ELSEVIER

International Journal of Pharmaceutics 129 (1996) 223–231

international journal of pharmaceutics

Permeability of 5-fluorouracil and prodrugs in Caco-2 cell monolayers.

Anders Buur[1a], Lars Trier[a], Christin Magnusson[b], Per Artursson[b,*]

[a]*Royal Danish School of Pharmacy, Dept. of Pharmaceutical Chemistry, 2-Universitetsparken, Copenhagen, Denmark*
[b]*Uppsala University, Department of Pharmaceutics, BMC, Box 580, S-751 23 Uppsala, Sweden*

Received 16 June 1995; revised 19 September 1995; accepted 24 September 1995

Abstract

The toxicity and permeability of 5-fluorouracil (5FU) and 13 5FU prodrugs was investigated in Caco-2 monolayers grown on permeable supports. Only one of the prodrugs (VII) was toxic to the cell monolayers at 1×10^{-4} M, as assessed by an increased permeability of the cell monolayers to the hydrophilic probe ^{14}C-mannitol. The monolayer integrity was restored after a 4-fold reduction in the concentration of this prodrug. The permeabilities of 5FU and prodrugs increased roughly with the apparent partition coefficients ($P_{\text{octanol-buffer}}$) ($r = 0.74$). Attempts to correlate the permeabilities with other single physicochemical parameters such as hydrogen-bonding capacity and solubility resulted in poorer correlations. A slightly better relationship was obtained when the permeability was correlated with the two physicochemical parameters ($P_{\text{octanol-buffer}}$) and aqueous solubility by multiple linear regression analysis ($r = 0.81$). The ranking of the permeabilities of four of the compounds previously studied in rabbit colon and rectum was comparable in the cell monolayers and in the intestinal segments. These results indicate that Caco-2 monolayers can be used to predict drug absorption, not only of conventional drugs but also of small prodrugs such as those used here.

Keywords: 5-Fluorouracil; Caco-2 monolayers; Prodrugs; Toxicity; Permeability; Drug absorption

1. Introduction

Monolayers of human intestinal epithelial Caco-2 cells have been introduced both as a screening tool for the prediction of drug absorption in humans and experimental animals (Artursson and Karlsson, 1991; Rubas et al., 1995) and for mechanistic studies of intestinal drug transport (e.g. Inui et al., 1992; Karlsson et al., 1993). However, few studies of the permeability of prodrugs have been performed in these cells (Lundin et al., 1991; Toth et al., 1994; Hovgaard et al., 1995). For instance, limited information is available on how the physicochemical properties of prodrug molecules influence their permeability in Caco-2 monolayers.

5FU is a hydrophilic drug which display low oral and rectal absorption (Cohen et al., 1974; Christophidis et al., 1978; Almersjö et al., 1980). Its absorption can be improved by a prodrug

[1] Present address: H. Lundbeck A/S, Preformulation, Ottiliavej 7-9, DK-2500 Valby-Copenhagen, Denmark.

* Corresponding author.

SSDI 0378-5173(95)04331-4

approach (Buur et al., 1990). 5FU is actively transported in the small intestine but the active transport mechanism is saturated at concentrations far below those employed in the oral delivery of 5FU (Smith et al., 1988). Thus, the oral absorption of 5FU is dominated by a passive mechanism.

The first aim of this study was to investigate the permeability of 5-fluorouracil (5FU) and 13 of its prodrugs in Caco-2 monolayers. The second aim was to study how single physicochemical factors correlated with absorption. Therefore, 5FU prodrugs displaying a large variability in hydrophobicity (partition coefficients; log $P_{\text{octanol-buffer}}$) and hydrogen-bonding capacity (Δ log P; log $P_{\text{octanol-buffer}}$ − log $P_{\text{cyclohexane-buffer}}$) were synthesised. In addition, the prodrugs differed in pKa-values, molecular weight and aqueous solubility.

2. Materials and methods

2.1. Chemicals

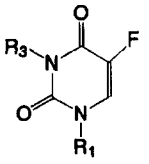
5-Fluorouracil was purchased from Fluka AG, Switzerland or Sigma Chemical Co., St. Louis, USA and was used as received. All other chemicals and solvents used were of reagent grade.

2.2. Preparation of the 5-fluorouracil derivatives

The chemical structures of the 5FU prodrugs are given in Fig. 1. The preparation of the 5FU prodrugs was performed as described previously (Buur and Bundgaard, 1986a; Buur and Bundgaard, 1986c).

2.3. HPLC assays

High-performance liquid chromatography (HPLC) was performed with a system consisting of a Merck Hitachi pump (Model L-6200), a variable UV detector (Merck Hitachi Model 4200), and an auto sampler Merck Hitachi (Model AS-4000). Data acquisition and processing were performed using a Merck Hitachi HPLC Manager (Model D-6000). The analytical column was a reversed-phase ChromSep column (4.6 × 100 mm) packed with Microsphere C18 (3μ particles, ChromPack, The Netherlands) protected with a pre column (2.1 × 10 mm) packed with pellicular material (30-40 μ particles, ChromPack). A gradient procedure was used in order to determine 5FU and prodrugs in a single system. Initially, the mobile phase consisted of 2% (v/v) acetonitrile in 0.05 M acetate buffer at pH 4.75. The concentration of acetonitrile was then increased in a linear fashion from 2 to 60% in 10 min, whereafter this concentration was maintained until the compounds in question were eluted from the column. The system was subsequently re-equilibrated using 2% acetonitrile before injection of the following sample. The column was operated at ambient temperature at a flow rate of 1.0 ml/min. The column effluent was monitored at 266 nm. Under these conditions the following retention times were observed: 5FU (compound I) 3.6 min, compound II 16.8 min; III 20.9 min, IV 22.6 min; VII 21.7 min; IX 14.6 min; VIII 17.4 min; X 32.5 min; V 27.6 min; and VI 33.6 min. The compounds XI, XII, XIII and XIV



	R_1	R_3
I	H	H
II	$COOCH_2CH_3$	H
III	$COOCH_2CH_2CH_2CH_3$	H
IV	$COOCH(CH_2CH_2)_2$	H
V	$COOCH(CH_2CH_2CH_3)_2$	H
VI	$COOCH(CH_2CH_2CH_2CH_3)_2$	H
VII	$COOCH_2C_6H_5$	H
VIII	$CH_2OCO(CH_2)_2CH_3$	H
IX	H	COC_5H_5N*
X	$CH_2OCOC(CH_3)_3$	$CH_2OCOC(CH_3)_3$
XI	CH_2OCOCH_3	COC_6H_5
XII	$CH_2OCO(CH_2)_2CH_3$	COC_6H_5
XIII	CH_2OCOCH_3	CH_2OCOCH_3
XIV	$CH_2OCO(CH_2)_2CH_3$	COC_5H_5N*

*Compounds IX and XIV are 3-nicotinoyl derivatives

Fig. 1. Structures of 5FU and its prodrugs.

were determined as the parent 5FU after quantitative hydrolysis in carbonate buffer (pH 11). No interference in the chromatic system was caused by the transport medium (Hanks balanced salt solution; HBSS) although a peak at 17.5 min occurred (due to the indicator (phenol red) used in the solution).

2.4. Determination of partition coefficients

The apparent partition coefficients (P) of 5FU and its prodrugs were determined in n-octanol buffer and cyclo-hexane buffer systems at pH 7.4 and 22°C essentially as described previously (Buur and Bundgaard, 1984).

2.5. Determination of ionisation constants

The ionisation constants for derivatives IV, V and VI were determined by UV-spectrophotometry as described previously (Buur and Bundgaard, 1986a). The solute concentration was 10^{-4} M and the UV absorbances were measured at pH 2 and 9.5, and at three further pH values within the range of 6–8. The measurements were performed immediately after preparation of the solutes.

2.6. Kinetic measurements

The hydrolysis of the 5FU prodrugs was studied in Hank's balanced salt solution containing 25 mM HEPES-buffer (HBSS) in the presence of Caco-2 cells. These studies were carried out in screw-capped test tubes and the reaction mixtures were kept in a waterbath at 37°C. The initial concentration of the compounds was 5×10^{-4} M. At appropriate times, samples of 200 μl were withdrawn and added to 800 μl of 96% ethanol and 100 μl of 10% v/v phosphoric acid. After mixing and centrifugation for about 3 minutes at 13 000 rev./min, 20 μl of the clear supernatant was analysed by HPLC as described above.

2.7. Caco-2 cells

Caco-2 cells were obtained from American Tissue Culture Collection, Rockville, MD. The cells were cultivated on polycarbonate filters (Transwell cell culture inserts; mean pore size 0.4 μm) as described elsewhere (Artursson, 1990). The cells were fed every second day and were allowed to grow and differentiate for 21–30 days before being used in the transport experiments. Cells of passage 91–105 were used throughout.

2.8. Transport studies

The transport experiments were performed at 37°C and 95% relative humidity in HBSS as described previously (Anderberg et al., 1992). In the first series of experiments [^{14}C]-mannitol, a marker of paracellular permeability, was used to investigate possible effects of 5FU and its prodrugs on epithelial integrity. The effects of the drugs on [^{14}C]-mannitol (New England Nuclear, Boston, MA) permeability were studied for 60 min. The radioactive samples were analysed immediately in a liquid scintillation counter.

In the second series of experiments, the transport of 5FU and its prodrugs was studied for 8, 15, and 60 min. The samples were stored at −70°C until analysed with HPLC. The drugs were dissolved in HBSS (final concentration 1×10^{-4} M or 2.5×10^{-5} M) and were added to the apical side of the cells. The corresponding medium without drug was added to the basolateral side. At regular time intervals, the cell culture inserts were moved to new basolateral chambers containing fresh HBSS. The initial drug concentration in the apical chamber was determined from triplicate samples of the drug solutions. The chambers were stirred as described previously in order to minimise the effects of the aqueous boundary layer (Karlsson and Artursson, 1991). All rate constants were obtained under 'sink conditions' (i.e., before $>10\%$ of the drug had diffused across the cell monolayers) from the linear drug-appearance curves in the basolateral chambers. The apparent permeability coefficients (P_{app}) (cm/s) were determined according to the following equation:

$$P_{app} = dQ/dt \; 1/(AC_0)$$

where dQ/dt is the permeability rate (steady state flux, mol/s), C_0 is the initial concentration in the donor chamber (mol/ml), and A is the surface area of the membrane (cm^2).

Table 1
Physicochemical properties of 5FU (I) and 5FU prodrugs (compounds II to XIV; see Fig. 1)

	Molecular Weight	p*K*a	log *P*					Aqueous solubility pH 7.4 (M)
			Cyclohexane-buffer		*n*-Octanol-buffer		Δ log *P*	
			Intrinsic	pH 7.4	Intrinsic	pH 7.4		
I	130	8.0, 13[b]	−4.0	< −4.0	−0.83[a]	−0.96[a]	3.0	0.11[a]
II	216	6.8[a]	−2.0	−2.7	0.20[a]	−0.50[a]	2.2	0.11[a]
III	243	6.8[a]	−1.7	−2.4	0.89[a]	0.19[a]	2.6	0.13[a]
IV	244	6.8[a]	−0.68	−1.70	1.30	0.60	2.3	n.d.
V	272	6.8	0.25	−0.45	2.2	1.50	2.0	0.0012
VI	300	6.8	1.50	0.80	2.95	2.25	1.5	0.00013
VII	264	6.8[a]	−1.40	−2.1	1.18[b]	0.48[a]	2.6	0.018[a]
VIII	230	7.3[c]	−2.2	−2.6	0.47[c]	0.06[c]	2.7	0.11[a]
IX	236	1.6;6.4[d]	n.d.	−2.3	−0.06[d]	−1.10[d]	1.2	0.13[d]
X	359	-	1.70	1.70	2.54[c]	2.54[c]	0.8	0.00012[c]
XI	306	-	−0.73	−0.73	1.02	1.02	1.8	n.d.
XII	336	-	0.72	0.72	2.1	2.1	1.4	n.d.
XIII	274	-	−0.25	−0.25	−0.37[c]	−0.37[c]	−0.08	0.016[c]
XIV	324	-	0.14	0.14	1.28	1.28	1.1	n.d.

[a]Buur and Bundgaard, 1986c.
[b]Buur and Bundgaard, 1986a.
[c]Buur et al., 1985.
[d]Buur and Bundgaard, 1986b.
[e]Buur et al., 1990.

3. Results

3.1. Characteristics of 5FU and prodrugs

The physico-chemical characteristics of 5FU and its prodrugs are given in Table 1. All the derivatives were more hydrophobic than the parent 5FU. An increase in hydrophobicity was not always accompanied by a corresponding decrease in aqueous solubility. Indeed, the aqueous solubilities of II, III, VIII and IX are either higher than or similar to that of 5FU. This may be explained by disruption, or decrease by derivatization, of the capability to form intermolecular hydrogen bonds between NH protons in one molecule and a carbonyl group in another molecule. This hypothesis is supported by the finding that replacement of the N-1 and/or N-3 proton(s) in the parent 5FU results in derivatives with decreased crystal lattice energy and with higher solubilities.

The log *P* values determined in the *n*-octanol aqueous buffer system were higher than those determined with cyclo-hexane aqueous buffer. This reflects the higher solubility of 5FU and its prodrugs in *n*-octanol than in cyclo-hexane, which in part is due to the capability of *n*-octanol to form hydrogen bonds with these compounds. Thus, the Δ-log *P* values given in Table 1 for monosubstituted 5FU derivatives are all positive, ranging from 1.2 for IX to 2.7 for VIII. The N_1-,N_3-disubstituted derivatives (X–XIV) do not possess the same capacity for hydrogen bond formation with *n*-octanol and, accordingly, the Δ-log *P* values for these compounds are generally lower than those for the monosubstituted derivatives.

3.2. Hydrolysis of prodrugs in HBSS and by Caco-2 cells

The hydrolysis of the prodrugs was investigated

Table 2
Apparent permeability coefficients (P_{app}) of [^{14}C]-mannitol before and after exposure to 5FU (I) or 5FU prodrugs (1×10^{-4} M) in Caco-2 cell monolayers

Compound	Permeability coefficient (P_{app}) ($\times 10^6$ cm/s)			
	^{14}C-Mannitol		^{14}C-Mannitol + 5-FU/Prodrug	
	P_{app}	± S.D. (n = 3)	P_{app}	± S.D. (n = 3)
I	0.13	0.01	0.16	0.02
II	0.14	0.01	0.15	0.01
III	0.14	0.01	0.14	0
IV	0.14	0.01	0.15	0
V	0.14	0.01	0.17	0.13
VI	0.14	0.01	0.11	0.01
VII[a]	0.09	0.02	0.12	0.03
VIII	0.14	0.01	0.12	0.01
IX	0.14	0.01	0.23	0.02
X	0.25	0.03	0.17	—
XI	0.09	0.02	0.07	0.01
XII	0.09	0.02	0.11	0.03
XIII	0.09	0.02	0.08	0.02
XIV	0.09	0.02	0.07	0.02

[a] $C_o = 2.5 \times 10^{-5}$ M.

in HBSS (the buffer used in the permeability studies) in the presence of Caco-2 cells. Within the time-frame of the permeability studies (8–60 min) only a small and insignificant fraction of each derivative was hydrolysed as evidenced by HPLC analysis of samples taken from the cell cultures (data not shown).

3.3. Effects of 5FU and prodrugs on monolayer integrity

In general, 5FU and its prodrugs (1×10^{-4} M) did not affect the integrity of the cell monolayers (Table 2). This was evidenced by a lack of increase in P_{app} of the hydrophilic paracellular marker molecule [^{14}C]-mannitol on addition of the drugs. The exception was compound VII which increased the permeability of [^{14}C]-mannitol significantly at 1×10^{-4} M (data not shown). Therefore, the effects of compound VII were re-examined at a lower concentration (2.5×10^{-5}M). The lower concentration did not affect the [^{14}C]-mannitol permeability as compared to control values. In addition, the mean P_{app}-value of VII itself was reduced from 40.7×10^{-5} cm/s to 6.0×10^{-5} cm/s or almost 7-fold (data not shown). These results show that the potential effects on epithelial integrity must always be investigated before reliable conclusions can be drawn from transport experiments in cell monolayers.

3.4. Permeability of 5FU and prodrugs in Caco-2 monolayers

The permeability of 5FU and its prodrugs ranged about 100-fold, from 1.4×10^{-6} cm/s for compound IX to 1.4×10^{-4} cm/s for compound X (Table 3). Since the transport of 5FU (due to saturation of carrier-mediated mechanisms at the high concentrations used in the present study, Smith et al. (1988)) and prodrugs in Caco-2 cell monolayers is dominated by passive diffusion, physicochemical properties such as molecular size, aqueous solubility, hydrophobicity and hydrogen-bonding capacity should influence the prodrug permeabilities. To investigate whether P_{app} was correlated with any of these parameters, linear regression analysis was performed. A rough correlation was obtained between permeability and hydrophobicity (r = 0.74), Fig. 2. Thus, a classical relationship was seen where the permeability increases with hydrophobicity until a plateau is

Table 3
Apparent permeability coefficients (P_{app}) of 5FU (I) and 5FU prodrugs (1×10^{-4} M) (II–XIV) in Caco-2 cell monolayers

Compound	Permeability coefficient (P_{app}) of 5-FU/Prodrug ($\times 10^6$ cm/s)	
	P_{app}	± S.D. (n = 3)
I	5.5	0.07
II	45.0	4.6
III	75.6	1.9
IV	63.6	2.5
V	45.0	2.3
VI	64.2	17.6
VII	407	46.0
VII[a]	60.0	-
VIII	24.7	0.9
IX	1.4	0
X	141	11
XI	57.2	6.4
XII	76.6	27
XIII	31.8	2.2
XIV	61.0	6

[a] $C_o = 2.5 \times 10^{-5}$ M.

reached. Correlations with the other physicochemical parameters did not occur or were much weaker ($r < 0.7$) (data not shown).

Since no single physicochemical parameter satisfactorily described the permeability of 5FU and prodrugs, we performed multiple regression analysis in order to correlate the permeability with

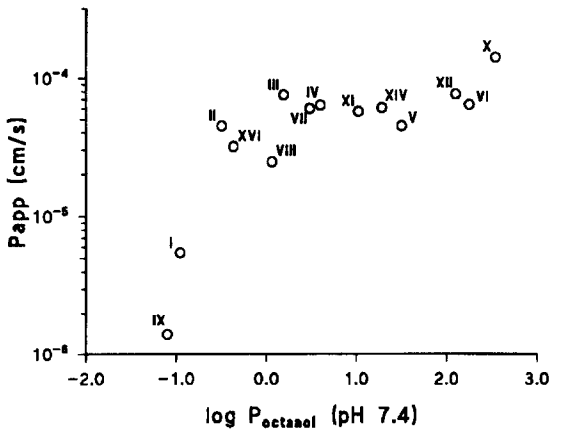


Fig. 2. Correlation between apparent permeability coefficients (P_{app}) and hydrophobicity ($P_{octanol\text{-}buffer;\ pH\ 7.4}$) for 5FU and its prodrugs (r = 0.74).

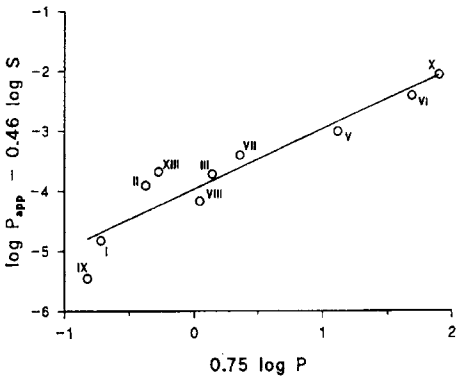


Fig. 3. Correlation between apparent permeability coefficients (P_{app}), hydrophobicity ($P_{octanol\text{-}buffer;\ pH\ 7.4}$) and aqueous solubility (S) for 5FU and its prodrugs (r = 0.81).

more than one physicochemical parameter. The best correlation was found between permeability and hydrophobicity + solubility (n = 10; r = 0.81), Fig. 3. The relationship is described by the following equation (standard deviations within brackets):

$$P_{app} = 0.75(0.29) \times \log P + 0.46(0.30) \times \log S - 3.97(0.48)$$

where P refers to the partition coefficient using n-octanol-aqueous buffer of pH 7.4. Similar results were obtained for log ($P_{cyclohexane\text{-}buffer}$) at pH 7.4 (data not shown) while attempts to correlate other physicochemical parameters to permeability resulted in much weaker correlations (data not shown).

3.5. Comparison with permeability in intestinal tissues

The permeability of 5FU and three of the prodrugs (II, III and VIII) has previously been investigated in rabbit colon (Buur et al., 1990). A comparison of the permeability coefficients in Caco-2 monolayers with those in rabbit colon and rectum is shown in Table 4. The ranking order of the permeabilities in the three models was comparable. The higher permeabilities of the two most permeable compounds (II and III) in the Caco-2 model may be related to better control of the stirring conditions (i.e. a smaller aqueous boundary layer) in the in vitro model.

Table 4
Comparison of permeability coefficients (P_{app}) of 5FU and prodrugs II, III and VII in Caco-2 cell monolayers, rabbit colon and rectum

Compound	Permeability coefficients ($P_{app} \times 10^6$ cm/s)		
	Caco-2	Rabbit colon[a]	Rabbit rectum[a]
5-FU (I)	5.5	1.9	2.3
II	45.0	23.5	12.7
III	75.6	22.7	14.9
VIII	24.7	8.7	11.4

[a]Buur et al., 1990.

4. Discussion

The results of this study show that the permeability of 5FU and its prodrugs in Caco-2 monolayers follows a classical relationship with hydrophobicity. This finding is in agreement with the hypothesis that the prodrugs are transported across the cell monolayers by a passive transcellular route. No, or much weaker correlations were seen with other physicochemical parameters such as molecular weight, hydrogen bonding capacity and solubility. The lack of correlation with molecular weight is not surprising since all of the investigated compounds were small and thus, their membrane permeabilities should not be limited by molecular size. The observation that the hydrogen-bonding capacity did not influence permeability was perhaps more surprising since the prodrugs displayed a wide range of hydrogen-bonding capacity and this parameter has previously been shown to determine the permeability of drugs and peptides in various tissues (Young et al., 1988; Conradi et al., 1991). However, it has recently been suggested that the relative importance of hydrophobicity and hydrogen-bonding capacity for the (blood-brain barrier) permeability of H_1-antihistaminic agents may vary from one compound to another (ter Laak et al., 1994). We conclude that hydrophobicity may be the most important physicochemical predictor of the permeability of the 5FU-prodrugs used in this study. Further studies are needed to show if this conclusion holds also for other types of prodrugs.

The rough correlation between hydrophobicity and permeability indicates that more than one physicochemical factor should be used in order to establish a better correlation with permeability. The simplest way to do this is to perform multiple regression analysis, taking two physicochemical factors into account. The result of this analysis was unexpected since the best correlation was found when permeability was correlated to hydrophobicity and aqueous solubility. These results should be interpreted with caution since a correlation between log P and log S was also observed ($r = 0.90$). However, they are supported by recent experiments in our laboratory indicating that the dynamic polar surface areas of drug molecules – a measure that incorporates several physicochemical factors – give an excellent correlation with drug permeability in Caco-2 monolayers and intestinal segments (Palm et al., 1996). Indeed, aqueous solubility has been shown to be related to molecular surface properties (Amidon et al., 1975). We conclude that the permeability of 5FU and prodrugs in Caco-2 monolayers is most likely dependent on several physicochemical factors.

A limited comparison between prodrug permeability in Caco-2 monolayers and in situ was possible since four of the compounds had been studied previously in rabbit colon and rectum (Buur et al., 1990). The finding that all three models ranked the permeabilities in a similar order is in good agreement with previous comparisons of drug permeability in Caco-2 monolayers and human jejunum, rat ileum and rat colon (Lennernäs et al., 1996; Artursson et al., 1993; Kim et al., 1993). We can therefore now extend our previous statement that Caco-2 monolayers can be used to predict the absorption of

conventional drugs in vivo (Artursson and Karlsson, 1991) to include hydrophobic prodrugs as well.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (9478), the Swedish Foundation for Scientific Research without Animal Experiments and Centrala försökdjursnämnden (93-11).

References

Almersjö, E.E., Gustavsson, B.G., Regårdh, C.-G. and Wålen, P., Pharmacokinetic studies of 5-fluorouracil after oral and intravenous administration in man. *Acta Pharmacol. Toxicol.*, 46 (1980) 326–336.

Amidon, G.L., Yalkowsky, S.H., Anik, S.T. and Valvani, S.C., Solubility of nonelectrolytes in polar solvents. V. Estimation of the solubility of aliphatic monofunctional compounds in water using a molecular surface area approach. *J. Phys. Chem.*, 79 (1975) 2239–2246.

Anderberg, E.K., Nyström, C. and Artursson, P., Epithelial transport of drugs in cell culture, VII. Effects of pharmaceutical surfactant excipients and bile acids on transepithelial permeability in monolayers of human intestinal epithelial (Caco-2) cell., *J. Pharm. Sci.*, 81 (1992) 879–887.

Artursson, P., Epithelial transport of drugs in cell culture. I. A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J. Pharm. Sci.*, 79 (1990) 476–482.

Artursson, P. and Karlsson, J., Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.*, 175 (1991) 880–885.

Artursson, P., Ungell, A.-L. and Löfroth, J.-E., Selective paracellular permeability in two models of intestinal absorption: cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharm. Res.*, 10 (1993) 1123–1129.

Buur, A. and Bundgaard, H., Prodrugs of 5-fluorouracil. I. Hydrolysis kinetics and physicochemical properties of various *N*-acyl derivatives of 5-fluorouracil. *Int. J. Pharm.*, 21 (1984) 349–364.

Buur, A., Bundgaard, H. and Falch, E., Prodrugs of 5-fluorouracil. IV. Hydrolysis kinetics, bioactivation and physiochemical properties of various *N*-acyloxymethyl derivatives of 5-fluorouracil. *Int. J. Pharm.*, 24 (1985) 43–60.

Buur, A. and Bundgaard, H., Prodrugs of 5-fluorouracil. V. 1-Alkoxycarbonyl derivatives as potential prodrug forms for improved rectal or oral delivery of 5-fluorouracil. *J. Pharm. Sci.*, 75 (1986a) 522–527.

Buur, A. and Bundgaard, H., Prodrugs of 5-fluorouracil. VI. Hydrolysis kinetics and bioactivation of 3-nicotinoyl-5-fluorouracil and various 1-substituted derivatives of 3-benzoyl-5-fluorouracil. *Arch. Pharm. Chem. Sci. Ed.*, 14 (1986b) 99–112.

Buur, A. and Bundgaard, H., Prodrugs of 5-fluorouracil. VIII. Improved rectal and oral delivery of 5-fluorouracil via various prodrugs. Structure-rectal absorption relationships. *Int. J. Pharm.*, 75 (1986c) 676–679.

Buur, A., Yamamoto, A. and Lee, V.H.L., Penetration of 5-fluorouracil and prodrugs across the intestine of the albino rabbit: evidence for shift in absorption site from the upper to the lower region of the gastrointestinal tract by prodrugs. *J. Controlled Release*, 14 (1990) 43–51.

Christophidis, N., Vajda, F.J.E., Lucas, I., Drummer, O., Moon, W.J. and Louis, W.J., Fluorouracil therapy in patients: a pharmacokinetic comparison of various rates and routes of administration. *Clin. Pharmacokinet.*, 3 (1978) 330–336.

Cohen, J.L., Irwin, L.E., Marshall, G.J., Darven, H. and Bateman, J.R., Clinical pharmacology of oral and intravenous 5-fluorouracil (NSC-19893). *Cancer Chemother. Rep.*, 58 (1974) 723–732.

Conradi, R.A., Hilgers, A.R., Ho, N.F.H. and Burton, P.S., The influence of peptide structure on transport across Caco-2 cells. *Pharm. Res.*, 8 (1991) 1453–1460.

Hovgaard, L., Brøndsted, H., Buur, A. and Bundgaard, H., Drug delivery studies in Caco-2 monolayers. Synthesis, hydrolysis, and transport of 0-cyclopropane carboxylic acid ester prodrugs of various β-blocking agents. *Pharm. Res.*, 12 (1995) 387–392.

Inui, K.-I., Yamamoto, M. and Saito, H., Transepithelial transport of oral cephalosporins by monolayers of intestinal epithelial cell line Caco-2: specific transport systems in apical and basolaterial membranes. *J. Pharmacol. Exp. Ther.*, 261 (1992) 195–201.

Karlsson, J. and Artursson, P., A method for the determination of cellular permeability coefficients and aqueous boundary layer thickness in monolayers of intestinal epithelial (Caco-2) cells grown in permeable filter chambers. *Int. J. Pharm.*, 71 (1991) 55–64.

Karlsson, J., Kuo, S.-M., Ziemniak, J. and Artursson, P., Transport of celiprolol across human intestinal epithelial (Caco-2) cells: mediation of secretion by multiple transporters including *P*-glycoprotein. *Br. J. Pharmacol.*, 110 (1993) 1009–1016.

Kim, D.-C., Burton, P.S. and Borchardt, R.T., A correlation between the permeability characteristics of a series of peptides using an in vitro cell culture model (Caco-2) and those using an in situ perfused rat ileum model of the intestinal mucosa. *Pharm. Res.*, 10 (1993) 12.

Lennernäs, H., Palm, K., Fagerholm, U. and Artursson, P., Comparison between active and passive drug transport in human intestinal epithelial (Caco-2) cells in vitro and human jejunum in vivo. *Int. J. Pharm.*, (in press, 1996).

Lundin, S., Möss, J., Bundgaard, H. and Artursson, P., Absorption of thyrotropin-releasing hormone (TRH) and TRH prodrug in a human intestinal cell line (Caco-2). *Int. J. Pharm.*, 76 (1991) R1–R4.

Palm, K., Luthman, K., Ungell, A-L., Strandlund, G. and Artursson, P., A new method for the prediction of intestinal drug absorption based on molecular surface properties. *J. Pharm. Sci.* (in press, 1996).

Rubas, W., Villagran, J., Cromwell, M., McLeod, A., Wassenberg, J. and Mrsny, R., Correlation of solute flux across Caco-2 monolayers and colonic tissue in vitro. *S.T.P. Pharm. Sci.*, 5 (1995) 93–37.

Smith, P., Mirabelli, C., Fondacaro, J., Ryan, F. and Dent, J., Intestinal 5-fluorouracil absorption: use of Ussing chambers to assess transport and metabolism. *Pharm. Res.* 5 (1988) 598–603.

ter Laak, A.M., Tsai, R.S., Donné-Op den Kelder, G.M., Carrupt, P.-A., Testa, B. and Timmerman, H., Lipophilicity and hydrogen-bonding capacity of H_1-antihistaminic agents in relation to their central sedative side-effects. *Eur. J. Pharm. Sci.*, 2 (1994) 373–384.

Toth, I., Hillery, A.M., Wood, I.P., Magnusson, C. and Artursson, P., Oral absorption of lipidic amino acid conjugates. *Int. J. Pharm.*, 102 (1994) 223–230.

Young, R.C., Mitchell, R.C., Brown, T.H., Ganellin, C.R., Griffiths, R., Jones, M., Rana, K.K., Saunders. D., Smith, I.R., Sore, N.E. and Wilks, T.J., Development of a new physicochemical model for brain penetration and its application to the design of centrally acting H_2 receptor histamine antagonists. *J. Med. Chem.*, 31 (1988) 656–671.