers were tested using 96 well Transwell™ transport system after dosing B->A direction, 100 nM of [3H]-quinidine (5 Ci/mmol) incubated for 30 – 120 min. Also, dose-response curve was prepared after 90 min for concentration ranging 0.1 – 30 µM. After permeabilizing and extracting the cellular contents with 1.1 water:acetorinile, the transported amount to receiving compartment and cellular concentrations were determined. Ccell,u was measured in the presence of high potassium buffer to eliminate the membrane potential-driven cellular accumulation. Based on the free drug hypothesis, which assumes the concentration of unbound drug measured from extracellular compartment equals Ccell,u, the intracellular fractional unbound (fu,cell) can be obtained by the ratio of the extracellular to the total cell concentration. Kinetic constants were plotted against dosing concentration (to result apparent parameter) vs. Ccell,u (intrinsic).

After 120 min incubation of 100 nM quinidine, pseudo steady-state was reached for transport rate, resulting decreased dosing concentration (0.046 µM), total cellular accumulation (~2.9 µM) and increased receiver concentration (~0.052 µM). If shorter incubation (30 min) was used, apparent permeability was over-predicted to 122%, which demonstrates the necessity of reaching steady-state for intracellular concentration. Quinidine fu,cell increased from 0.005 to 0.016 over total cellular concentration ranging 20 to 2000 µM (cell volume 1.6 µL/cm). In order to correctly measure kinetic constants for apically expressed P-gp, intracellular unbound concentration, time course study as well as dose response study should be carried out. Simulation result of transcellular disposition for quinidine using intrinsic kinetic parameters demonstrated more reliable prediction than the apparent values.

MECHANISTIC UNDERSTANDING OF TRANSCELLULAR TRANSPORT

- B-A transcellular transport equals to cross-apical membrane transport. This is affected by the apical components (e.g. apical membrane potential and receiver concentration) and the intracellular unbound concentration.
- Basal components (e.g. dosing concentration) may indirectly affect transcellular transport through affecting intracellular concentration.
- Although concentration gradient may drive the net flux from B->A direction, ionized molecules entering (basal membrane) and leaving the cell (apical membrane) will have different intrinsic permeability (Pint) as electric potential gradient in two membranes are opposite.
- In order to measure the active efflux (Pint active efflux) of the multi-drug resistant transporter P-gp, the intracellular drug concentration should be determined.

METHODS

1. MDRI-MDCK cells were cultured in PWF-membrane Transwell™ inserts (0.4 µm in pore size) for three days to reach confluence.
2. Dose [3H]-Quinidine to basal dosing reservoir (300 µl) in the presence of paracellular diffusion marker [14C]-mannitol. Paracellular transport rate was separately incorporate to increase the accuracy for transcellular transport.
3. Based on the experimental values cell membrane potential was approximated to -28 mV.
4. Passive permeability for quinidine was estimated from basal uptake permeability, approximating 61 x10¹⁰ cm/s. Experimentally 59 x10¹⁰ cm/s was measured without the membrane potential (use of intracellular buffer).
5. Other simulation condition matched the B->A MDCK-MDR1 transcellular experiment settings.

RESULTS

1. Transcellular Quinidine Transport: Apparent vs. Intrinsic Kinetics
2. Time Course, 100 nM Quinidine

FORWARD PREDICTION USING OPTI-SIM

- Paracellular transport of [3H]-Quinidine was estimated by the paracellular diffusion marker [14C]-mannitol. Paracellular transport rate was separately incorporated to increase the accuracy for transcellular transport.
- Based on the experimental values cell membrane potential was approximated to -28 mV.
- Passive permeability for quinidine was estimated from basal uptake permeability, approximating 61 x10¹⁰ cm/s. Experimentally 59 x10¹⁰ cm/s was measured without the membrane potential (use of intracellular buffer).
- Other simulation condition matched the B->A MDCK-MDR1 transcellular experiment settings.

CONCLUSION

- Highly protein bound drug quinidine has about 0.5% fu,cell, which may be increased as cellular accumulation continues.
- Intrinsic p-gp activity (clearance) was approximately 47 µl/min/cm², that is about 8.4x greater than the apparent value of 5.6 µl/min/cm².
- Utilizing the unbound intracellular concentration in transcellular transport assay better explains high efficiency of Pgp in quinidine efflux, demonstrating the importance of intrinsic permeability (Pint) over Papp.
- Preliminary simulation using intrinsic membrane passive permeability, intrinsic efflux kinetic parameters, and series of differential equations predicted cellular disposition processes within 30% accuracy.