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Characterisation of a newly isolated Caco-2 clone (TC-7), as a model of transport processes and biotransformation of drugs

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Abstract

Three clones isolated from early (clone PD-7 and PF-11) and late (TC-7) passages of the parental Caco-2 epithelial cell line, were characterized for their ability to transport and metabolize endogenous compounds as well as xenobiotics. All three clones were able to form a homogeneous well-differentiated epithelial monolayer as demonstrated by the presence of microvilli at the apical pole of the cells and a high transepithelial electrical resistance. These cell monolayers were further characterized for their ability to transport different probes such as mannitol for the paracellular route, testosterone for passive diffusion and taurocholic acid for the presence of active biliary acids transporters. Only small differences were observed between the parental cell line Caco-2 and the different clones in terms of transepithelial electrical resistance, mannitol paracellular transport and testosterone passive diffusion. However, large differences were observed in the active transport of taurocholic acid with V_{max}/K_m values of 0.037, 0.048, 0.060 and 0.178 for Caco-2 parental cell line, clones PD-7, PF-11 and TC-7, respectively. Among transport processes, clones were also characterized for the expression of various enzyme systems involved in the biotransformation of endogenous compounds and xenobiotics, such as cytochromes P450 and UDP-glucuronosyltransferases. All cell types expressed cytochrome P450IA1, as demonstrated by the *O*-deethylation of 7-ethoxyresorufin. However, a 3 day β-naphthoflavone pretreatment induced 10.1 ± 3.0- and 10.4 ± 5.9-fold increases in 7-ethoxyresorufin *O*-deethylation in Caco-2 cells and PF-11 clone, respectively, while 24.7 ± 9.6- and 22.7 ± 8.1-fold increases were observed for PD-7 and TC-7 clones, respectively. These two clones also exhibited a much higher catalytic activity towards 1-naphthol, a substrate for UDP-glucuronosyltransferases. Since the intestinal epithelium plays an important role in the rate of absorption of intact drugs following their oral administration, both transport and metabolic characteristics make the Caco-2/TC-7 clone a suitable in vitro model for studying the intestinal disposition of drugs.

Keywords: Cell culture; Caco-2; Taurocholate; Transport; Epithelial monolayer; Enzyme activity

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1. Introduction

The human adenocarcinoma cell line, Caco-2, reproducibly displays a number of the properties of differentiated intestinal cells and has been widely used to study intestinal epithelial differentiation because of its ability to express morphological and biochemical features of adult differentiated enterocytes (Pinto et al., 1983; Zweibaum et al., 1983, 1984; Rousset et al., 1985; Zweibaum, 1991). This differentiation process is growth-dependent and after confluency, brush border hydrolase activities are similar to those reported for normal villous enterocytes (Pinto et al., 1983; Grasset et al., 1985; Zweibaum, 1991). Caco-2 cells grown onto collagen-coated membranes (Hidalgo et al., 1989; Wilson et al., 1990; Borchardt et al., 1991) form a tight monolayer of polarized epithelial cells similar to that described for the simple, columnar epithelium of the small intestine. Thus, the cells appears to undergo differentiation from 'crypt-type cells' in exponential phase of growth, to 'villus-type cells' in stationary phase. As the Caco-2 cell line displays the most highly differentiated properties under standard culture conditions (Artursson, 1991; Borchardt et al., 1991; Wilson, 1991), it appears to be the most relevant in vitro system for investigating transepithelial transport processes (Hidalgo et al., 1989; Artursson, 1990; Hidalgo and Borchardt, 1990; Hilgers et al., 1990).

However, it has been reported that some characteristics of this cell line, such as sucrase activities and glucose utilization varied from early (passage 15–30) to late passages (P110–200) (Zweibaum et al., 1983), reflecting the heterogeneity of the parental population. Although several groups have isolated Caco-2 cell clones (Beaulieu and Quaroni, 1991; Woodcock et al., 1991; Peterson and Mooseker, 1992), they were all derived from early passages of the cell line. Clones isolated from early (P29) and late (P198) passages of the Caco-2 cell line have recently been characterized (Chantret et al., 1994; Mahraoui et al., 1994). When analyzed at post-confluency, they were found to be similar as to cell polarity, presence of an apical brush border and levels of villin, dipeptidyl-IV, aminopeptidase N and alkaline phosphatase, but dramatically different as to their rates of glucose consumption and levels of expression of sucrase-isomaltase.

In the present work, three of these clones, selected on the basis of their different metabolic properties, were further characterized for: (i) transepithelial electrical resistance (TEER) and the ability to transport various drugs by different processes such as mannitol via the paracellular route, testosterone by passive diffusion and taurocholic acid through an active transport carrier; and (ii) the ability to express different enzyme systems involved in the biotransformation of xenobiotics (drugs, pollutants, environmental compounds) as well as endogenous compounds. On the basis of such data, also including morphological and biochemical characteristics, a single Caco-2 cell clone, TC-7, with functional properties of terminally differentiated small intestinal enterocytes, could be proposed as a more appropriate drug absorption model than the Caco-2 parental cell line.

2. Materials and methods

2.1. Chemicals

[^{3}H]Mannitol (specific activity 30 Ci/mmol) and [24-^{14}C]taurocholic acid (specific activity 46.3 mCi/mmol) were purchased from New England Nuclear Products (Boston, USA). [4-^{14}C]Testosterone (specific activity 57.3 mCi/mmol) was obtained from Dositek, Orsay, France.

1-Naphthol, 1-naphthyl-β-D-glucuronide, testosterone, taurocholic acid, UDP-glucuronic acid and taurocholic acid were of analytical grade and purchased from Sigma. 7-Ethoxyresorufin and resorufin were obtained from Pierce.

2.2. Cell culture

Parental Caco-2 cells, originating from a human colorectal carcinoma (Fogh et al., 1977), were obtained from Dr J. Fogh (Sloan Kettering Institute for Cancer Research, Rye, NY). Caco-2 cells were grown in 75 cm^2 flasks at 37°C in an

atmosphere of 10% CO_2 using Dulbecco's modified Eagle medium (DMEM) supplemented with 15% heat-inactivated fetal calf serum (FCS), 1% nonessential amino acids, 10 mM L-glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin. D-Glucose concentration in the culture medium was 4.5 g/l. The medium was changed every other day until the flasks reached 90% confluence and daily thereafter. Under these culture conditions, cells became confluent 5–6 days after seeding. The cells were detached with trypsin (0.25%)-0.2% EDTA in phosphate-buffered saline at pH 7.4 for 10 min at 37°C. All tissue culture media were obtained from Eurobio Laboratories (Paris, France). Cells used in this study were between passage 80 and 120 for Caco-2 cells and between passage 20 and 35 for the different clones. Previous studies already demonstrated that different parameters such as growth rate and sucrase isomaltase remained constant from early passage to passage 40 for the different clones (Chantret et al., 1994). For transport studies Caco-2 cells were seeded onto 3 μm pore Transwell-col (Costar) at 120000 cells/cm^2. Cell seeding was also similar for the different clones, except for clone PF-11 where 240000 cells/cm^2 were seeded.

In some experiments, Caco-2 parental cell line and the different clones were treated with β-naphthoflavone (50 μM final concentration), a specific and potent inducer of the cytochrome P450IA gene subfamily. Under these incubation conditions, the dimethyl sulfoxide final concentration never exceeded 0.1%.

2.3. Cell cloning

The isolation, characterization and stability of the Caco-2 clones obtained from early and late passages of the cell line have previously been reported (Chantret et al., 1994; Mahraoui et al., 1994). Of the three clones used in this study, two originated from passage 29 (PD-7 and PF-11) and one from passage 198 (TC-7).

2.4. Integrity of the monolayers

The integrity of the monolayers was determined by the measurement of the potential difference (TEER) (Boulenc et al., 1993) and by following the transepithelial transport of a macromolecular marker, polyethylene glycol 4000 (Boulenc et al., 1993). The potential difference was expressed as transmembrane resistance (Ω/cm^2) after subtraction of the intrinsic resistance of the model (i.e., the resistance obtained over the cell-free inserts). A monolayer with low TEER was assumed to exhibit extensive leakage through imperfect occluding junctions or holes in the monolayer.

2.5. Measurement of drug transport

Drug solutions were prepared from the radiolabeled isotopes and the corresponding unlabeled compounds in Hanks buffer to give final concentrations up to 10^{-3} M. 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 D-glucose per l and 10 mM Hepes buffer. The monolayers were continuously agitated on a mixer during the transport experiments. The radiolabeled drug solutions were added either to the apical or the basolateral side of the monolayer and aliquot parts, usually 0.2 ml, were withdrawn at previously determined intervals. Usually, radiolabeled drug solutions were added to the apical compartment, except if specified, and the rate of appearance of radiolabeled drug in the basal compartment was monitored. After withdrawing an aliquot, the same volume of buffer was added in the basal compartment in order to keep the receiver fluid volume constant. A maximum of four samples were taken from each chamber at regular time intervals. All inserts were checked for monolayer integrity by evaluating the TEER before each experiment.

2.6. Radioactive scintillation counting

Radioactivity was determined by liquid scintillation counting of 0.05–0.2 ml aliquot parts of the incubation medium in a Tricarb liquid scintillation spectrometer (Packard Instruments). Results were corrected to dpm by comparison with standard quench curves.

2.7. Calculations

The apparent permeability coefficient (P_{app}) was expressed in cm/s and determined as previously reported (Boulenc et al., 1993):

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

where dQ/dt is the transport rate ($\mu g/s$) and corresponds to the slope of the regression line determined on at least four different time points, C_o denotes the initial concentration in the donor chamber ($\mu g/ml$ or $\mu g/cm^3$), and A is the area of the membrane (4.2 cm^2).

2.8. Enzyme activity

Brush border preparation of Caco-2 cells was performed as previously described (Boulenc et al., 1992). Protein concentrations were determined by the Bio-Rad assay according to Pollard et al. (1978) using bovine serum albumin as standard protein.

Sucrase-isomaltase (EC 3.2.1.48) activity (Messer and Dahlqvist, 1966) and alkaline phosphatase (EC 3.1.3.1), with *p*-nitrophenyl phosphate as substrate (Garen and Levinthal, 1960), were determined as previously described.

UDP-glucuronosyltransferase activity was evaluated using 1-naphthol as substrate. Enzyme activity was evaluated according to the method of Lett et al. (1992). Briefly, 1-naphthol (500 μM final concentration) was incubated for 30 min with the membrane preparation (0.2 mg/ml) in the presence of both UDP-glucuronic acid as cofactor (3 mM final concentration) and Brij-58 as activator of the enzyme reaction (0.2 mg/mg protein). Enzyme reaction was stopped by addition of a trichloroacetic acid (0.4 M)/glycine (0.6 M) mixture. Unmetabolized drug was extracted with dichloromethane and the 1-naphthol-β-D-glucuronide was quantified by fluorescence (290 and 330 nm; excitation and emission wavelengths, respectively) following addition of Na_2HPO_4 (0.35 M) buffer.

Cytochrome P450 activity towards 7-ethoxyresorufin (*O*-deethylation process) was measured by a modification of the direct fluorimetric assay described by Burke and Mayer (1983). Microsomal fractions (0.2 mg/ml) were incubated over 10 min at 37°C in KH_2PO_4 buffer (0.1 M; pH 7.4) in the presence of 1 mM reduced nicotinamide adenine dinucleotide phosphate, reduced form and 5 μM 7-ethoxyresorufin. Parameters for fluorescence detection were 530 nm for the excitation wavelength and 585 nm for the emission wavelength.

All experiments were run under conditions (protein concentration, substrate concentration, excess of cofactor and incubation duration) in which the appearance of the metabolite was linear and never exceeded 30% of the initial substrate concentration.

3. Results

3.1. Ultrastructural and biochemical characteristics of Caco-2 clones

Cell growth characteristics, phase contrast microscopy of the cell monolayers, ultrastructural morphology and brush border enzyme activities were tested (data not shown) and were found to be consistent with results already reported (Chantret et al., 1994).

3.2. Metabolic properties of Caco-2 clones

The expression of different enzymes involved in the biotransformation of endogenous compounds as well as xenobiotics was further investigated.

3.2.1. Expression of cytochrome P450 isozymes

Different studies have reported (Boulenc et al., 1992; Rosenberg and Leff, 1993) that the parental cell line Caco-2 expressed the cytochrome P450IA1 isozyme as demonstrated by catalytic activities as well as Western and Northern blot analyses. They also demonstrated the specific induction of cytochrome P450IA1 by β-naphthoflavone. In the present study, both cytochrome P450IA1 expression and inducibility by β-naphthoflavone were investigated in the different clones. The results are illustrated in Fig. 1. Whatever the cell line investigated, basal 7-ethoxyresorufin *O*-deethylase activity was detected

and could be largely increased following a 3 day treatment with 50 μM β-naphthoflavone. Basal activities in the parental cell line and in the different clones were 18.0 ± 8.0, 7.5 ± 2.1, 24.0 ± 16.0 and 9.0 ± 1.0 pmol/min per mg, respectively (n = 2–4), which suggests a low inter-clone variability in 7-ethoxyresorufin O-deethylation. However, the induction of this enzyme activity by β-naphthoflavone was different from one clone to another. Hence, 7-ethoxyresorufin O-deethylation was 10.1 ± 3.0 (n = 3) and 10.4 ± 5.9-fold (n = 3) increased in Caco-2 cells and PF-11 clone, respectively, and 24.7 ± 9.6 (n = 4) and 22.7 ± 8.1-fold (n = 3) increased in PD-7 and TC-7 clones, respectively.

3.2.2. *Expression of UDP-glucuronosyltransferase isozymes*

The ability of cells to conjugate a specific probe of UDP-glucuronosyltransferase isozyme(s), i.e., 1-naphthol, with glucuronic acid, was also investigated. The ability of the different cells to conjugate 1-naphthol with glucuronic acid was examined as a function of culture time (Fig. 2).

Glucuronidation of 1-naphthol in Caco-2 cells occurred only very slowly over the entire period

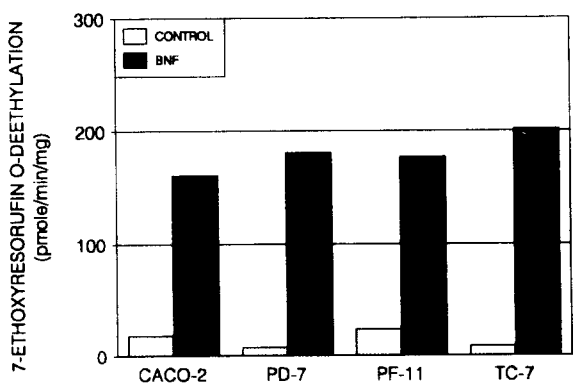

Fig. 1. Expression and inducibility of cytochrome P450IA1 activity towards 7-ethoxyresorufin. Caco-2 cells and the different clones were cultured on plastic dishes for 10 days. 3 days before scraping, half of the dishes were treated with 50 μM β-naphthoflavone. At day 10, cell monolayers were scraped in ice-cold pH 7.4 phosphate buffer and brush border preparation performed. 7-Ethoxyresorufin O-deethylase activity was monitored as described in section 2. Results were obtained in at least three different experiments run separately.

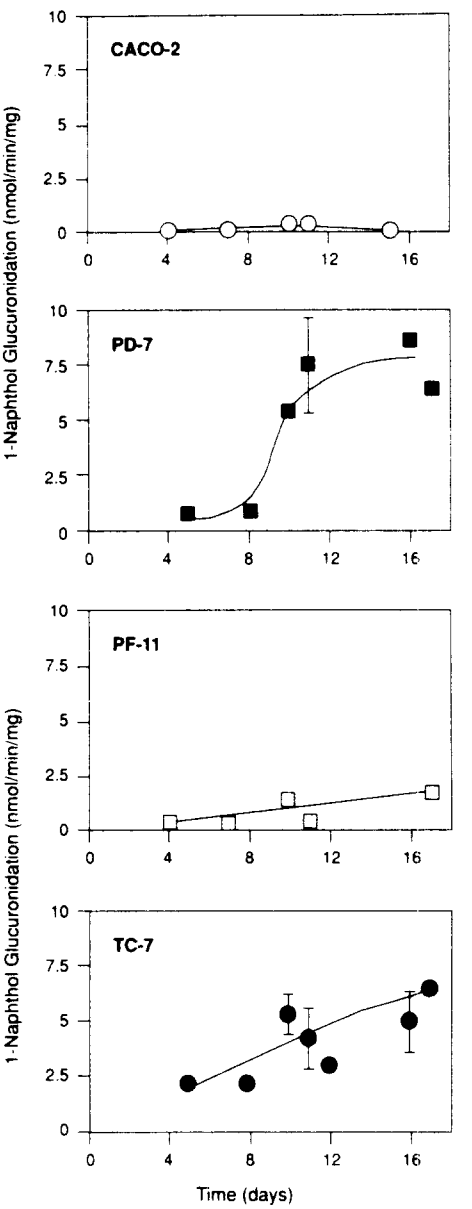

Fig. 2. Expression of UDP-glucuronosyltransferase activity towards 1-naphthol. Caco-2 cells and the different clones were cultured on plastic dishes up to day 16–17. At specified days, cell monolayers were scraped in ice-cold pH 7.4 phosphate buffer and brush border preparation performed. 1-Naphthol glucuronide formation was monitored as described in section 2. Results were obtained in at least three different experiments run separately.

of observation, achieving a turnover rate of 0.13 ± 0.04 nmol/min per mg at day 11 ($n = 5$). 1-Naphthol glucuronidation was greater in the three different clones with turnover rates of 7.50 ± 2.13 nmol/min per mg ($n = 3$), 0.46 ± 0.31 nmol/min per mg ($n = 5$) and 4.21 ± 1.41 nmol/min per mg ($n = 3$) for clone PD-7, clone PF-11 and clone TC-7, respectively.

3.3. Transport properties of Caco-2 clones

3.3.1. TEER measurements

The different clones and parental Caco-2 cells were cultured on filters and assayed for TEER at day 16 when a tight well-differentiated monolayer was formed (Artursson, 1990, 1991; Boulenc et al., 1992). Due to the efficacy of the junctional complex, epithelial cell monolayers were only slightly permeable to the test compound. Clones PD-7, PF-11 and TC-7 had a TEER of 196 ± 38 Ω/cm^2 ($n = 8$), 218 ± 42 Ω/cm^2 ($n = 6$) and 174 ± 23 Ω/cm^2 ($n = 5$), respectively, values which can be compared to 229 ± 44 Ω/cm^2 ($n = 6$) for Caco-2 cells. These clones, as well as parental Caco-2 cells displayed a TEER consistent with an integral monolayer.

3.3.2. Mannitol transport

Mannitol a marker for paracellular transport, has been intensively used to evaluate the permeability of the Caco-2 cell monolayers (Artursson, 1990, 1991; Boulenc et al., 1993). Due to the efficacy of the junctional complex, epithelial cell monolayers were only slightly permeable to the test compound. Parental Caco-2 cells and the different clones, cultured on polycarbonate filters, were tested for their ability to transport mannitol, 16 days after seeding. Rates of [^{3}H]mannitol transport across the monolayers were monitored every 30 min over 2 h. Under these experimental conditions, the rates of appearance of radiolabel in the basal compartment were linear with time, whatever the cell line investigated (data not shown). Permeability coefficients for mannitol are illustrated in Fig. 3. No statistically significant difference was observed for Caco-2 cells, clone PF-11 and clone TC-7 with P_{app} of 1.54 ± 0.18 × 10^7 cm/s ($n = 3$), 1.42 ± 0.16 × 10^7 cm/s ($n = 3$) and 1.29 ± 0.13 × 10^7 cm/s ($n = 3$), respectively. Clone PD-7 monolayers were slightly but significantly more permeable to mannitol with a P_{app} of 4.06 ± 2.00 × 10^7 cm/sec ($n = 3$).

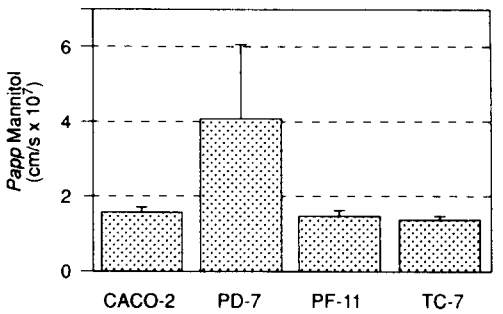

Fig. 3. Permeability coefficient of mannitol. Permeability coefficient of mannitol was determined on monolayers of Caco-2 cells or the different clones seeded for 16 days. After addition of [^{3}H]mannitol in the apical compartment, the appearance of radiolabel in the basal compartment was determined every 30 min over 2 h and the rate of transport across monolayers determined. Each kinetic run was performed in triplicate and on three different inserts. Results are expressed as the mean ± S.D. of three different experiments.

3.3.3. Testosterone transport

Testosterone transport across monolayers was evaluated 16 days after cell seeding. [^{14}C]Testosterone was added in the apical compartment of inserts and the kinetics of appearance of radiolabel in the basal compartment were monitored (Fig. 4). No obvious difference was observed between parental Caco-2 cells and the different clones. Permeability coefficients determined after 10 min were 1.80 ± 0.12 × 10^5 cm/s ($n = 3$), 1.32 ± 0.10 × 10^5 cm/s ($n = 3$), 1.69 ± 0.35 × 10^5 cm/s ($n = 3$) and 1.24 ± 0.25 × 10^5 cm/s ($n = 3$) for Caco-2 cells, clone PD-7, clone PF-11 and clone TC-7, respectively. A steady state in testosterone concentration between both the apical and the basal compartments was achieved within 1 h (data not shown).

3.3.4. Taurocholic acid transport

Transport of [^{14}C]taurocholic acid (final concentration in the apical compartment = 1 μM) was investigated over a 30 min period in the

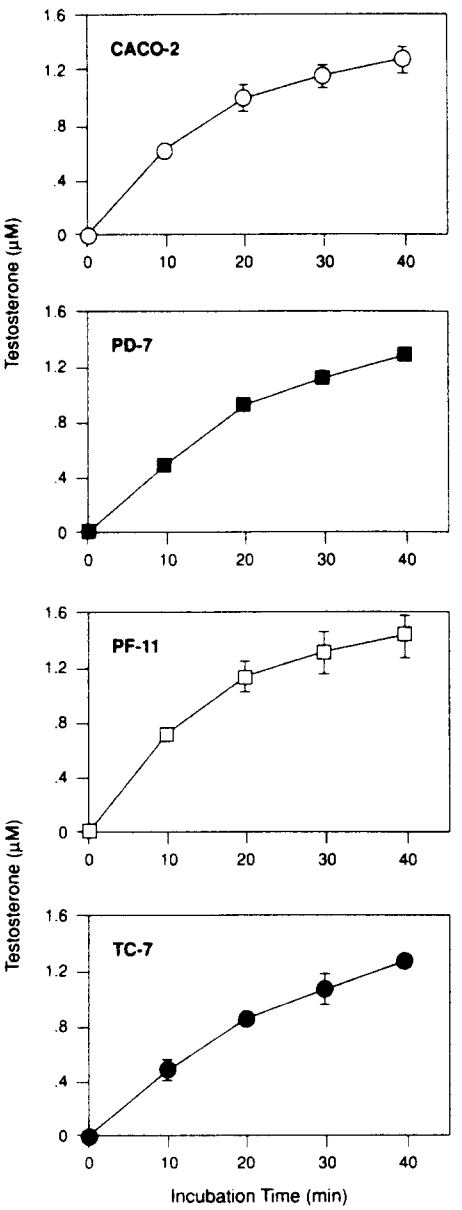

Fig. 4. Kinetics of testosterone transport across monolayers. Kinetics of testosterone were determined on cell monolayers obtained 16 days after seeding. [^{14}C]Testosterone was added in the apical compartment and the concentration of testosterone was determined every 10 min over 40 min in the basal compartment. Results are the mean ± S.D. of three different experiments, each one being performed on three different inserts.

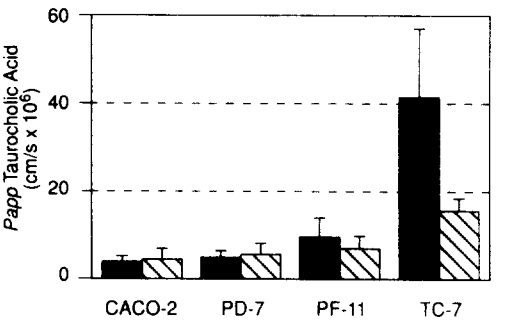

Fig. 5. Transport of [^{14}C]taurocholic acid across monolayers. Transport of [^{14}C]taurocholic acid across monolayers of Caco-2 cells and of the different clones was investigated at day 16 in the absence (filled bars) or presence (hatched bars) of excess unlabeled taurocholic acid in the apical compartment. Permeability coefficient for taurocholic acid was determined on the rate of taurocholic acid transport, every 30 min and over a 2 h period. Results are the mean ± S.D. of three single experiments performed on triplicate inserts.

absence or the presence of an excess, i.e., 1.9 mM, of unlabeled taurocholic acid. Preliminary studies demonstrated that under these incubation conditions, the appearance of radiolabeled drug in the basal compartment was linear with time. Permeability coefficients obtained for parental Caco-2 cells and the different clones are illustrated in Fig. 5. Marked differences in the amount of taurocholic acid transported from the apical to basolateral side were observed among the different cells. Caco-2 cells, clone PD-7 and clone PF-11 did not specifically transport taurocholic acid in the apical to basolateral direction. In contrast, clone TC-7 actively transported taurocholic acid. Since Caco-2 cells as well as the different clones displayed a TEER consistent with an integral monolayer, this suggested that Caco-2 cells, clone PD-7 and clone PF-11 lacked an inherent part of the taurocholic acid transport machinery compared to the TC-7 clone.

Michaelis-Menten constants for taurocholic acid transport were also determined for the different cells. Increased [^{14}C]taurocholic acid concentrations ranging between 15 and 300 μM were added to the apical compartment of the chambers, in which cells were seeded for 16 days. Radiolabel appearance in the basal compartment was monitored over 2 h and the rate of tauro-

cholic acid transport across the cell monolayer determined. In Fig. 6 are illustrated the double reciprocal plots of taurocholic acid transport rates as a function of initial taurocholic acid concentration added to the apical compartment. Results are plotted according to Lineweaver-Burk representation. Whatever the cell line studied, a straight line was observed consistent with an active transport involving a single carrier protein, or a group of very closely related proteins. Michaelis-Menten constants (K_m) for taurocholic acid transport were 38.9 ± 17.4 μM ($n = 3$), 36.2 ± 0.3 μM ($n = 2$), 69.3 ± 4.8 μM ($n = 3$) and 29.7 ± 11.7 μM ($n = 2$) for Caco-2 parental cell population, clone PD-7, clone PF-11 and clone TC-7, respectively, while V_{max} values were, respectively, 1.43 ± 0.65 nmol/min ($n = 3$), 0.74 ± 0.07 nmol/min ($n = 2$), 4.15 ± 1.70 nmol/min ($n = 3$) and 5.31 ± 0.12 nmol/min ($n = 2$). These apparent K_m values are consistent with the results of Barnard and Ghishan (1983) who reported a K_m for taurocholic acid uptake into human brush-border membrane vesicles of 37 ± 7 μM and those of Wilson et al. (1990) who reported a K_m of 42.5 ± 2.8 μM in the human intestinal Caco-2 cell line. The catalytic efficiency ('V_{max}/K_m' ratio) of the different cells to transport taurocholic acid across integral monolayers are illustrated in Fig. 7. Consistent with the coefficient permeability data, clone TC-7 transported taurocholic acid more efficiently than other cell types.

4. Discussion

The human intestinal epithelial cell line, Caco-2, provides a cellular model to study the differentiated functions of intestinal enterocytes. Caco-2 cells spontaneously differentiate in culture into polar cells possessing microvilli and enterocytic properties. Confluent monolayers form tight junctions between cells. The region of the normal human GI tract represented by the Caco-2 model is still under investigation. Indeed, while the presence of brush border hydrolases and transport pathways for bile acids and cobalamin are

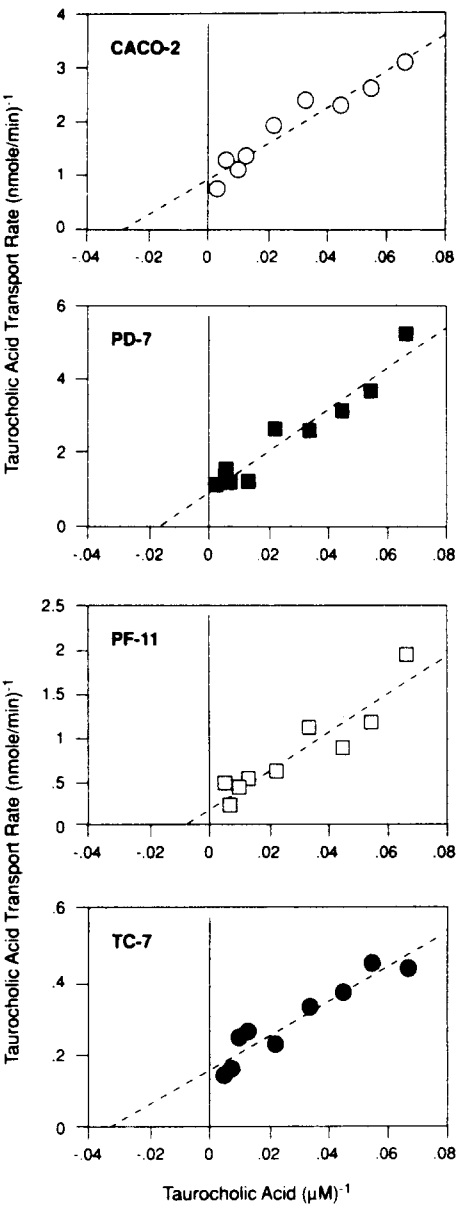

Fig. 6. Saturation of [^{14}C]taurocholic acid transport across monolayers. Increasing isotopic concentrations of [^{14}C]taurocholic acid were added in the apical compartment of inserts seeded for 16 days with Caco-2 cells or the different clones. After a 1 h incubation period, taurocholic acid concentration was determined in the basal compartment and the rate of taurocholic acid transport determined. Results are plotted according to Lineweaver-Burk and are the mean of three to four single experiments performed on duplicate inserts.

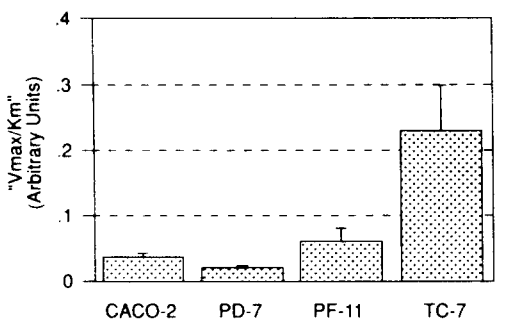

Fig. 7. Catalytic efficiency of taurocholic acid transport. Catalytic efficiency, i.e., V_{max}/K_m, of Caco-2 cells and the different clones to transport taurocholic acid across the monolayers was derived from the Lineweaver-Burk plot shown in Fig. 6.

properties of the distal ileum, the electrical properties are more indicative of a colonic epithelium.

The parental Caco-2 model was largely used to characterize transport processes of numerous xenobiotics such as L-α-methyldopa (Hu and Borchardt, 1990), cephalosporins (Inui et al., 1992), vasopressin analogues (Lundin and Artursson, 1990), peptides (Conradi et al., 1991) and bisphosphonates (Boulenc et al., 1993) as well as endogenous compounds such as bile acids (Hidalgo et al., 1989), i.e., taurocholic acid (Woodcock et al., 1991).

Moreover, numerous attempts were made to correlate such in vitro data to the in vivo situation. Hence, Artursson and Karlsson (1991) reported a close correlation between the oral absorption of various β-blockers in humans and the apparent permeation coefficients determined on Caco-2 cells model. Boulenc et al. (1993), using the same cellular model, also related the low bioavailability of hydrophilic bisphosphonates to the use of the paracellular route to cross the intestinal epithelium.

However, as the parental cell line Caco-2 appears to be morphologically heterogeneous (Wilson et al., 1990), the transport properties of the monolayers are probably the sum of the contributions of the different cell types. Woodcock et al. (1991) isolated various clones from the parental population that displayed higher taurocholic acid transport and were homogeneous with respect to certain morphological parameters. It was clear from their data that the amount of taurocholic acid transported by the parental population represented the sum from sub-populations. Recently, Chantret et al. (1994) isolated 26 clones from early and late passages of the Caco-2 cell line. These clones differed dramatically in the levels of sucrase isomaltase expression, rates of glucose consumption and expression of the fructose transporter GLUT5 (Mahraoui et al., 1994). Our data demonstrated that in the parental Caco-2 cell line, as well as in the different clones, the binding and uptake of [^{14}C]taurocholic acid was saturable with apparent K_m for the transcellular transport of [^{14}C]taurocholate ranging between 30 and 70 μM for the different cells. These results are in agreement with those reported by different authors such as 42.5 ± 2.8 μM for Wilson et al. (1990). Moreover, these different apparent K_m values obtained on Caco-2 parental cells or on the various clones, are consistent with the results of Barnard and Ghishan (1983) who reported a K_m for taurocholic acid uptake into human ileal brush border of 37 ± 7 μM. These different studies demonstrate that irrespective of the cell line investigated, or the different parts of the intestine analyzed, the membrane transporter(s) exhibited similar affinity for taurocholate. However, except for the present study, the quantitative presence of this transporter (or group of transporters), which could be estimated by the determination of V_{max}, was not determined. Hence, the comparison of the different cell lines as well as the extrapolation to the in vivo situation could not be performed.

Since the original observations in which everted intestinal segments from the ileum but not from the jejunum were able to transport bile acids against a concentration gradient, it has been recognized that bile acids are passively absorbed across the jejunum and are actively absorbed across the ileum (Lack and Weiner, 1961). Different in vivo and in situ experiments, have then demonstrated that the proximal small intestine was a major site for bile acid absorption in the rat (Sklan et al., 1976) and in human (Angelin et al., 1976). Similarly, cells isolated from jejunum and ileum have demonstrated the capacity to discriminate between passive and active processes for bile

acids. Specifically, bile acid uptake by cells isolated from ileum but not from jejunum exhibited all characteristics of an active transport (Wilson and Treanor, 1975). Our results suggest that despite its colon origin, Caco-2 cell line and more particularly TC-7 clone, exhibited characteristics of epithelial cells from ileum origin.

Recent studies have demonstrated that Caco-2 cultured under defined conditions or in the presence of various compounds such as enzyme inducers, i.e., phenobarbital, dexamethasone, β-naphthoflavone, expressed different enzyme systems including cytochrome P450 monooxygenases (Boulenc et al., 1992; Rosenberg and Leff, 1993), glutathione-*S*-transferases (Peters and Roelofs, 1989), sulfotransferases (Baranczyk-Kuzma et al., 1991) and UDP-glucuronosyltransferases (Bjorge et al., 1991). Our results demonstrate a similar enzyme activity in the different clones towards 7-ethoxyresorufin, an enzyme activity specifically catalyzed by cytochrome P450IA1 isozyme. However, the clones differ as to the inducibility of this enzyme; hence, a 22-fold increase was achieved for PD-7 and TC-7 clones, while only a 10-fold increase was observed for PF-11 and the parental cell line.

Previous in vivo studies demonstrated that UDP-glucuronosyltransferase activity was particularly important in the major extrahepatic organs directly exposed to the external environment. Hence, intestinal mucosa obviously played an important role in the glucuronidation of ingested xenobiotics. Intestinal glucuronidation was also reported to decrease the bioavailability of some drugs, such as enterally administered morphine. In rat, the specific activity of UDP glucuronosyltransferase isozymes appears to decline from proximal to distal small intestine (Koster et al., 1985). Peters et al. (1989) investigated the longitudinal distribution of various UDP-glucuronosyltransferase activities in the human small intestine. They reported that activity towards two planar substrates, i.e., 4-nitrophenol and 4-methylumbelliferone, increased or remained constant, respectively, from the duodenum to ileum. Glucuronidation of 1-naphthol, another planar compound, was investigated on Caco-2 cells and on the different clones. Large inter-clone difference was observed from one clone to another and to the parental cell line. This enzyme activity was almost not detected in Caco-2 parental cell line and in PF-11 clone, while PD-7 and TC-7 clones exhibited a very high activity.

In this study, we have demonstrated large differences in the biochemical characteristics of the parental Caco-2 cell line and its various clones. Indeed, although the different clones differentiated in well-defined polarized monolayers with a high trans-membrane epithelial electrical resistance and a low permeability to hydrophilic compounds such as mannitol, some characteristics varied intensely from one clone to another; in contrast to other clones and to the parental cell line, TC-7 clone which exhibited higher expression of the brush border-associated hydrolase sucrase isomaltase (Chantret et al., 1994) (i) displayed greater taurocholic acid transport (K_m = 29.7 ± 11.7 μM and V_{max} = 5.31 ± 0.12 nmol/min) than other cell types, (ii) was highly inducible by β-naphthoflavone for the cytochrome P450IA1 gene subfamily and (iii) exhibited a high UDP-glucuronosyltranferase activity toward 1-naphthol.

These different characteristics make the TC-7 clone a valuable in vitro intestinal tool, not only to study the transport processes of drugs across the intestinal wall but also to evaluate the role of the intestine in the biotransformation of drugs.

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