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# Comparison between active and passive drug transport in human intestinal epithelial (Caco-2) cells in vitro and human jejunum in vivo

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**Abstract**

Drug transport rates in Caco-2 monolayers were compared with those obtained in the human jejunum in vivo. Permeability coefficients unbiased by the hydrodynamic conditions were calculated in order to allow direct comparison of the two models. The rapidly (passively) transported drugs naproxen, antipyrine and metoprolol had comparable permeability coefficients in Caco-2 cells and in human jejunum. The permeability coefficients of the slowly (passively) transported, hydrophilic drugs, terbutaline and atenolol, 79- and 27-fold lower, respectively, in Caco-2 cells than in jejunum. The carrier-mediated transport rates of L-dopa, L-leucine and D-glucose were also much slower in Caco-2 cells than in human jejunum. The lower permeability of the actively transported compounds and of atenolol and terbutaline is consistent with the colonic origin of the Caco-2 cells. The results indicate that Caco-2 monolayers can be used to predict passive drug transport in humans, while prediction of transport by carrier-mediated systems may require a scaling factor, due to a low expression of carriers in this cell line.

*Keywords:* Human intestinal permeability; Caco-2 cell; Drug transport; Membrane transport; In vitro–in vivo correlation; Permeability coefficient; Drug absorption

## 1. Introduction

The rate-limiting barrier to drug absorption across the intestinal mucosa is the single layer of intestinal epithelial cells. Transport rates of passively absorbed drugs in the Caco-2 cells have been shown to correlate with the fraction absorbed in humans (Artursson and Karlsson, 1991). However, no comparative studies on transmucosal transport rates in Caco-2 monolayers and human intestine have previously been performed. Data on human drug permeability are rare, but a new regional perfusion technique has made it possible to determine effective jejunal permeability coefficients ($P_{eff}$) for both passive

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and carrier-mediated transport (Lennernäs et al., 1992; Lennernäs et al., 1993; Lennernäs, 1994).

The aim in this report is to compare the permeability coefficients of compounds with different absorption mechanisms obtained in Caco-2 cell monolayers with the human effective jejunal permeability values.

## 2. Material and methods

Caco-2 cells (American Cell Culture Collection, Rockville, USA) of passage number 95-100 were used as described previously (Artursson, 1990). The transepithelial resistance of the monolayers were 202 $\pm$ 18 $\Omega$ cm$^2$ at 37°C. The drug solutions were added to the apical side of 21- to 32-day-old monolayers and samples were withdrawn from the basolateral side at regular time intervals. The apparent permeability coefficients ($P_{app}$) were determined at stirring rates ($V$) of 135 and 767 rpm, and then the cellular permeability coefficients ($P_c$) were calculated as previously described (Karlsson and Artursson, 1991).

The proximal jejunum was perfused in human volunteers as described earlier, and is based on a double balloon technique with a 10-cm long segment of the jejunum. The segment is perfused by single-pass approach at a flow rate of 2.0–3.0 ml/min. The effective permeability coefficients ($P_{eff}$) were calculated using the well-stirred model, $P_{eff} = \{(C_{in} - C_{out})/C_{out}\} \cdot Q_{in}/2\pi rl$ (Lennernäs et al., 1994). A human drug permeability data base is currently being established by using this human perfusion approach. The purpose is to establish a new biopharmaceutical classification scheme based on drug dissolution and human effective intestinal permeability (Amidon et al., 1995).

All chemicals used were of analytical grade. The drugs were analysed by HPLC. D-Glucose and L-leucine were measured using routine clinical chemistry methods (Lennernäs et al., 1993). $^{14}$C-D-glucose and $^{14}$C-L-leucine were analysed in a liquid scintillation counter (Caco-2 experiments). Variability is expressed as standard deviation.

## 3. Results and discussion

Permeability coefficients of six structurally diverse drugs were investigated. The rapidly and completely absorbed model drugs (naproxen, antipyrine and metoprolol) are sufficiently lipophilic to be absorbed by the most common route for orally administered drugs, i.e. passive transcellular diffusion (Lennernäs et al., 1993, 1994; unpublished results). Atenolol and terbutaline are slowly and incompletely absorbed in humans (Fagerholm et al., 1995; Lennernäs et al., 1994). Their hydrophilicity limits their partitioning into the lipophilic cell membranes and it is assumed that these drugs are absorbed mainly by the paracellular route. L-Dopa is rapidly transported in humans by the carrier for large neutral amino acids, and was therefore used as a model drug for active transcellular transport (Lennernäs et al., 1993). L-Leucine and D-glucose were included for comparative reasons since they are endogenous substrates for the carrier-mediated transport of amino acids and hexoses, respectively.

The results of the transport studies in Caco-2 monolayers and in human jejunum are summarised in Table 1. The permeability coefficients of the rapidly transported drugs were dependent on the hydrodynamics in the Caco-2 monolayers. However, the true $P_c$-estimate for antipyrine of 267 $\pm$ 7.0 $\times$ $10^{-6}$ cm/s was calculated according to Karlsson and Artursson, 1991. This value, which is directly comparable to that obtained in the well-stirred in vivo situation, was only 2-fold lower than the permeability coefficient for antipyrine in the human jejunum (Table 1). Similar results were obtained for the other two model drugs for passive transcellular drug absorption, naproxen and metoprolol (Table 1, Fig. 1). The slightly lower passive transcellular permeability in the Caco-2 monolayers was within the interindividual variability of the human data. It could also be related to a slight underestimation of the available absorptive surface area in the human jejunum (Lennernäs et al., 1992). These results give further support to the hypothesis that the intestinal epithelium and not the adjacent unstirred water layer is the rate-limiting barrier to absorption of rapidly transported drugs (such as

Table 1
Permeability coefficients obtained in Caco-2 monolayers ($P_{app}$, $P_c$) and from regional perfusions of the human proximal jejunum ($P_{eff}$), and fraction absorbed ($f_a$) observed in vivo in man. The permeability values are mean ± S.D. The pH was 7.4 in all experiments except for metoprolol (human model) and naproxen (both models) which used a pH of 6.5.

| Solute | Caco-2 monolayer data | | | | Human data | | |
|---|---|---|---|---|---|---|---|
| | Conc. (mM) | $P_{app}$, 135 rpm ($\cdot 10^{-6}$ cm/s) ($n$=3–4) | $P_{app}$, 767 rpm ($\cdot 10^{-6}$ cm/s) ($n$=3–4) | $P_c$ ($\cdot 10^{-6}$ cm/s) ($n$=7–8) | Conc (mM) | $P_{eff}$ ($\cdot 10^{-4}$ cm/s) ($n \geq 8$) | $f_a$ (%) |
| Antipyrine | 0.1 | 82±4 | 167±4 | 267±7 | 0.1 | 5.6±1.6 | 100[b] |
| Naproxen | 1.8 | 89±2 | 148±7 | 206±7 | 1.8 | 8.0±4.1 | 100[c] |
| Metoprolol | 1.0 | 64±4 | 80±5 | 92±4 | 0.6 | 1.5±0.9 | 95(≥95)[c,d,h] |
| Terbutaline | 0.001[a] | — | — | 0.38±0.03[a] | 0.01 | 0.3±0.3 | 60(25-80)[e,f,g] |
| Atenolol | 2.0 | 0.36±0.03 | 0.51±0.07 | 0.55±0.05 | 0.83 | 0.15±0.2 | 50(40-70)[c,h] |
| D-Glucose | 10 | 20±2 | 24±1 | 25±1 | 10 | 11.0±8.2 | 100 |
| L-Dopa | 2.5 | 0.82±0.23 | 1.1±0.2 | 1.0±0.2 | 2.5 | 3.4±1.7 | 100[i] |
| L-Leucine | 40 | 0.30±0.03 | 0.47±0.09 | 0.51±0.08 | 40 | 6.2±2.9 | 100 |

The human $P_{eff}$-values are obtained from the following references: Lennernäs. H., et al., 1992, 1993, 1994; Fagerholm, U., et al., 1995. [a]Artursson. P. and Karlsson. J., 1991 ($n$=3). [b]Eichelbaum. M., et al., 1982. [c]Therapeutic Drugs, ed. A. Wade, 1991. [d]Sandberg. A., Doctorial thesis, 1994. [e]Martindale, 27th edition, 1977. [f]Borgström. L., et al., 1989. [g]Nyberg. L., 1984. [h]Clarke's Isolation and Identification of Drugs, 2nd edition, 1986. [i]Yeh. K.C. et al., 1989.

antipyrine, naproxen and metoprolol). (Karlsson and Artursson, 1991; Lennernäs et al., 1992). We therefore conclude that the passive transcellular transport rate of drugs in human jejunum in vivo can be predicted in Caco-2 monolayers.

The permeability values of the incompletely absorbed drugs, atenolol and terbutaline, were 27 and 79 times lower, respectively, in the Caco-2 monolayers than in the human jejunum (Table 1, Fig. 1). Although the permeability values in the Caco-2 monolayers are within the wide range of those observed in the human jejunum, it is unlikely that the difference in paracellular permeability is related to inter- or intra-individual variability. More likely, the lower mean permeability in the Caco-2 monolayers is related to the low paracellular permeability of this colon-derived cell line (Artursson et al., 1993). Thus, the permeability values of hydrophilic compounds in the Caco-2 monolayers are closer to those seen in the human colon. This further supports the notion that Caco-2 monolayers can be used to identify passively absorbed drugs with potential absorption problems after oral administration.

The carrier-mediated transport rate of L-dopa was approximately 340-fold slower in Caco-2 monolayers than in human jejunum. This finding was supported by an even slower transport of L-leucine in the Caco-2 monolayers. The slower transport of the natural substrate for the large neutral amino acid carrier is probably explained by saturation of the carrier, since a higher concentration of L-leucine was used than for the drug L-dopa (Lennernäs et al., 1993). A similar, if not so pronounced, difference in the permeability of D-glucose was observed between the two models. Since relatively high concentrations of L-dopa, L-leucine and D-glucose were used, we cannot exclude that the compounds were partly transported also by the passive paracellular route in the Caco-2 monolayers. Nevertheless, the results are in agreement with previous studies in Caco-2 monolayers which show that this cell line displays a variable and generally lower expression of carrier-mediated transport than seen in vivo (Hu and Borchardt, 1990). Prediction of human active drug transport in Caco-2 monolayers will therefore only be possible after characterisation of each transport system and subsequent introduction of a scaling factor to compensate for the different expression of the carrier in Caco-2 cells from that seen in vivo.

The human effective permeability coefficient ($P_{eff}$) is measured at steady state in the perfusion

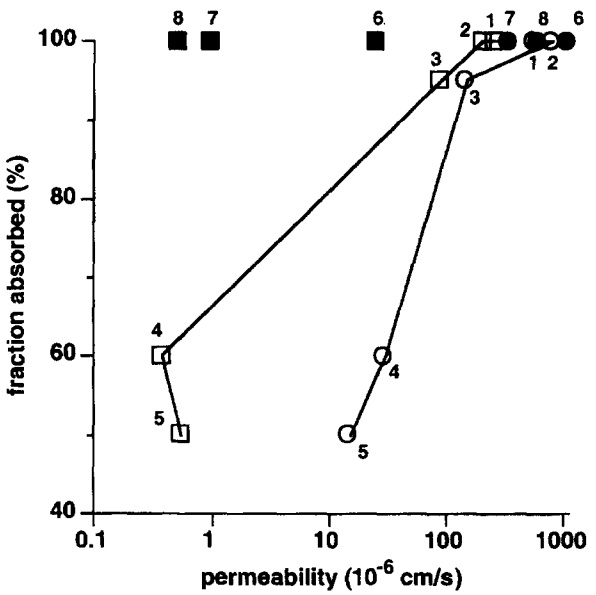


Fig. 1. Fraction absorbed in humans after oral administration as a function of permeability coefficients in the Caco-2 model (□, passive transport; ■, active transport) and human jejunum (○, passive transport; ●, active transport). For identification of each compound: 1, antipyrine; 2, naproxen; 3, metoprolol; 4, terbutaline; 5, atenolol; 6, D-glucose; 7, L-dopa; 8, L-leucine.

system, and is calculated from the disappearance rate of the drug from the segment, which then is related to the smooth cylinder area of the jejunal segment. This value should not be considered as the 'true' permeability value of the intestinal wall, instead the effective permeability obtained in man reflect the transport across the mucosa under the conditions used with this perfusion approach. However, the human effective permeability has been shown to correlate very well with the fraction absorbed in vivo for a broad range of structurally different compounds, which suggests a mass balance between disappearance and appearance rate (Lennernäs et al., 1992; Lennernäs, 1994). Furthermore, the effective absorbing area is not possible to include in the calculations, but its numerical value is probably similar to the cylinder area, since it is well established that solute and fluid absorption occurs mainly in the upper part of the villus (Chang and Rao, 1994).

In conclusion, these results show that Caco-2 monolayers can be used to predict passive drug transport in humans. However, prediction of carrier-mediated transport will require a scaling factor, due to the low expression of carriers in this cell line. The almost quantitative relationship between the permeability coefficients for rapidly (passively) transported drugs in the two models indicates that the intestinal epithelial cell layer is the rate-limiting barrier to drug absorption.

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## References

Amidon., G.L., Lennernäs, H., Shah, V. and Crison, J., Theoretical considerations in the correlation of in vitro drug product dissolution and in vivo bioavailability: A basis for a biopharmaceutics drug classification. *Pharm. Res.*, 12 (1995) 413–420.

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. Comm.*, 3 (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.

Borgström, L., Nyberg, L., Jönsson, S., Lindberg, C. and Paulson, J., Pharmacokinetic evaluation in man of terbutaline given as separate enantiomers and as the racemate. *Br. J. Clin. Pharmacol.*, 27 (1989) 49–56.

Chang, E.B. and Rao, M.C., Intestinal water and electrolyte transport: mechanisms of physiological and adaptive responses. In Johnson L.R. (Ed.), *Physiology of the Gastrointestinal Tract*, Raven Press, New York, 1994, pp. 2027–2081.

*Clarke's Isolation and Identifying of Drugs*, 2nd edition. Ed. A.C. Moffat. The Pharmaceutical Press, London (1986).

Eichelbaum, M., Ochs, H.R., Roberts, G. and Somogyi, A., Pharmacokinetics and metabolism of antipyrine

(phenazone) after intravenous and oral administration. *Arzneim.-Forsch.*, 32 (1982) 575–8.

Fagerholm, U., Borgström, L., Ahrenstedt, Ö., Lennernäs, H., The influence of net water absorption on the small intestinal permeability of terbutaline, studied in vivo in man. *J. Drug Targeting.*, 3 (1995) 191–200.

Hu, M. and Borchardt, R.T., Mechanism of L-α-methyldopa transport through a monolayer of polarized human intestinal epithelial cells (Caco-2). *Pharm. Res.*, 7 (1990) 1313–1319.

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

Lennernäs, H., Ahrenstedt, Ö., Hällgren, R., Knutson, L., Ryde, M. and Paalzow, L.K., Regional jejunal perfusion, a new in vivo approach to study oral drug absorption in man. *Pharm. Res.*, 9 (1992) 1243–1251.

Lennernäs, H., Nilsson, D., Aquilonius, S.-M., Ahrenstedt, Ö., Knutson, L. and Paalzow, L.K., The effect of L-leucine on the absorption of levodopa, studied by regional jejunal perfusion in man. *Br. J. Clin. Pharmacol.*, 35 (1993) 243–250.

Lennernäs, H., Ahrenstedt, Ö. and Ungell, A.-L., Intestinal drug absorption during induced net water absorption in man; A mechanistic study using antipyrine, atenolol and enalaprilat. *Br. J. Clin. Pharmacol.*, 37 (1994) 589–596.

Lennernäs, H., Gastrointestinal absorption mechanisms: A comparison between animal and human models. *Eur J. Pharm. Sci.*, 2 (1994) 39–43.

*Martindale*, 27th edition. Ed. A. Wade, The Pharmaceutical Press, London (1977).

Nyberg, L. Pharmacokinetic parameters of terbutaline in healthy man. An overview. *Eur. J. Resp. Dis.*, 65 (1984) 149–60.

Sandberg, A. *Extended-release metoprolol.* Doctorial thesis, Uppsala (1994).

*Therapeutic Drugs.* Ed. Sir C. Dollery. Churchill Livingstone, New York (1991).

Yeh, K.C.H., August, T.F., Bush, D.F., Lasseter, K.C., Musson, D.G., Schwartz, S., Smith, M.E., and Titus, D.C., Pharmacokinetics and bioavailability of Sinemet CR: A summary of human studies. *Neurol.*, 39 (1989) 25–35.