Correlation Between Oral Drug Absorption in Humans, and Apparent Drug Permeability in TC-7 Cells, A Human Epithelial Intestinal Cell Line: Comparison with the Parental Caco-2 Cell Line

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Purpose. To determine and compare the relationship between *in vivo* oral absorption in humans and the apparent permeability coefficients (P_{app}) obtained *in vitro* on two human intestinal epithelial cell lines, the parental Caco-2 and the TC-7 clone.

Methods. Both cell lines were grown for 5–35 days on tissue culture-treated inserts. Cell monolayers were analysed for their morphology by transmission electron micrography, and for their integrity with respect to transepithelial electrical resistance, mannitol and PEG-4000 transport, and cyclosporin efflux. P_{app} were determined for 20 compounds exhibiting large differences in chemical structure, molecular weight, transport mechanisms, and percentage of absorption in humans.

Results. The TC-7 clone exhibits morphological characteristics similar to those of the parental Caco-2 cell line, concerning apical brush border, microvilli, tight junctions and polarisation of the cell line. The TC-7 clone however appeared more homogenous in terms of cell size. Both cell lines achieved a similar monolayer integrity towards mannitol and PEG-4000. Monolayer integrity was achieved earlier for the TC-7 clone, mainly due to its shorter doubling time, i.e. 26 versus 30 hours for parental Caco-2 cells. When using cyclosporin A as a P-glycoprotein substrate, active efflux was lower in the TC-7 clone than in the parental Caco-2 cells. The P_{app} and mechanisms of transport (paracellular or transcellular routes, passive diffusion and active transport) were determined for 20 drugs. A relationship was established between the *in vivo* oral absorption in humans and P_{app} values, allowing to determine a threshold value for P_{app} of 2 10^{-6} cm/sec, above for which a 100% oral absorption could be expected in humans. Both correlation curves obtained with the two cell types, were almost completely superimposable. These studies also confirmed that the dipeptide transporter is underexpressed in both cell lines.

Conclusions. On the basis of morphological parameters, biochemical activity and drug transport characteristics, the TC-7 clone appeared to be a valuable alternative to the use of parental Caco-2 cells for drug absorption studies.

KEY WORDS: Caco-2 cells, *in vitro* absorption, *in vitro-in vivo* relationship.

INTRODUCTION

In order to successfully mimic a biological barrier like the intestinal wall with an *in vitro* cell culture system, the selection, and then the properties of the cell line are of paramount importance (1). Among the different human colon carcinoma cell lines, the Caco-2 cells isolated by Fogh *et al.* (2) were reported to undergo spontaneous morphological and biochemical enterocytic differentiation in culture (3) and developed a well-defined brush border on the apical surface as well as tight cellular junctions (4). When cultured on filter inserts, they form a tight differentiated monolayer, thus constituting an *in vitro* model which allows the rapid evaluation of the permeability of drugs, the study of drug absorption mechanisms under controlled conditions, and the screening of approaches for improving drug absorption, e.g., the use of prodrugs, absorption enhancers, or other pharmaceutical additives (1,5–7). Compared to *in vivo* animal models, such studies present the advantage of being performed on human cells. In addition, they also minimise the use of time-consuming, expensive, and sometimes controversial animal studies (8).

Among the drugs which are transported across the gastrointestinal barrier, some do so via a passive diffusion process, either through the cells (transcellular absorption) or between the cells (paracellular absorption). Certain drugs however, (1,9–10) are able to utilise active transport pathways which are present in the small intestine, for the uptake of amino acids, di-and tri-peptides or sugars. These pathways require energy and can operate against a concentration gradient. Moreover, when carrier mediated transport occurs, a dose-related decrease in absorption can often be observed because of the carrier capacity limitation (i.e. saturating process). Structural analogues within a series of xenobiotics, as well as endogenous compounds can compete for such active transport pathways.

More recently, in order to validate the use of this cell culture model for anticipating *in vivo* absorption in humans, various attempts have been made at establishing a relationship between *in vitro* permeability of Caco-2 monolayers and human absorption *in vivo* (11–14). Although these various authors all established such relationships, the threshold P_c values reported, i.e. the minimum apparent permeability coefficient required to anticipate a 100% absorption in humans, varied between 1×10^{-6} cm/sec (12) and 6×10^{-5} cm/sec (14). These differences could result either from cell culture conditions (cell seeding, confluency, cellular differentiation, presence or absence of essential nutrients and/or growth factors and/or a metabolic source of energy or even ions), from cell characteristics, or, from varying experimental conditions. All these factors need to be carefully optimised and controlled so as to mimic as best as possible the *in vivo* biological barrier.

Moreover, various authors have isolated numerous subclones from the Caco-2 parental cell line. Woodcock *et al.* (15) selected different subclones which displayed increased taurocholic acid transport, while Chantret *et al.* (16) isolated different clones, from early and late passages of the parental Caco-2 cell line, which differed mainly in the levels of expression of sucrase isomaltase and glucose transporters (17). One of these clones, the TC-7 clone, also displayed increased taurocholic acid transport compared to Caco-2 parental cell line (18).

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The aim of this study was i) to develop an *in vitro* approach in order to investigate the transport process of different drugs exhibiting specific physico-chemical characteristics, ii) to analyse the relationship between oral absorption in humans and *in vitro* permeability through the monolayers, and iii) to compare the properties of the parental Caco-2 cell line with its TC-7 clone selected from late parental Caco-2 passage.

MATERIALS AND METHODS

Chemicals

The drug substrates used in this study were obtained from different sources; [1-methyl-^{14}C]-caffeine (53.3 mCi/mmol), *L*-[^{14}C-(U)]-glutamine (264.6 mCi/mmol), [1,2,6,7-^{3}H-(N)-]-hydrocortisone (78.4 Ci/mmol), [^{3}H(G)]-inuline (32.1 Ci/mmol), *D*-[1-^{3}H(N)]-mannitol (22.4 Ci/mmol), [^{14}C]-PEG-400 (15.3 Ci/mg), [1,2-^{3}H]-PEG-4000 (2.27 mCi/mg), L-[4-^{3}H]-propranolol (15 mCi/mmol), [^{14}C(U)]-sucrose (4.78 mCi/mmol) and [^{3}H(G)]-taurocholic acid (2 Ci/mmol) were purchased from New England Nuclear products (Boston, U.S.A.). [4-^{14}C]-Testosterone (57.3 mCi/mmol) was obtained from Dositek, Orsay, France. [^{3}H]-*L*-phenylalanine (10 Ci/mmol) and [^{3}H]-cyclosporin A (8.9 Ci/mmol) were purchased from ICN (Orsay, France) and Amersham (Les Ulis, France), respectively.

Amoxicillin, antipyrine, atenolol, caffeine, cefalexin, cyclosporin A, *L*-glutamine, hydrocortisone, inuline, (±)-metoprolol tartrate salt, *D*-mannitol, *L*-phenylalanine, sucrose, terbutaline (hemisulfate salt), testosterone and taurocholic acid were purchased from Sigma. PEG-400 and PEG-4000 were obtained from Aldrich (St Quentin Fallavier, France) and propranolol from Interchim (Montluçon, France). Enalaprilate was a kind gift of Merck. All compounds used were of analytical grade.

Culture Medium

Phosphate buffered saline (PBS), trypsine, ethyleneglycol-bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), ethylene diamine tetraacetic acid (EDTA), Dulbecco's modified Eagle medium (DMEM), nonessential amino acids, L-glutamine, penicillin, streptomycin, fungizone, *D*-glucose, Hanks' buffered salt solution (HBSS) and foetal calf serum (FCS) were purchased from EUROBIO Laboratories (Paris, FRANCE).

Cell Culture

Parental Caco-2 cells, originating from a human colorectal carcinoma (2), were obtained from Dr J. Fogh (Sloan Kettering Institute for Cancer Research, Rye, NY). TC-7 cells selected from a late passage (P-198) of Caco-2 parental cells (16), were obtained from Dr M. Rousset (INSERM U-178, Villejuif, FRANCE). Cells used in this study were between passages 70 and 110 for Caco-2 cells and between 40 and 80 for TC-7 clone. Caco-2 and TC-7 cells were seeded at a density of 1.3 $\times$ 10^4 cells/cm^2, and grown in 75 cm^2-flasks at 37°C in an atmosphere of 10% CO_2, using DMEM supplemented with 1% nonessential amino acids, 20% heat-inactivated FCS, 10 mM L-glutamine, 45 IU/mL penicillin, 45 μg/mL streptomycin and 1 μg/mL fungizone. D-Glucose concentration in culture medium was 4.5 g/L (25 mM). The medium was changed every other day until the flasks reached 90% confluence. Under these culture conditions cells became confluent 5 to 6 days after seeding (27,28). After an initial washing step with 10 mL PBS, cells were detached with 2 mL Trypsin (0.25%)−0.2% EDTA in Ca^{2+}- and Mg^{2+}-free PBS at pH 7.4 for 10 minutes at 37°C.

For transport studies Caco-2 cells were seeded onto 3 μm-pore Transwell-clear (Costar) at 2 $\times$ 10^5 cells/cm^2. The filters were inserted into the wells of 6 well-plates (Costar).

Drug Transport

Integrity of the Monolayers

Before each experiment, monolayers integrity was determined either by measuring the transepithelial electrical resistance, *i.e.* TEER (19), or by following the transepithelial transport of a poorly absorbed marker, mannitol. The potential difference was expressed by the TEER (ohms.cm^2), after subtraction of the intrinsic resistance of the model (*i.e.* the resistance obtained over the cell-free inserts). A monolayer with a low TEER, or with a high mannitol transport rate, *i.e.* above 1% per hour, was assumed to exhibit extensive leakage through imperfect occluding junctions or holes in the monolayer.

Measurement of Drug Transport

Drug solutions were prepared in DMSO in order to achieve a 50 mM final concentration. For transport studies, drugs were then diluted to 0.1% in HBSS, in order to achieve a final concentration of 50 μM, and a final solvent concentration of 0.1%. When [^{14}C]- or [^{3}H]-radiolabelled compounds were used, final specific activity was 0.5 μCi/mL. All transport experiments were performed in a 10%-CO_2 incubator at 95% relative humidity and 37°C in serum-free Hanks buffer (pH 7.4) containing 1 g/L *D*-glucose and 10 mM HEPES buffer. The monolayers were continuously agitated on a plate shaker during the transport experiments.

Protocol for Drug Transport Experiments

For each drug investigated, both "Apical-to-Basal" and "Basal-to-Apical" transports were investigated in order to demonstrate either a carrier-mediated transport, or a carrier-mediated efflux. "Apical-to-Basal" passage was also investigated after addition of 2.5 mM EGTA in both compartments, in order to demonstrate a transport of drugs through the paracellular route. Usually 0.2 mL-aliquots were withdrawn from the receiving compartment at determined time intervals (0.5, 1, 2 and 4 hours). After sample withdrawal, an equivalent volume of the buffer was added to the receiving compartment to keep the receiver fluid volume constant. The rate of appearance of the drug in the receiving compartment was monitored. Apparent permeability coefficients were determined following duplicate experiments (n = 3 inserts, each).

Drug Analysis

For [^{14}C]- and [^{3}H]-radiolabelled drugs, radioactivity was determined in a Tricarb liquid scintillation spectrometer (Packard Instruments), by liquid scintillation counting (Ready Safe; Beckman, U.S.A.) of 0.2 mL aliquots of the incubation medium. Results were corrected to dpm by comparison with standard quench curves. For non-radiolabelled compounds, drugs were quantified by HPLC methods.

Calculations

The apparent permeability coefficients (P_{app}) were expressed in "10^{-7} cm/sec" and determined as previously reported (19):

$$P_{app} = dQ/[dt \times A \times C_o],$$

where

dQ/dt was the transport rate (μg/sec)

C_o the initial concentration in the donor chamber (μg/mL # μg/cm^3),

A, the area of the membrane (4.7 cm^2)

Transmission Electron Microscopy

Cells grown on filters were washed in fresh medium and fixed with a solution containing 2% glutaraldehyde in 0.1 M cacodylate buffer, for 45 minutes at 25°C. The cells were then fixed with a solution containing 2% glutaraldehyde in 0.1 M cacodylate buffer (2 volumes) and ruthenium red (1 volume) for 2 hours at 4°C. The cells were fixed again with a solution containing 1% osmium tetroxide in 0.1 M cacodylate buffer (2 volumes) and ruthenium red at 0.4% in distilled water (1 volume) for 3 hours at room temperature. The cells were finally rinsed three times (for twice 2 hours, then overnight) in 0.1 M cacodylate buffer. Filters were removed from the plastic holder, cut into quarters and placed in cacodylate buffer. Filters were dehydrated through an ascending series of ethanol washes (30% for 6 minutes, 70% for 6 minutes, then 100% for 15 minutes twice), infiltrated, embedded in Epon resin and cured in an oven at 60°C for 48 hours. Cells were sectioned on a Reichert OM4 microtome, stained with uranyl acetate and bismuth, visualised and photographed on a Philips CM10 electron microscope.

Scanning Electron Microscopy

Cells grown on filters were washed in fresh medium and fixed with a solution containing 2% glutaraldehyde in 0.1 M cacodylate buffer, for 3 hours at 4°C. The cells were fixed with a solution containing 1% osmium tetroxide in 0.1 M cacodylate buffer for 1.5 hours at room temperature. The cells were finally rinsed three times (for 2 hours twice, then over one week) in 0.1 M cacodylate buffer. Filters were dehydrated through an ascending series of ethanol washes (30% for 6 minutes, 50% for 6 minutes, 70% for 6 minutes, 80% for 6 minutes, 50% for 6 minutes then 100% for 15 minutes twice).

RESULTS AND DISCUSSION

Morphological Characterisation

The morphology of both cell lines is illustrated in Figure 1. Morphological measurements on the parental Caco-2 and the TC-7 clone cell monolayers indicate similar characteristics in both cell lines, the TC-7 clone exhibiting a more homogenous scheme. The cell height was similar for both cell lines, 13.8 ± 2.4 μm and 15.4 ± 1.2 μm for parental Caco-2 and TC-7 clone, respectively. Values for parental Caco-2 were close to the value of 25 μm reported by Neutra and Padykula (20) for the normal human intestinal epithelium, and the values of 29.6 μm (21) and 14.2 μm (6) reported at day 15–16 for Caco-2 cells grown on polycarbonate filters.

One of the factors existing in the monolayer, that could influence the permeability of drugs through the intestine, is the size and density of tight junctions, possibly resulting from a difference in the packing density of the cells. Growth curves for both the parental Caco-2 (passage 88) and the TC-7 clone (passage 80), demonstrated significant differences between the two cell lines, especially regarding growth rate (Figure 2) and cell density at late confluency. Doubling time for parental Caco-2 and TC-7 clone was 30 hours versus 26 hours, respectively. Cell density determined during the stationary phase, i.e. between day 9 and day 15, was 13 ± 3 × 10^6 and 18 ± 2 × 10^6 cells per 25 cm^2, for parental Caco-2 and TC-7 clone, respectively. These values are similar to those of Chantret *et al.* (16) who reported a doubling time of 20–24 hours for TC-7 clone (passage 6-20) and a cell density of 16 ± 1.5 × 10^6 cells per 25 cm^2 at late confluency. For comparison, doubling times for parental Caco-2 were 24 hours and 34 hours for passages 198 and 29, respectively, while cell density values were 25 and 12 × 10^6 cells per 25 cm^2, respectively.

Monolayer Transport Characteristics

Cells grown on polycarbonate filters for 23 days, formed monolayers with tight junctions connecting cells at their apical surface, and well-developed apical brush borders secreting glycoproteins labelled with ruthenium red, as observed for a normal intestinal epithelium (Figure 1). The functionality of the cell monolayer, was demonstrated by the evaluation of the resistance across tight junctions, i.e. transepithelial electrical resistance, and by the measurement of the transepithelial passage of either mannitol or PEG-4000 (Figure 2) over a 7 to 35-day period. Low concentrations of [^{3}H]-mannitol (flux of 0.6% of applied dose/hour) and [^{3}H]-PEG-4000 (flux of 0.3% of applied dose/hour) were detected in the basolateral culture fluid. A constant TEER value of 377.5 ± 25.5 ohm.cm^2 and 624.5 ± 83.6 ohm.cm^2 was achieved between days 18 and 35 for parental Caco-2 and TC-7 clone cells, respectively. Under the same experimental conditions, cell monolayers achieved a constant permeability to mannitol and PEG-4000, 8 and 7 days after cell seeding, respectively (Figure 2).

Transport of cyclosporin A in both the "Apical-to-Basal" and "Basal-to-Apical" directions was also evaluated over the period of cell differentiation, for both parental Caco-2 and TC-7 cells (Figure 3). The P_{app} of CsA in the "Basal-to-Apical" direction increased with the culture age and was always larger than that seen in the "Apical-to-Basal" direction. A large difference was observed between the two cell lines, regarding the "Basal-to-Apical" transport, which was at least 2-fold higher in the parental cell line, suggesting a higher expression of P-glycoprotein in the parental cell line. For both cell lines, 0.3 mM verapamil both, decreased the "Basal-to-Apical" and increased the "Apical-to-Basal" transport of CsA.

Apparent Permeability of Reference Drugs

P_{app} obtained for the "Apical-to-Basal" route, for both parental Caco-2 and TC-7 clone are reported in Table I. The values for fraction absorbed in humans were obtained from the literature and ranged from less than 1% for PEG-4000, to 100%

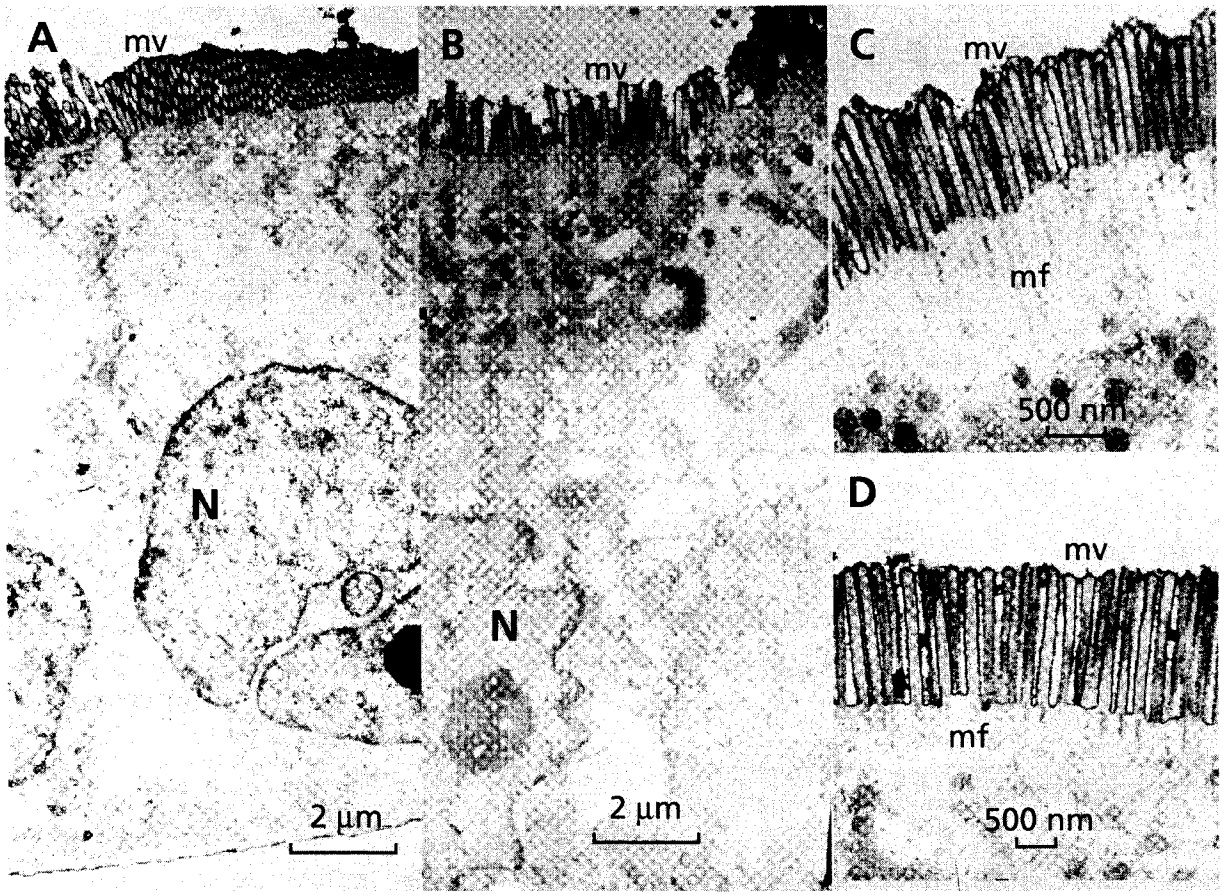

Fig. 1. Transmission electron micrograph of day 23 TC-7 clone (Passage 77; A) and Caco-2 parental (passage 86; B) cells. Electron micrograph of a section through cell monolayers grown on polycarbonate filters, showing extensive apical microvilli (mv) with microfilaments (mf), and basal Nuclear (N). In panels C and D an enlargement of the apical membrane is illustrated for TC-7 clone and parental Caco-2, respectively.

for various compounds, such as amoxicilllin, caffeine, cephalexin, *L*-phenylalanine, taurocholic acid or testosterone. As reported in Table I, values for fraction absorbed *in vivo* were highly variable due to physico-chemical characteristics, e.g. dose, dissolution rate limits, as well as inter-individual variability in metabolism, gastric emptying, food regimen, intestinal transit. . . .

All drugs evaluated were transported through the Caco-2 parental and TC-7 clone monolayers (Table I). Thus, it was possible to obtain apparent permeability coefficients even for compounds for which absorption in humans was very poor. Apparent permeability coefficients ranged from $1.45 \pm 0.2 \times 10^{-7}$ cm/sec for PEG-4000 to $842.9 \pm 33.5 \times 10^{-7}$ cm/sec for caffeine in parental Caco-2 cells, and from $1.52 \pm 0.1 \times 10^{-7}$ cm/sec for PEG-4000 to $758.4 \pm 32.1 \times 10^{-7}$ cm/sec for caffeine in TC-7 clone.

Characterisation of Transport Mechanisms

For each compound, the specific route of transport was also investigated. Hence, compounds were divided into two groups, those using a carrier-mediated transport and those transported across the intestinal epithelial barrier by passive diffusion, either by the transcellular or the paracellular route. Among the different compounds using a carrier-mediated route were amoxicillin, cephalexin, *L*-phenylalanine, sucrose and taurocholate. For these compounds the "Apical-to-Basal"/"Basal-to-Apical" ratios were 3, 10.5, 2, 2.5 and 7.6 for parental Caco-2 cells and 4, 8, 1.5, 2.5 and 6.3 for TC-7 clone, respectively. Although these differences in "Apical-to-Basal" and "Basal-to-Apical" unambiguously demonstrated the involvement of a specific polarised carrier, this single experiment did not allow comparison of the two cell lines in terms of transporter concentrations. For other compounds, a passive diffusion was demonstrated. The paracellular route is the largest component of the overall transport at the specific concentration investigated for atenolol, inuline, mannitol, PEG-400, PEG-4000, sucrose (22) and terbutaline (28).

In Vitro-In Vivo Relationship

The correlation obtained between the percentage of absorbed drug after oral administration in humans, and apparent permeability coefficients obtained in parental Caco-2 and TC-7 clone monolayers, is illustrated in Figure 4. Among the different drugs investigated, when compared to other drugs that are equally well absorbed *in vivo* by passive diffusion, amoxicillin showed relatively poor permeability across the monolayers, regardless of the cell line. For drugs using a carrier-mediated transport, this difference could be explained at least in part,

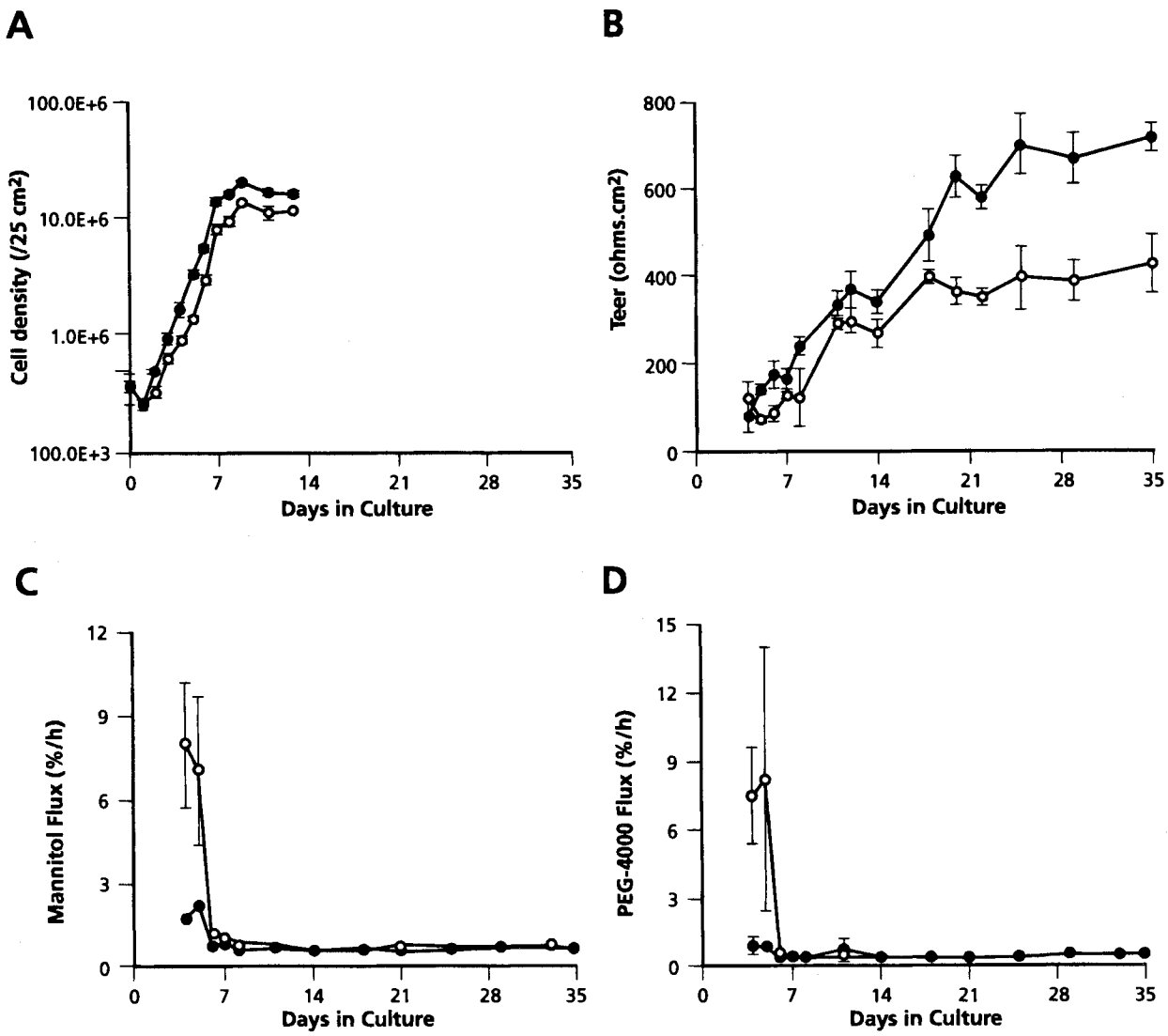

Fig. 2. Growth curves for parental Caco-2 (open circles) and TC-7 clone (close circles) cells obtained on 25 cm^2-plastic dishes are illustrated in panel 1. Parental Caco-2 (passage 88) and TC-7 clone (passage 80) cells were also grown on polycarbonate filters and different parameters of the permeability of the monolayer were determined as a function of day of culture. Parameters monitored were the transepithelial electrical resistance, i.e. TEER (B), mannitol flux (C), PEG-4000 flux (D). Results (± S.E.M. for 3 samples) are expressed in ohm.cm^2 for TEER and %/hour for mannitol and PEG-4000 fluxes.

either by the saturation of the carrier, or more likely by the fact that Caco-2 cells displayed a variable and generally lower expression of carrier-mediated transport than that seen *in vivo* (28). These observations were in agreement with data from Chong *et al.* (25) who reported that the permeability coefficients of an heterologous series of ten passively absorbed compounds, covering a wide range of physico-chemical properties (e.g., size, charge and lipophilicity) correlated fairly well with the extent of absorption in humans. However, based on the current permeability-absorption relationship defined for passively transported drugs, the predicted *in vivo* absorption for the four carrier-mediated drugs tested was as much as tenfold lower than the actual *in vivo* absorption value (24). This study demonstrated that the dipeptide transporter system in the Caco-2 cells monolayer was either saturated at the investigated concentration or more likely was quantitatively under-expressed when compared to *in vivo*.

More recent studies compared drug transport rates in Caco-2 monolayers, with those obtained in the human jejunum *in vivo* (28). The rapidly passively absorbed drugs, including naproxen, antipyrine and metoprolol, had comparable permeability in both models. However, some discrepancies were observed for carrier-mediated transported drugs (*L*-dopa, *L*-leucine and *D*-glucose). For these drugs, permeability coefficients obtained with the Caco-2 cells, were much lower than in the jejunum.

A parameter of still greater importance is the determination of the threshold P_c value, allowing to anticipate a 100% absorption in humans. For both cell lines, a threshold P_c value of 2×10^{-6} cm/sec was determined. Using 20 drugs and peptides with different structural properties, and with a 0–100% absorption in humans, Artursson and Karlsson (12) reported apparent permeability coefficients ranging from approximately 5×10^{-8} to 5×10^{-5} cm/sec. Drugs that were completely absorbed in humans, had permeability coefficients above 1×10^{-6} cm/sec.

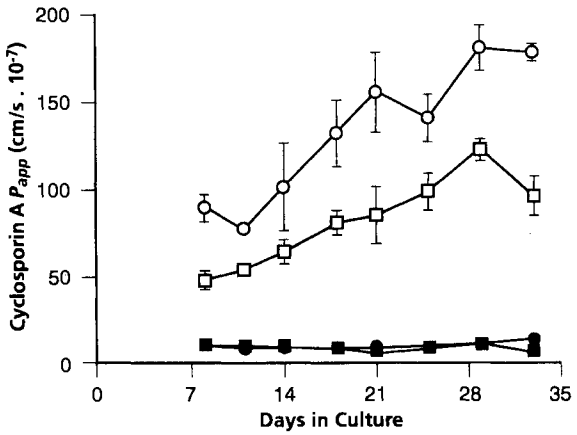

Fig. 3. Cyclosporin A (0.5 μM final concentration) was added either in the "Apical" or the "Basal" compartment and the rates of transport in the "Apical-to-Basal" (closed symbols) and "Basal-to-Apical" (open symbols) directions were monitored for both parental Caco-2 (passage 88; circles) and TC-7 clone (passage 80, squares). Results (± S.E.M. for 3 inserts) are expressed in cm/s.

Our data are also consistent with the results published by Stewart *et al.* (13), in which Caco-2 permeability were determined for a set of 6 compounds, and where a 100% absorption in humans was associated with an apparent permeability coefficient of approximately 2×10^{-5} cm/sec. Controversially, Rubas *et al.* (14) showed that 100% absorption in humans was only achieved for permeability values higher than 7×10^{-5} cm/sec.

CONCLUSIONS

These studies were performed to quantitatively correlate oral drug absorption in humans and apparent drug permeability coefficients in the parental human intestinal epithelial cell line Caco-2, and its TC-7 clone. Altogether, these data showed that both the TC-7 clone and the parental Caco-2 cell monolayers are a valuable tool to predict passive drug transport in humans. Some parameters helped to demonstrate that the TC-7 clone would be a more valuable tool for investigating transport characteristics, compared to parental Caco-2 cells. Hence (i) the TC-7 clone expresses similar levels of sucrase isomaltase compared to human jejunum, while levels are much lower in parental Caco-2 (16), (ii) enzymes involved in phase II metabolic reactions, i.e. UDP-glucuronyltransferases, are also present in the TC-7 clone as in human jejunum, while their activity is very low in Caco-2 cells (18), (iii) active transport of taurocholic acid is much faster in the TC-7 clone compared to parental Caco-2 (18), (iv) P-glycoprotein-mediated cyclosporin active efflux was less strongly expressed in the TC-7 clone compared to parental Caco-2, and (v) a relationship between *in vivo* oral absorption in humans and Papp *in vitro* is observed for both the TC-7 clone and the parental Caco-2 cell line.

Since large differences exist in the quantitative determination of apparent permeability coefficients from one laboratory to another, it is of the utmost importance to analyse permeability coefficients of new chemical entities in relation to a reference curve established with compounds exhibiting a large range of permeability coefficients and for which absorption from an orally administered dose in humans is known.

Table I. List of Compounds Tested and, *In Vitro* and *In Vivo* Absorption Characteristics

Drug	Codes	MW[a]	P_{app}[b] Caco-2	P_{app}[b] TC-7	f_a in Humans[c]
Amoxicillin	A	365.4	3.3±0.3	3.2±0.7	100
Antipyrine	B	188.2	490.1±37.4	505.0±32.1	97
Atenolol	C	266.3	11.6	3.4	40-70,50
Caffeine	D	194.2	842.9±34.4	758.4±64.9	100
Cephalexin	E	347.4	26.9±4.1	20.1±4.2	100
Cyclosporin A	F	1206	8.6±1.8	7.5±1.8	30
Enalaprilate	G	384.4	6.2±0.6	6.0±1.5	<10
L-Glutamine	H	146.2	8.5±2.2	10.2±1.3	60-90
Hydrocortisone	I	362.5	121.9±23.9	77.0±22.3	80,89,95
Inuline	J	5000	10.4±1.1	10.6±0.8	<1
D-Mannitol	K	182.2	11.7±1.4	9.2±1.0	5,16,17
Metoprolol	L	267.4	180.0±24.8	216.9±28.1	95
L-Phenylalanine	M	165.2	69.1±14.9	59.6±11.6	100
PEG-400	N	400	4.8±0.6	4.1±0.3	8-52,55.6
PEG-4000	O	4000	1.5±0.2	1.5±0.2	<1,0
Propranolol	P	259.3	344.3±22.6	346.8±30.3	90
Sucrose	Q	342.3	7.1±2.8	3.9±0.9	42
Taurocholate	R	515.7	40.2±15.5	37.4±3.3	100
Terbutaline	S	225.3	10.4±3.9	12.8±3.7	25-80,73
Testoterone	T	288.4	445.0±38.8	438.3±39.4	100

[a] Molecular weight.
[b] P_{app} correspond to the apparent permeability coefficients determined for the "Apical-to-Basal" route, and expressed in $\times 10^{-7}$ cm/sec.
[c] The human f_a values correspond to the percent absorbed of an orally administered dose, and were obtained from the literature (12,13,23–34).

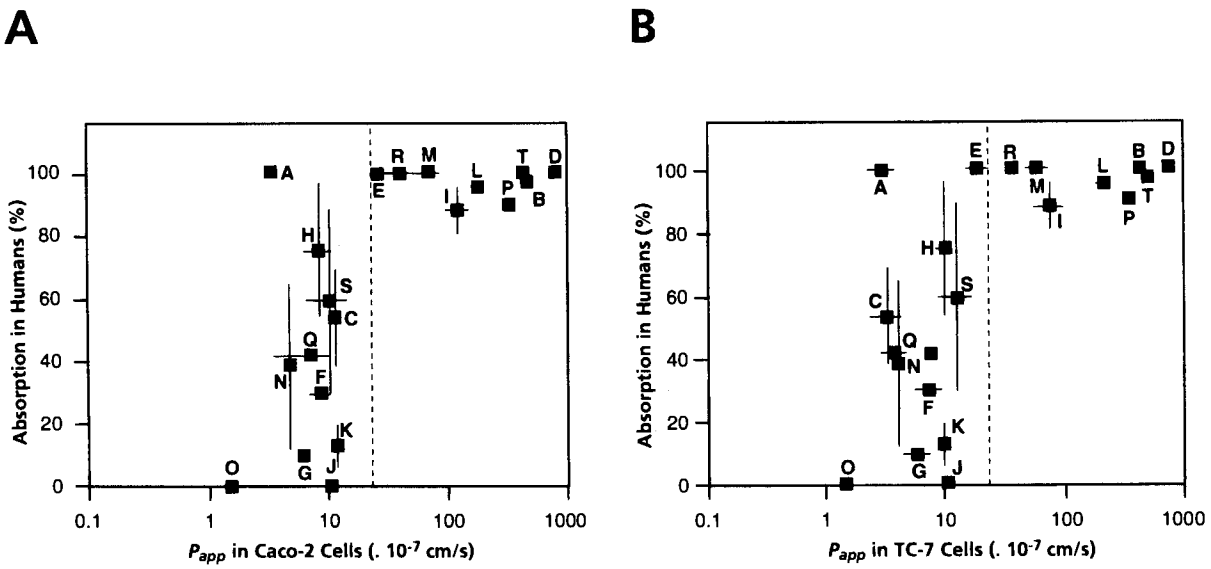

Fig. 4. Fraction absorbed in humans after oral administration as a function of apparent permeability coefficients in either parental Caco-2 (passages 70-110; A) or TC-7 clone (passage 40-80; B) cells. For identification of each compound, see Table I.

The different drawbacks reported for parental Caco-2 cells, include the high expression of P-glycoproteins and the low expression of different transporters (dipeptides. .). Hence, prediction of carrier-mediated transports will require a scaling factor, due to the low or over-expression of carriers in these cell lines.

In support to the drug discovery process, the ideal screening tool has to anticipate the oral absorption of drugs in humans based on the early knowledge of permeability coefficients obtained with the TC-7 clone model. Since this cell line quantitatively under- or over-expresses different carriers, the new chemical entities have to be screened with more rigorous studies, such as characterisation of transport processes used, in addition to the determination of the apparent permeability coefficients.

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