

BD GentestSM Contract Research Services

Combine our expertise and exclusive technologies to help you identify better drugs faster.

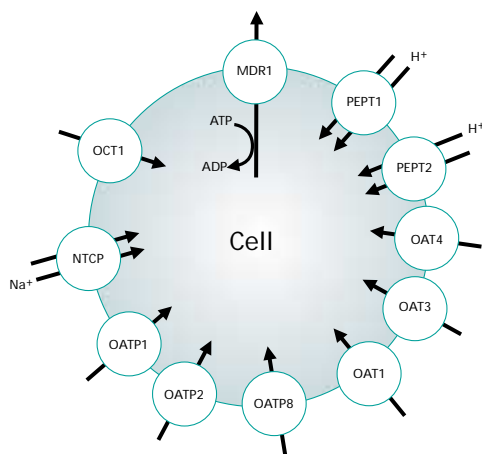


Absorption and Transport Studies

INTRODUCTION

The preferred method of drug delivery is a non-invasive orally administered drug. Understanding the bioavailability of new drug candidates early in the drug development process will help to rank order and select compounds for lead optimization.

BD GentestSM Contract Research Services offers epithelial cell barrier systems using Caco-2, LLC-PK1, or MDCK cells, widely accepted *in vitro* models used to rank order absorption of drug candidates. BD Biosciences is a leader in the development of many novel systems and services for *in vitro* analysis of xenobiotics. In addition to these standard models used for drug absorption, BD Gentest Contract Research Services offers unique capabilities to specifically study human PGP-mediated drug transport using cDNA transfected LLC-PK1 porcine cell lines. These human PGP expressing cell lines offer the advantage of studying this important efflux transporter without interference from other expressed transporters.



An "idealized" cell showing the mechanism and direction of transport for many influx and efflux transporters available from BD Biosciences. Polarization of cell not shown.

Membrane transporters play a key role in determining the exposure of liver, kidney, brain, and other tissues to a variety of solutes, including nutrients, cellular by-products, environmental toxins, drugs, and other xenobiotics. Membrane transporters are also essential in the delivery and excretory processes of drugs and their metabolites. BD Biosciences offers BD GentestSM Transportocytes, *Xenopus* oocytes pre-injected with OATP, OCT, NTCP, or other transporter cRNA. *Xenopus* oocytes efficiently express these membrane-bound transporters and can

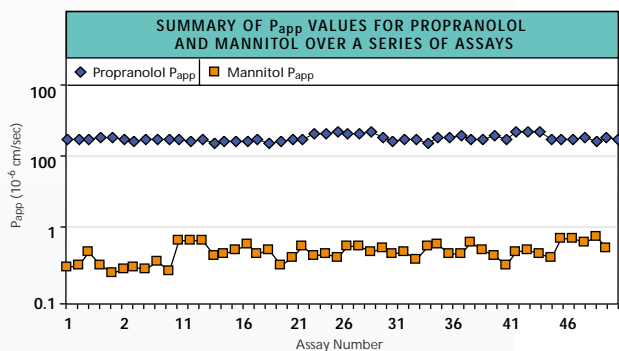
be used as a convenient model system in pharmaceutical lead discovery to predict drug disposition, drug clearance, and drug-drug interactions.

EXPERIMENTAL OUTLINE

- 1 Determine study design based on client needs
- 2 Generate samples using multi-tiered, time-dependence, and affinity assays using intestinal permeability or drug transporter-expressing models incorporating FDA-recommended model drugs
- 3 Analyze test article samples using LC/MS and/or liquid scintillation counting
- 4 Determine test article permeability, transporter interaction (substrate and/or inhibitor of efflux or uptake transporters) through calculation of apparent kinetic parameters
- 5 Expert data analysis is provided in a signed, detailed report from one of our Study Directors



DRUG PERMEABILITY MEASUREMENT IN CACO-2, LLC-PK1, OR MDCK CELL MONOLAYERS



Drug permeability through cell monolayers correlates well with intestinal permeability and oral bioavailability. Several mammalian cell lines are appropriate for this measurement. Apical to basolateral diffusion is measured using a standard set of time points and drug concentrations or adapted to a high throughput mode. Analysis is by LC/MS or LC/MS/MS. Alternatively, the incubations can be returned to the sponsor for analysis. FDA recommended permeability comparators and controls for membrane integrity are included. Data are reported as apparent permeability (P_{app}) or percent flux under fixed conditions.

DRUG PERMEABILITY DETERMINED IN 21-DAY CACO-2 CELL MONOLAYERS

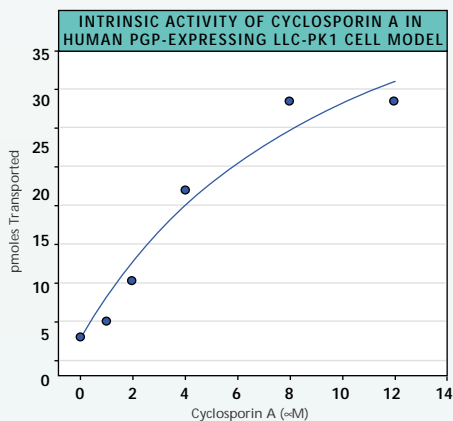
Compound	A to B P_{app} Value ($\times 10^{-6}$ cm/sec)	% Human Absorption*
Erythromycin	0.53	
Vinblastine	0.62	poor
Mannitol	0.63	15
Atenolol	0.33	44
Cimetidine	0.75	62
Cyclosporin A	1.4	40
Digoxin	1.3	66-90
Indinavir	3.6	60-70
Paclitaxel	3.4	
Ritonavir	7.4	60-70
Verapamil	21	90
Propranolol	24	90
AZT	28	100
Testosterone	36	100
Dexamethasone	40	100

*Human % oral absorption values were obtained from published values.

P-GLYCOPROTEIN (PGP)-MEDIATED DRUG TRANSPORT IN POLARIZED CELL MONOLAYERS

PGP (encoded by MDR1) is a member of the ABC transporter superfamily and is expressed in the human intestine, liver, and other tissues. Localized to the cell membrane, PGP functions as an ATP-dependent efflux pump, capable of transporting many structurally unrelated xenobiotics out of cells. Intestinal expression of PGP may affect the oral bioavailability of drug molecules that are substrates for this transporter. Determine if your compounds are PGP substrates by a direct measure of transport across polarized cell monolayers. Bidirectional transport (apical to basolateral and basolateral to apical) is measured in Caco-2 cells, or in LLC-PK1 cells expressing human PGP cDNA and corresponding control cells. The concentration-dependence of intrinsic activity of human PGP is analyzed for saturation of PGP-mediated transport, and apparent kinetic parameters (P_{app} , K_m , and V_{max}) are calculated. Alternatively, the incubations can be returned to the sponsor for analysis. Controls for membrane integrity and comparator compounds are included.

New: BD Biosciences has developed LLC-PK1 cell lines expressing mouse, dog, cynomolgus, and rhesus monkey PGP cDNAs. Determine if your compounds are substrates for PGP transport in control and polarized cell monolayers.



HUMAN PGP SCREEN

Determine if your compounds interact with the xenobiotic transporter PGP. ATP hydrolysis is required for *in vivo* drug efflux by PGP. The *in vitro* PGP-ATPase assay measures the phosphate liberated from drug-stimulated ATP hydrolysis in our human PGP membranes (*Cat. No. 453228, formerly K228*).

INHIBITION OF PGP-MEDIATED DRUG TRANSPORT IN POLARIZED CELL MONOLAYERS

Drugs that inhibit PGP can alter the absorption, disposition, and elimination of co-administered drugs and can enhance bioavailability or cause unwanted drug-drug interactions. Determine if your compounds inhibit PGP-mediated transport of a model substrate across polarized cell monolayers. Typically, multiple test compound concentrations are examined. Data are reported as IC₅₀ values or percent inhibition. Positive controls for PGP-inhibition and membrane integrity are included.

INHIBITION OF PGP-MEDIATED DIGOXIN TRANSPORT

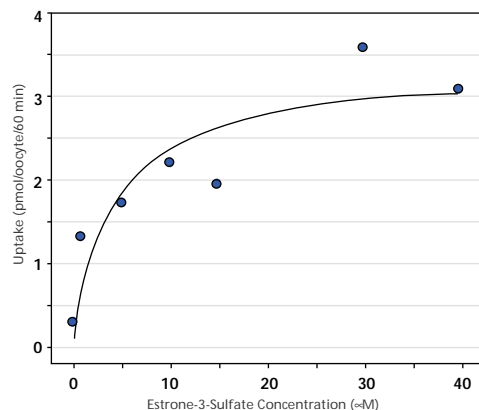
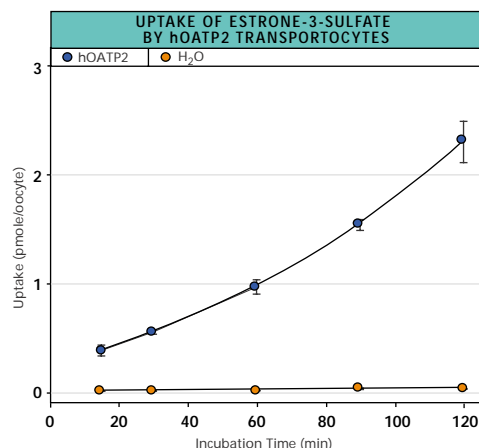
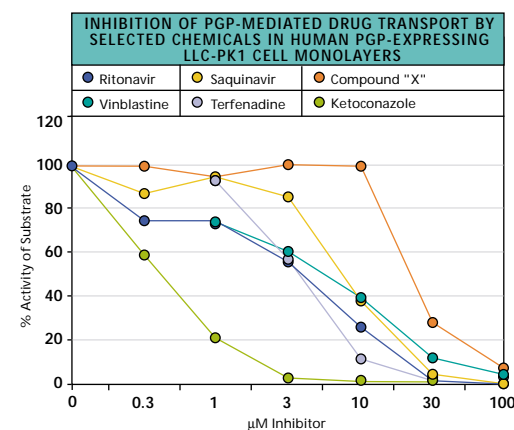
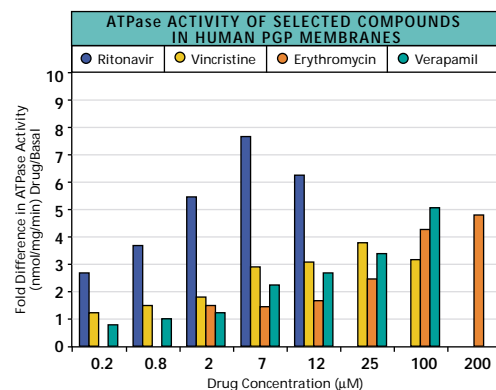
Substrate: Digoxin Control Inhibitor: Ketoconazole Test Inhibitor: Ritonavir Cell Line: MDR1 LLC-PK1

Controls	Digoxin Polarization Ratio (B-A/A-B)	% Inhibition of Digoxin Transport
digoxin + inhibitor	1.0	101
digoxin w/o inhibitor	6.8	0
Ritonavir Concentration (μM)		
0.003	6.5	5
0.01	5.0	31
0.03	5.6	21
0.1	5.0	31
0.3	5.2	27
1	5.2	27
3	4.2	45
10	2.4	75
30	1.1	98
100	1.1	99
	IC ₅₀	4.2 μM

TRANSPORTER-MEDIATED DRUG UPTAKE IN *XENOPUS LAEVIS* OOCYTES

Membrane transporters play a key role in determining the exposure of liver, kidney, brain, and other tissues to a variety of solutes, including nutrients, cellular by-products, environmental toxins, drugs, and other xenobiotics.

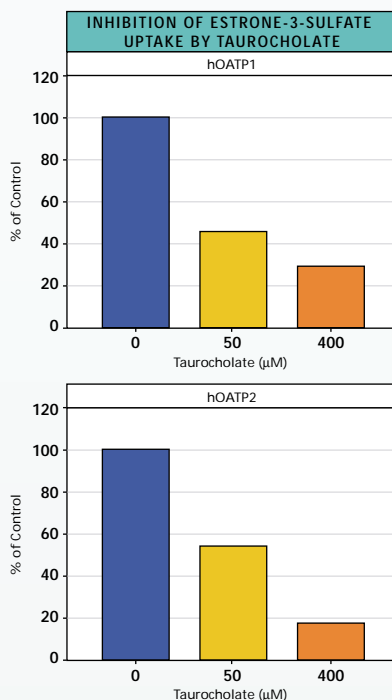
Human Organic Cation Transporter 1 (hOCT1, *SLC22A1*) is present in liver, kidney, and other tissues. It transports TEA, NMN, MPP and many other cations.³ Human Na⁻-Taurocholate Cotransport Protein (hNTCP, *SLC10A1*) is found in liver and localized to the sinusoidal membrane. It transports many bile salts.⁴ Human Organic Anion Transporting Polypeptide 1 (hOATP1, *SLC21A3*) is predominantly found in brain. Human Organic Anion Transporting Polypeptide 2 (hOATP2, *SLC21A6*) is a liver-specific transporter and localized to the sinusoidal membrane. Both hOATP1 and hOATP2 have broad substrate specificity, including conjugated and unconjugated bile salts, steroids and steroid conjugates, organic compounds, and thyroid hormones.⁵ Rat Organic Anion Transporting Polypeptide 1 (rOATP1, *SLC21A1*) is localized to the sinusoidal membrane in liver, or to the brush border membrane in kidney. It also has broad substrate specificity.^{6,7} All of these transporters have been expressed in our *Xenopus laevis* oocyte model. Determine if your compounds are substrates of these transporters by a direct uptake assay.



In the direct uptake assay, the tested compound is incubated with transporter-cRNA injected oocytes; the compound taken up inside the oocytes is quantified by scintillation counting or by LC/MS. The concentration-dependence is analyzed for saturation of transporter-mediated transport and apparent kinetic parameters calculated. Time dependence of transporter-mediated transport is analyzed by incubating the test compound with oocytes for different time periods. Negative controls are performed under the same conditions using water-injected or uninjected oocytes.

INHIBITION OF TRANSPORTER-MEDIATED DRUG UPTAKE IN *XENOPUS LAEVIS* OOCYTES

Drugs that inhibit membrane transporters can alter the absorption, disposition, and elimination of co-administered drugs and can enhance bioavailability or cause unwanted drug-drug interactions. Inhibition of transporter-mediated drug uptake can be measured with indirect assays. In this test, we determine if your compounds can inhibit the transporter-mediated uptake of a model substrate. Typically, multiple test substance concentrations are examined. Data are reported as IC₅₀ values, or percent inhibition when using only one or two concentrations of test compound.



CONTACT INFORMATION

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Your local BD GentestSM Products and Services Sales Specialist will provide you with initial study and price information. Your project will be assigned to a Study Director who will coordinate and tailor your Absorption and Transport Study to your total satisfaction. A finished detailed report will be provided.

References

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