Permeability values and efflux ratios of test compounds atenolol, propranolol & loperamide in differentiated CACO-2 cultures

CACO-2 Master Cell Bank (09042001)

	A-to-B Papp ($x10^{-6}$ cms^{-1})					B-to-A Papp ($x10^{-6}$ cms^{-1})					Efflux
Compound	Papp	Papp	Mean	SD	N	Papp	Papp	Mean	SD	N	ratio
Loperamide	2.7	2.2	2.45	0.25	2	8.96	8.41	8.685	0.39	2	3.54
Atenolol	0.435	0.799	0.62	0.18	2	0.83	0.58	0.655	0.11	2	1.06
Propanolol	19.3	19.95	19.63	0.33	2	15.4	16	15.7	0.42	2	0.80

CACO-2 Working Cell Bank (09042001)

	A-to-B Papp ($x10^{-6}$ cms^{-1})					B-to-A Papp ($x10^{-6}$ cms^{-1})					Efflux
Compound	Papp	Papp	Mean	SD	N	Papp	Papp	Mean	SD	N	ratio
Loperamide	1.08	1.23	1.155	0.075	2	4.8	4.96	4.88	0.11	2	4.23
Atenolol	0.65	1.14	0.90	0.25	2	0.98	0.77	0.875	0.15	2	0.98
Propanolol	17.1	19.95	18.53	1.43	2	15.4	15	15	0.28	2	0.81

Method

CACO-2 cells, ECACC catalogue number 09042001, (Master and Working banks) were used between passage numbers 44-48. Cells were seeded on to Millipore Millicell® cell culture inserts (0.4µm pore size) in 24 well plates at 1 x 10^5 cells/cm^2. They were cultured for 20 days in DMEM with media changes every two or three days. TEER measurements were taken at Day 5, 14 and 20 prior to assay. On day 20 the permeability study was performed.

Hanks Balanced Salt Solution (HBSS), phenol red-free, pH 7.4 buffer with 25 mM HEPES and 4.45 mM glucose at 37°C was used as the medium in the permeability studies. Incubations were carried out in an atmosphere of 5% CO_2 with a relative humidity of 95% at 37°C. HBSS was then removed from the apical compartment and replaced with test compound dosing solutions. The solutions were made by diluting 10 mM test compound in DMSO with HBSS to give a final test compound concentration of 10 µM (final DMSO concentration 1%). The fluorescent integrity marker lucifer yellow was also included in the dosing solution. Analytical standards were made from dosing solutions. The apical compartment inserts were then placed into 'companion' plates containing fresh HBSS. For basolateral to apical (B-A) permeability determination the experiment was initiated by replacing buffer in the inserts then placing them in companion plates containing dosing solutions. At 120 min the companion plate is removed and apical and basolateral samples diluted for analysis by LC-MS/MS. Test compound permeabilities were assessed in duplicate. Test and control compounds were quantified by LC-MS/MS cassette analysis using a 5-point calibration with appropriate dilution of the samples.

Results

In the Table above we provide data for three reference compounds which assess the functions of paracellular, passive transcellular and efflux transport in differentiated CACO-2 cells. Permeability data will vary quantitatively between laboratories, however there should be general agreement of the qualitative activity for different compounds in differentiated CACO-2 cultures in their ability to demonstrate these transport functions.

There are several ways in which the permeability data can be used. Firstly, the compounds can be ranked in terms of their CACO-2 P_{app} values. Two reference compounds, atenolol (paracellular transport) and propranolol (passive transcellular transport) are screened alongside the test compounds. Atenolol and propranolol have known human absorption of 50% and 90% respectively (1, 2), and can be used as markers for ranking the test compounds. Secondly, the permeability data can be used in conjunction with other *in vitro* parameters to predict the oral pharmacokinetics of a compound *in vivo* using the simulation software. The efflux transporter protein, P-glycoprotein, a member of the adenosine triphosphate-binding cassette superfamily, is a major determinant of the pharmacokinetics and pharmacodynamics of the opioid loperamide, a well-recognized antidiarrheal agent (3). Loperamide is an FDA recommended P-glycoprotein substrate, loperamide (4).

References

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3. Wandel C, *et.al*., Interaction of morphine, fentanyl, sufentanil, alfentanil, and loperamide with the efflux drug transporter P-glycoprotein. Anesthesiology. 2002 Apr; 96(4):913-20.

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