A Comparison of Assay Methods for the Assessment of Transporter-Mediated Drug Interactions

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Abstract

Transporters are membrane-bound proteins that facilitate the movement of substances across biological membranes. These transporters regulate the absorption, distribution and elimination of many xenobiotic compounds and may have clinically relevant effects on the pharmacokinetics of a compound, including the potential to cause drug-drug interactions. Regulatory agencies have issued guidance identifying P-gp, BCRP, OATP1B1, OATP1B3, OAT 1, OAT 3, OCT2 and more recently MATE 1 and MATE-2K as key transporters with potential clinical implications. The purpose of these investigations was to compare the test systems and assay detection methods in the assessment of a platform of regulatory recommended transporters.

Materials and Methods

Thawing and Plating TransPortoCells™
Comring® HEK293 TransPortoCells™ were thawed and resuspended in DMEM media (supplemented with 1X MEM non-essential amino acids and 10% FBS). Cells were seeded onto Poly-D-Lysine coated plates at a density of 400,000 cells/well. Following incubation for 3-4 hours, the media was replenished and the cells were incubated overnight at 37°C, 5%CO2. To ensure optimal uptake activity for OATP1B1, the DMEM media was refreshed with 2 mM Sodium Butyrate.

Uptake Assay
Cells were initially washed three times with pre-warmed HBSS buffer (containing 10 mM HEPES, pH 7.4). Following the third wash, cells were incubated for 10 minutes at 37°C, 5%CO2 to equilibrate. Uptake was initiated by the addition of a probe substrate prepared in HBSS buffer. For inhibition studies, the probe substrate was incubated in the presence of a prototypical inhibitor. For OATP1B1, a 30-minute pre-incubation was also performed prior to addition of substrate. Cells were incubated for the required period on the transporter. Uptake was terminated by the removal of substrate followed by addition of ice-cold HBSS buffer. Cells were washed on a further two occasions with ice-cold HBSS buffer. Cells were lysed by addition of M-PER protein extraction reagent or 80% acetone for 5 minutes at room temperature and analysed by LSC or LC-MS. Protein analysis using the BCA assay was undertaken on a portion of the cell extract from each well for normalisation of uptake activity for protein content.

CacoReady™ Caco-2 Cells
Pre-plated 21-day post-split cultures of Caco-2 cells (passage 50-60) grown on permeable support filters in 24-wells were purchased. Plates were received on day 17 of cell differentiation. On day 18 the shipping medium was liquefied and replaced with DMEM media (1g/L glucose, 2 mM glutamine, 10% FBS, 100 U/mL penicillin 100 µg/mL streptomycin). Cells were further maintained in an incubator at 37°C, 5%CO2 until use on day 21.

EvoTech Plateable Caco-2 Cells
A vial of cells (passage 50) was defrosted and seeded in DMEM media (high glucose, 2 mM glutamine, 10% FBS, 100 U/mL penicillin 100 µg/mL streptomycin) onto 24-well polycarbonate transwell plates. Cells were maintained in an incubator at 37°C, 5%CO2 with media refreshed on alternative days.

Efflux Assay
Prior to commencing the assay, the transcellular electrical resistance was measured to determine acceptable tight junction formation. Briefly cells were washed on three occasions with pre-warmed HBSS buffer (25mM HEPES, pH 7.4). Following the addition of the third wash, cells were incubated for 30 minutes at 37°C, 5%CO2. Following equilibration, dosing solutions containing probe substrate and prototypical inhibitor where applicable were added to either the apical or basal compartment of the Caco-2 cell monolayer. HBSS buffer was added to the opposing apical or basal compartment to enable apical to basal (A-B) or basal to apical (B-A) flux to be measured. Following incubation at 37°C, 5%CO2 for 2 hours, samples were removed from the apical and basolateral compartments for analysis.

Analysis
Samples were analysed by either LC-MS/MS in the presence of Warfarin as an internal standard to determine the peak area and peak area ratio to LSC.

Results

Figure 1. Comparison of pre-plated and plateable Caco-2 cells following incubation with probe substrates

Figure 6. Inhibition profiles of OAT 3 prototypical inhibitor Probenecid following endpoint analysis by LC-MS analysis. Cells were incubated for 5 minutes and analysed by (a) liquid scintillation counting with radiolabelled substrate and (b) LC-MS analysis following incubation with non-radiolabelled substrate. The results are the mean ± SD of 3 replicates.

Figure 5. Uptake activity of 1 µM Estradiol-17β-Glucuronide in HEK 293 cells following endpoint analysis by liquid scintillation counting and LC-MS. Bidirectional transport and efflux ratios were determined following incubation of commercially available pre-plated and plateable Caco-2 cells incubated for 2 hours at 37°C with probe substrates 1µM digoxin (P-gp) and 0.1µM Estrone Sulphate (BCRP). Digoxin and Estrone sulphate were additionally incubated in the presence of prototypical inhibitors 2 µM Zosu呱lo (P-gp inhibitor) and 1 µM Ko143 (BCRP inhibitor) respectively. Cells were lysed for 3 minutes and analysis was performed using either LSC (a) or LC-MS (b) for calculation of the efflux ratios. The results are the mean ± SD of three replicates.

Conclusions

Following incubation with pre-plated or plateable Caco-2 cells, Digoxin and Estrone sulphate probe substrates produced efflux ratios >4 and this ratio was abolished to approximately 1 in the presence of prototypical inhibitors.

Mannitol and Caffeine substrates were incubated concurrently as paracellular and transcellular permeability markers and produced Papp values of >1x10⁻⁶ cm/s and >20 x10⁻⁶ cm/s respectively.

LC-MS and LC-MS analysis both produced reproducible results to assess apparent permeability and Efflux Ratios indicating both are suitable models for evaluating efflux transporter-mediated drug interactions.

LSC and LC-MS analysis both produced reproducible results to assess P-gp and BCRP mediated transporter interactions.

Preliminary data indicated analysis by LC-MS produced higher efflux ratios compared to LSC using the same batch of cells.

Uptake of Estrone Sulphate by cells overexpressing OAT 3 was comparable following analysis by LSC or LC-MS analysis. IC₅₀ determinations for inhibition of OAT 3 were also comparable following analysis by LSC or LC-MS.

Pre-incubation of HEK 293 cells overexpressing OATP1B1 with Cyclosporin A indicated time-dependent inhibition of this transporter.

Caco-2 models and Comring® HEK TransportoCells™ in conjunction with robust LSC and LC-MS analysis methods have been developed to assess the potential for a new molecular entity to be a substrate or inhibitor of a transporter.

Introduction

There is increasing evidence of transporter mediated drug-drug interactions (DDIs) and the role of transporters in drug safety has become an area of intense regulatory review during recent years. EMA, FDA and Japanese PMDA Drug Interaction Guidelines highlight the requirement to investigate a number of clinically relevant uptake and efflux transporters in vitro to provide information regarding the contribution of transporters to the clinical efficacy and safety of therapeutic agents.1,2,3 SLC uptake and ABC efflux transporters play an important role in the absorption, distribution, metabolism and elimination of compounds. There are several cell-based in vitro models available for evaluating new chemical entities as either a substrate or inhibitor of a drug transporter. The Caco-2 (human colon carcinoma) cell model is one of the industry standards for investigating intestinal absorption, permeability and drug-drug interactions. HEK (Human embryonic kidney) 293 cells that transiently overexpress a single human transporter are also considered a suitable in vitro model for assessing whether a new chemical entity is a substrate or inhibitor of the SLC uptake transporter family. Currently, endpoint analysis includes liquid scintillation counting and LC-MS. The purpose of this investigation was to establish suitable in vitro test systems for the assessment of transporter drug interactions using liquid scintillation counting (LSC) and LC-MS analytical methods.

References