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Involvement of P-glycoprotein in restricting the absorption of cefuroxime axetil across CACO-2 cells

M. A. BARRETT, A. J. HUTT AND A. B. LANSLEY

Department of Pharmacy, King's College London, Manresa Road, London SW3 6LX

The P-glycoprotein efflux pump (P-gp) is abundant in the epithelium of the small intestine (Cordon-Cardo et al 1990) and might be expected to limit the absorption of drug molecules using the transcellular route. As such, it was hypothesised that P-gp-mediated efflux might contribute to the incomplete oral bioavailability (< 50%) of the prodrug of cefuroxime, cefuroxime axetil. Since P-gp-like activity has been demonstrated in Caco-2 cells (Burton et al 1993; Augustijns et al 1993; Hunter et al 1993) these were used to study the absorption of cefuroxime axetil in the absence and presence of verapamil, a classic inhibitor of P-gp-mediated efflux. Cefuroxime axetil is a 50:50 mixture of two diastereomers (1'*R*, 6*R*, 7*R*) and (1'*S*, 6*R*, 7*R*) (designated below as *R* and *S*) and the absorption of the individual diastereomers was investigated.

Caco-2 cells (passage nos. 85-105) were cultured on permeable supports (0.4 μm pore size; Transwell, Costar) in Dulbecco's modified Eagle's medium supplemented with foetal calf serum (10% v/v), non-essential amino acids (1% v/v), penicillin (100 U/mL) and streptomycin (100 μg/mL). After 17 days the absorption of cefuroxime axetil (*R* and *S* applied at 0.5 mM in Hank's balanced salt solution) was examined in the absence and presence of apically-applied verapamil (0.1 mM). The drug solution, which also contained [^{14}C]-mannitol (5.5 kBq/ mL), was added to the apical or basolateral (donor) chamber and samples removed from the opposite (receptor) chamber hourly for four hours. The samples were analysed for cefuroxime and the axetil prodrug diastereomers by reversed-phase HPLC and [^{14}C]-mannitol by liquid scintillation counting. The apparent permeability coefficient (Papp) of each compound was calculated from: Papp = (dQ/dt).(1/ACo) where dQ/dt is the accumulation rate in the receptor chamber; A is the cell monolayer surface area and Co is the initial concentration of the applied solution.

The permeability of the *S*-axetil prodrug was greater than that of the *R*-diastereomer irrespective of transport direction and presence or absence of verapamil. Similarly, the Papp of cefuroxime was always greater following application of the *R*-compared to the *S*-diastereomer (Barrett et al 1997). The apparent permeability of (*S*)-cefuroxime axetil across Caco-2 cells was four-fold greater in the basolateral to apical (B-A) direction than in the opposite, apical to basolateral (A-B), direction (Table) ($P<0.05$; Student's unpaired *t*-test). The apical application of verapamil significantly reduced the rate of secretion (B-A) and increased the rate of absorption (A-B) ($P<0.05$) of both diastereomers. The rate of accumulation of cefuroxime following application of cefuroxime axetil was greater in the B-A direction than in the A-B direction (Table) and was unaffected by the presence of 0.1 mM verapamil ($P>0.05$). The permeability of the cells to mannitol, a marker of monolayer integrity, was unaffected by the application of cefuroxime axetil or verapamil (Papp = 0.5 - 0.7 x 10^{-6} cm/s; $P>0.05$).

Table The apparent permeability coefficients of cefuroxime and cefuroxime axetil after application of the individual diastereomers (*R* or *S*) of cefuroxime axetil (n=4).

Compound	Form	Papp x 10^6 cm/s (mean ±sd)			
		- verapamil		+ verapamil	
		A-B	B-A	A-B	B-A
Cefuroxime	*R*	0.7 ± 0.06	2.3 ± 0.10	0.8 ± 0.06	1.5 ± 0.12
	S	0.5 ± 0.05	0.7 ± 0.13	0.6 ± 0.02	0.9 ± 0.07
Cefuroxime axetil	*R*	1.8 ± 0.19	6.2 ± 0.40	2.7 ± 0.20	4.4 ± 0.07
	S	2.4 ± 0.12	10.0 ± 0.42	4.2 ± 0.45	7.3 ± 0.72

The results obtained provide strong evidence that cefuroxime axetil is a substrate for P-gp-mediated efflux which may contribute to the incomplete bioavailability of the drug.

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