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Differentiated intestinal epithelial cell lines as *in vitro* models for predicting the intestinal absorption of drugs

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Abstract

The oral absorption of a compound is a critical factor for the future of the compound as a drug. This absorption is mainly controlled by the passage across the intestinal epithelium. Thus, the prediction of the intestinal absorption by means of an *in vitro* model may represent a powerful tool for the early selection of molecules during the process of drug development. In the present study, the differentiated human intestinal epithelial cell line HT29-18-C_1, was grown on permeable filters in dual chambers. These cells formed tight monolayers that were used to measure *in vitro* the transepithelial permeability coefficient (P_c) of various molecules. The results were compared with *in vivo* data of oral absorption. A threshold value of *in vitro* permeability of 2×10^{-6} cm/s was found. Molecules having a permeability coefficient higher than this value were absorbed orally more than 80%, while drugs with P_c values lower than 2×10^{-6} cm/s were poorly absorbed. By mathematical simulation, it was found that this P_c value, when extrapolated to the surface area and volume of the small intestine, corresponds to an absorption of 80% for a compound with a transit time through the small intestine of 5 h. This demonstrates the predictive utility of the threshold value of the permeability coefficient derived from the *in vitro* model of intestinal epithelium.

Abbreviations: P_c, transepithelial permeability coefficient; MTX, methotrexate

Introduction

The oral route is the most convenient and the most widely used in drug delivery. During the process of drug development, oral bioavailability of a molecule is thus a critical factor for the future of this compound as a drug. This oral bioavailability is primarily controlled by the process of drug absorption across the intestinal epithelium. The determination of the intestinal absorption is traditionally obtained from *in vivo* experiments that use large amounts of product and are time-consuming. *In vitro* models are increasingly being developed for studying drug transport and metabolism across the intestinal epithelium (Hidalgo et al., 1989; Hilgers et al.,

1990; Artursson and Karlsson, 1991; Wils et al., 1993). These studies have been made possible by the ability of human colon carcinoma cell lines such as Caco-2 or HT29 to differentiate in culture and to express many features of mature enterocytes. The Caco-2 cell line and the subclone HT29-18-C_1 both exhibit the differentiated phenotype of polarized absorptive cells (Pinto et al., 1983; Huet et al., 1987), while methotrexate (MTX)-adapted HT29 cells differentiate into mucus-secreting cells (Lesuffleur et al., 1991). In the present study we used HT29-18-C_1 cells grown on permeable filters in dual chambers to determine the transepithelial permeability coefficient of different molecules and the results were compared with *in vivo* data of oral absorption. Furthermore, the influence of mucus on the transepithelial passage of some drugs has been studied with MTX-adapted HT29 cells. These results demonstrate the predictive value of *in vitro* measurements of the intestinal permeability.

Materials and methods

Radiolabeled compounds

The following radiolabeled drugs were obtained from the sources indicated: [^{14}C]mannitol (49.3 mCi/mmol), [^{3}H]propranolol (23.4 Ci/mmol) and [^{3}H]testosterone (24.6 Ci/mmol) from NEN (Les Ulis, France); [^{14}C]diazepam (53.8 mCi/mmol) from Amersham (Les Ulis, France); [^{14}C]RP12535 (pristinamycin IA, 20 mCi/mmol), [^{14}C]RP12536 (pristinamycin IIA, 20 mCi/mmol), [^{14}C]RP62203, 2-(3-(4-(4-fluorophenyl)-1-piperazinyl)-propyl) naphto(1,8-c,d)-iso-thiazole-1,1-dioxide (59 mCi/mmol) and [^{14}C]RP62955, 2-(7-chloronaphthyridine)-3-(5-methyl-2-oxohexyl)1-isoindolinone (92 mCi/mmol) from CEA, Service des Molécules Marquées (Gif-sur-Yvette, France); [^{14}C]RP64477, *N*-(2-methylthio-5-n-butylamino-carbonyl-phenyl)-4-decyloxybenzamide (10.55 mCi/mmol) from Rhône-Poulenc Rorer, Radiochemistry Section (Dagenham, UK). The radiochemical purity of the compounds was higher than 90% as determined by HPLC. Labeled drugs were stored in ethanol or dimethylformamide, and were diluted extemporaneously in the medium used for transport experiments.

Cell culture

HT29-18-C_1 cells were obtained from Dr C. Huet (CNRS, URA1149, Institut Pasteur, Paris, France) and cultured as described elsewhere (Wils et al., 1993). HT29 cells adapted to 10^{-5} mol/L methotrexate (referred to as HT29-MTX) were obtained from Dr A. Zweibaum (INSERM U178, Villejuif, France) and grown as described (Lesuffleur et al., 1991). They were used between passages 15 and 30 (in the absence of methotrexate). For transport experiments, cells were seeded at 100 000 cells/cm^2 on polycarbonate membranes (Transwell, 0.4 μm pore size, from Costar, Brumath, France). Medium was changed daily and filters were used at day 14 after seeding. Electron microscopy was performed as described (Wils et al., 1993).

Transport experiments

Transport studies were performed as described by Wils et al. (1993) except that the incubation medium was BHK21 (Glasgow Modified Eagle's Medium)–25 mmol/L Hepes, pH 7.4. Briefly, the apical medium was replaced with medium containing the radiolabeled compound. At various times, 100 μl were removed from the basolateral chamber and counted by liquid scintillation. The permeability coefficients were calculated from the equation:

$$P = dQ/dt \times 1/C \times 1/S$$

where dQ/dt is the permeability rate across the monolayer (dpm/s), C is the initial concentration

of drug in the donor compartment (dpm/µl) and S is the surface area of the monolayer. For HT29-MTX cells, on the day before the experiment, the complete culture medium was replaced by a serum-free medium and the wells were not washed before the experiment in order to keep the secreted mucous layer intact.

Results and discussion

The transepithelial permeability coefficient (P_c) of various compounds across HT29-18-C_1 monolayers was determined. Among the different compounds tested, RP64477 exhibited the lowest P_c value, 0.38 × 10^{-6} cm/s; testosterone and diazepam were the most rapidly transported through the epithelial monolayer, with P_c values around 40 × 10^{-6} cm/s. When the percentage of absorption of the drugs after oral administration in rats was plotted against the P_c value obtained *in vitro* (Figure 1), a P_c value of 2.5 × 10^{-6} cm/s seemed to be a threshold: above this value, drugs are absorbed more than 75%,

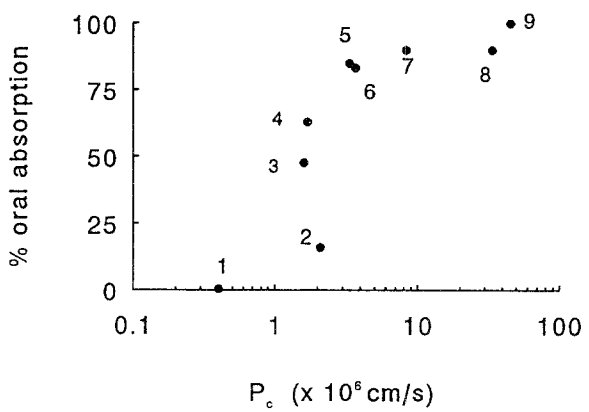

Figure 1. Oral absorption of various compounds in rats as a function of the *in vitro* permeability coefficient (P_c) across HT29-18-C_1 cells. The drugs are **1**, RP64477; **2**, mannitol; **3**, RP12536; **4**, RP12535; **5**, RP62955; **6**, RP62203; **7**, propranolol; **8**, diazepam; **9**, testosterone. For compounds 1, 2, 7, 8 and 9 the oral absorption was obtained from the literature (Artursson and Karlsson, 1991; Ashton et al., 1991). For the other molecules the oral absorption data are a personal communication from Dr M. Marlard, Rhône-Poulenc Rorer, Vitry-sur-Seine, France.

while drugs that have P_c values lower than this are poorly absorbed in animals or humans. Therefore, the results obtained in the present *in vitro* model agree perfectly with the *in vivo* situation. This confirms the work of Artursson and Karlsson (1991), who reported for the Caco-2 cell line that a threshold P_c value of 1 × 10^{-6} cm/s was necessary for maximal oral absorption. Our P_c values agree with those reported by these authors, except for mannitol, which has a P_c value ten times higher in HT29-18-C_1 cells. This could be due to the fact that HT29-18-C_1 monolayers are less tight than those obtained with Caco-2 cells.

The *in vitro* P_c value can be extrapolated to the physiological situation and can thus be used to calculate the *in vivo* absorption of a compound. The absorption surface area of the small intestine is considered to be 10 m^2 (which yields a PS value of 12 ml/min) and the volume of the intestinal lumen is 2.5 L (Bernier and Rey, 1983). Using first-order exponential kinetics for drug absorption, one can calculate that a P_c value of 2 × 10^{-6} cm/s corresponds to an *in vivo* absorption in the small intestine of 80% of the compound within 5.5 h (Figure 2), which is relevant to the transit time through the jejunum and the ileum in humans. For a drug with a P_c value four times lower (0.5 × 10^{-6} cm/s), the time necessary to have 80% absorption will be more than 20 h (Figure 2). Thus, only drugs having a P_c value higher than the threshold value of 2 × 10^{-6} cm/s will be absorbed within their transit time along the small intestine.

The results obtained demonstrate that intestinal epithelial cell monolayers are a relevant model for studying intestinal drug absorption, and that the *in vitro* measurement of the transepithelial permeability coefficient can be used to predict the oral absorption of a drug. Moreover, this model represents a powerful tool for analysing the relationship between the physicochemical characteristics of drugs and their transepithelial passage (Wils et al., 1994).

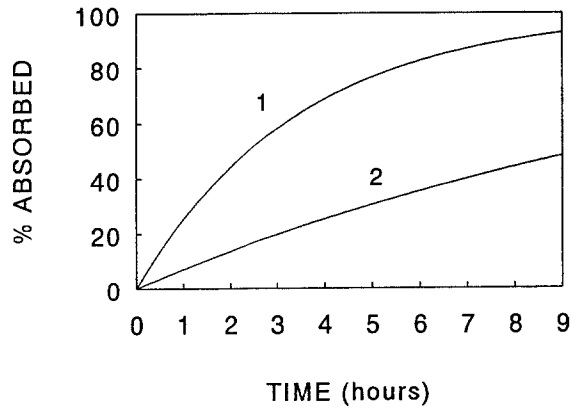

Figure 2. Computation of drug absorption across the intestinal epithelium with first-order exponential kinetics. Curves 1 and 2 correspond to molecules having *in vitro* permeability coefficient values of 2×10^{-6} cm/s and 0.5×10^{-6} cm/s, respectively.

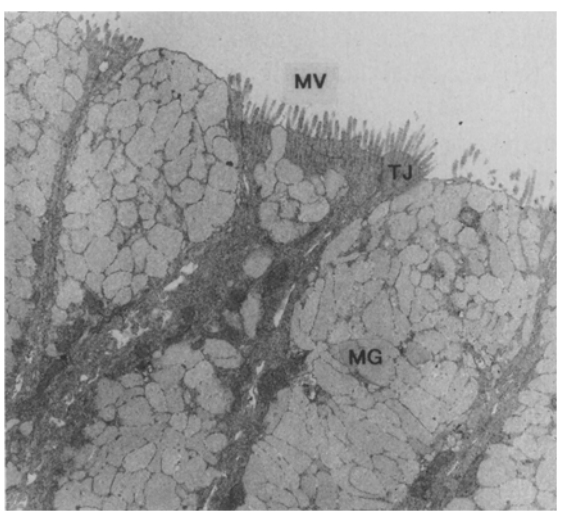

Figure 3. Transmission electron micrograph of HT29-MTX cells grown on permeable filters. The cytoplasm is filled with mucin granules (MG) and cells are joined together by tight junctions (TJ). Microvilli (MV) and are also clearly visible on the apical surface. Bar = 1 μm.

We have also used the HT29-MTX cells to study the influence of mucus on the permeability of the drugs and to obtain a model that mimics more closely the physiological situation. These cells, when grown on filters, form a tight monolayer of mucus-secreting cells. After 14 days in culture, the mucus appears as a viscoelastic gel on the apical surface of the monolayer and can be visualized by electron microscopy (Figure 3). As shown in Table 1, this cell line can be used as a mucus-secreting model for studying the transepithelial passage of various molecules. The permeability coefficient of the three compounds studied was not significantly different on the mucus-secreting cells as compared with those obtained on the absorptive HT29-18-C_1 cells. Using another mucus-secreting cell line, Karlsson et al. (1993) showed that the mucus layer was a significant barrier to the absorption of very lipophilic compounds such as testosterone. The present results suggest that this is not the case for other lipophilic molecules such as propranolol and diazepam.

Table 1. Transepithelial permeability coefficients of mannitol, propranolol and diazepam across absorptive HT29-18-C_1 and mucus-secreting HT29-MTX cell monolayers

Drug	$P_c \times 10^6$ (cm/s)[a]	
	HT29-18-C_1	HT29-MTX
Mannitol	0.8 ± 0.3 (3)	0.7 ± 0.2 (4)
Propranolol	10.2 ± 0.3 (3)	11.5 ± 0.6 (3)
Diazepam	39.5 ± 1.5 (6)	41.0 ± 1.0 (3)

[a]Data are mean ± SD (number of wells)

In conclusion, the various cell lines derived from colon carcinoma cells HT29 are good models for studying the different barrier functions of the intestinal epithelium. The *in vitro* determination of the transepithelial permeability coefficient of a molecule allows a good prediction of its oral absorption.

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