

Prediction of Drug Transport through the Blood-Brain Barrier *in Vivo*: A Comparison between Two *in Vitro* Cell Models

Stefan Lundquist,¹ Mila Renftel,¹ Julien Brillault,² Laurence Fenart,³ Roméo Cecchelli,² and Marie-Pierre Dehouck^{2,4}

Received January 28, 2002; accepted April 1, 2002

Purpose. Studies were conducted to evaluate whether the use of an *in vitro* model of the blood-brain barrier (BBB) resulted in more accurate predictions of the *in vivo* transport of compounds compared to the use of a human intestinal cell line (Caco-2).

Methods. The *in vitro* BBB model employs bovine brain capillary endothelial cells co-cultured with primary rat astrocytes. The Caco-2 cells originate from a human colorectal carcinoma. The rat was used as experimental animal for the *in vivo* studies.

Results. Strong correlations ($r = 0.93\text{--}0.95$) were found between the results generated by the *in vitro* model of the BBB and two different methodologies to measure the permeability across the BBB *in vivo*. In contrast, a poor correlation ($r = 0.68$) was obtained between Caco-2 cell data and *in vivo* BBB transport. A relatively poor correlation ($r = 0.74$) was also found between the two *in vitro* models.

Conclusion. The present study illustrates the limitations of the Caco-2 model to predict BBB permeability of compounds *in vivo*. The results emphasize the fact that the BBB and the intestinal mucosa are two fundamentally different biologic barriers, and to be able to make accurate predictions about the *in vivo* CNS penetration of potential drug candidates, it is important that the *in vitro* model possesses the main characteristics of the *in vivo* BBB.

KEY WORDS: blood-brain barrier; drug delivery; *in vitro* models; *in vivo* studies; intestinal barrier.

INTRODUCTION

The BBB is situated at the cerebral capillary endothelium and represents the principal route for the entry of most solutes into the central nervous system (CNS). The cerebral capillary endothelial cells are joined by continuous belts of tight junctions and are devoid of fenestrations. The critical zonularity of the tight junctions of the cerebral vasculature sets them apart from that of capillaries of other organs in the body and restricts the brain entry of most nontransported

hydrophilic compounds (1). The brain microvasculature also contains a variety of enzymes that may either inactivate or activate compounds that traverse the brain capillary wall (2–5). In addition, high levels of ATP-dependent transporters are localized in the brain capillary wall like e.g. several nutrient carrier systems and P-glycoprotein (6–10) that regulates the influx or efflux of a variety of compounds. Consequently, the BBB is a formidable obstacle to the effective treatment of many neurologic disorders, and unless a BBB drug delivery strategy is adopted many neuropharmaceuticals may exhibit excellent activity *in vitro*. Their therapeutic efficacy will often be significantly diminished when administered to appropriate animal models, since they are unable to gain access to the diseased site at a sufficient concentration for an appropriate time.

Another key biologic barrier is the intestinal epithelium. The anatomy and physiology of the gastrointestinal tract (GI) is as complex as the processes governing the absorption and transport of drug molecules across this important barrier. The overall bioavailability of an orally administered drug depends on many factors some of which are: (i) physicochemical properties of the drug molecules; (ii) susceptibility to metabolic transformation by enzymes in the intestinal lumen, intestinal epithelium and the liver; (iii) specificity for various transport systems in the epithelium; (iv) specificity for the P-glycoprotein efflux pump; and (v) the anatomic and physiological state of the GI tract.

Obtaining early information about the transport characteristics of potential drug candidates across these two barriers is therefore important within the pharmaceutical industry. However, since animal-based assays tend to be time-consuming and require bioanalytical input or access to radio-labeled compounds, such studies are generally performed at a relatively late stage of development and are not particularly suitable for dealing with the flow of compounds generated by combinatorial chemistry and high throughput screening.

To overcome the limitations of *in vivo* studies, many laboratories have developed *in vitro* techniques for determining the BBB permeability or intestinal permeability of potential drug candidates. In comparison to animal-based assays, *in vitro* studies provide several advantages:

less compound needed for evaluation; rapid technique and few or no animals needed; allows more compounds to be screened; mechanism of transport can be evaluated; metabolism during transport can be evaluated; early signs of cell toxicity can be recorded; pathologic conditions can be induced and molecular mechanisms evaluated; development of structure-transport relationships can be supported; no bioanalytical input needed since most drugs can be assayed directly in buffer.

The two *in vitro* models described in this study are (i) a co-culture model of brain capillary endothelial cells and astrocytes for mimicking the BBB and (ii) a human colon carcinoma cell line (Caco-2) as a model for the intestinal epithelium. Both models have been extensively used in the pharmaceutical industry to estimate the BBB permeability or intestinal permeability of drug candidates and have become an integral part of many drug discovery programs that are targeted toward the CNS and the oral route of administration.

Despite the fact that the BBB and the intestinal mucosa

¹ Astra Zeneca R & D, Discovery, DMPK S151, 85, Södertälje, Sweden.

² Laboratoire Mixte Institut, Pasteur de Lille—Université d'Artois, Faculté Jean Perrin, rue Jean Souvraz, 62307 Lens, France.

³ Cellial Technologies, Faculté Jean Perrin, rue Jean Souvraz 62307, Lens, France.

⁴ To whom correspondence should be addressed. (e-mail: mpierre.dehouck@univ-artois.fr)

ABBREVIATIONS: BBB, blood-brain barrier; BUI, brain uptake index; CNS, central nervous system; GI, gastrointestinal tract; HPLC, high-performance liquid chromatography; HEPES, N-[2-hydroxyethyl]piperazine-N'-2[2-ethanesulfonic acid]; PS, permeability surface area product.

are two fundamentally different biologic barriers as regards e.g., membrane lipids, enzymes and transporters it has been suggested that the Caco-2 cell model is able to give accurate predictions of BBB transport *in vivo*. The objective of this study was to evaluate if the *in vitro* model of the blood-brain barrier (in routine use at AstraZeneca, Södertälje) gave more accurate predictions of *in vivo* BBB transport than the Caco-2 model and furthermore, if there was a good correlation between these two *in vitro* models.

MATERIALS AND METHODS

Test Compounds and Formulation for BBB Studies

Radiolabeled sucrose, mannitol, vincristine, dexamethasone, hydrocortisone, dopamine, fenytoin, antipyrine, propranolol, diazepam, and nicotine were purchased from Amersham. Radiolabeled urea, pirenzepine, caffeine and lidocaine were purchased from NEN-Dupont. The radiochemical purity of all labeled compounds was found to be greater than 97% as assayed by HPLC. Aliquots of radiolabeled isotopes were added to solutions with corresponding unlabeled compounds. Morphine, codeine, pindolol, acetylsalicylic acid, and terbutaline were unlabeled; the two first compounds were obtained from University of Uppsala and the others were from Sigma (Stockholm, Sweden). The final concentrations of the test compounds were between 0.01–0.1 mM.

All test compounds were dissolved in HEPES buffered Ringer's solution (NaCl 150mM, KCl 5.2 mM, CaCl₂ 2.2 mM, MgCl₂ 0.2mM, NaHCO₃ 6mM, Glucose 2.8 mM, HEPES 5 mM, water for injection). All reagents were obtained from Sigma.

In Vitro Model of the Blood-Brain Barrier

Cell Culture

The method of Dehouck *et al.* and Cecchelli *et al.* was used (11,12). The rats were supplied by B&K Universal AB (Stockholm, Sweden). The astrocytes were isolated according to the method of Booher and Sensenbrenner (13) and plated on the bottom of cell culture clusters containing six wells each. The endothelial cells were seeded onto polycarbonate filters (Costar, Transwell), that were placed in the wells containing astrocytes.

Under these conditions the endothelial cells retain all the usual endothelial cell markers as well as the characteristics of the barrier which include e.g. complex tight junctions, low rate of pinocytosis, gamma glutamyl transpeptidase, monoamine oxidase (2,11) and P-glycoprotein (14). Experiments were initiated after 12 days of co-culture.

Fluorescence Microscopy

Endothelial cells grown on porous filter were fixed with 4% paraformaldehyde and permeabilized with cold acetone (–20°C). The samples were washed with PBS and soaked in the blocking solution: Tris-buffered saline (20mM Tris-HCl, 0.5M NaCl, pH 7) containing 5% ovalbumin and 1% heat-inactivated normal goat serum. They were then incubated with the mouse anti-vimentin antibody (from Zymed). After rinsing, the cells were incubated with the secondary antibody, CyTM3-conjugated goat anti-mouse IgG.

For the localization of tight junction-associated protein claudin-1, the endothelial cells were fixed with cold methanol (–20°C) and permeabilized with 0.1% Triton X-100 in PBS. The cells were soaked in the blocking solution and then incubated with the rabbit anti-claudin-1 antibody (from Zymed). The Alexa Fluor 568-conjugated goat anti-rabbit IgG was used as secondary antibody.

The specimens were visualized with a Leica DMR fluorescence.

Transport Experiments

The compounds were dissolved in buffered Ringer's solution. The experiments were carried out at pH = 7.4 and 37°C. At the initiation of the transport experiments, buffered Ringer's solution was added to wells of a 6-well plate without astrocytes. One insert, containing a confluent monolayer of brain capillary endothelial cells, was subsequently placed in this 6-well plate. The drug solution was added to the cell monolayer. The plate was then placed on an orbital shaker and at 10, 15, 20, 30, 45, and 60 min after the addition of the drug, the insert was moved to other wells of the plate to minimize back diffusion of compound to the upper compartment. Three inserts with cells and three without cells, were assayed for each test compound. The samples were analyzed by HPLC or liquid scintillation spectroscopy.

Data Analysis and Calculation

The cleared volume was calculated, as described by Siflinger-Birnboim *et al.* (15), by dividing the amount of compound in the receiver compartment by the drug concentration in the donor compartment at each time point. The average cumulative volume cleared was plotted vs. time and the slope was estimated by linear regression analysis (EXCEL 5.0) to give the mean and standard deviation of the estimate. The slope of the clearance curve with inserts alone and inserts with cells is equal to PS_f and PS_t respectively, where PS = the permeability surface area product. The units of PS and S are in microliters/minute and square centimeters, respectively. The PS-value for the endothelial monolayer (PSe) was computed as follows:

$$1/PSe = 1/PS_t - 1/PS_f$$

To generate the endothelial permeability coefficient, Pe (cm/min), the PSe-value was divided by the surface area of the insert. Pe-values were calculated for 20 compounds.

Caco-2 Cell Model

Test Compounds and Formulations for Caco-2 Studies

All data concerning the Caco-2 cells were obtained from a study published by Yazdani *et al.* (16). In their study, drug solutions were prepared in Hank's balanced salt solution (pH = 7.4) at final concentrations between 0.01 to 0.1 mM. All experiments were performed at 37°C.

Cell Culture

In short, the Caco-2 cells were seeded at a density of 80 000 cells/cm² on polycarbonate filters in 6 well plates (Costar, Transwell, Costar Europe Ltd., Badhoevedoxp, Nether-

lands). Cell culture inserts were coated with rat-tail collagen type I. The cells were allowed to grow and differentiate for up to 25 days. They were used between passage 23 to 50.

Transport Experiments

To initiate transport experiments, the apical side of the cell monolayer received 1.5 ml of drug solution. The plates were then placed on an orbital shaker. The amount of solute in the receiver chamber was determined by either moving the inserts to new wells containing fresh medium or by taking samples from the receiver chamber and replacing it with fresh medium. The samples were assayed by HPLC or liquid scintillation spectroscopy.

Data Analysis and Calculation

The apparent permeability coefficient (Papp) was determined according to the equation:

$$P_{app} = J/AC_0$$

where J is the rate of appearance of the drug in the receiver chamber, C_0 is the initial concentration of the solute in the donor chamber and A, the surface area of the filter.

In Vivo Methods

The *in vivo* data used in this study were obtained from the literature.

Single-Pass Uptake

The relative permeabilities to moderate and rapid penetrating compounds across the BBB can be determined in the rat by the brain uptake index (BUI) technique (17–21). A 0.2-ml bolus of buffered saline containing known concentrations of the test substance and a reference substance is injected rapidly (<1s) into the carotid artery. After 5–15 s the rat is decapitated and the brain analyzed for tracer contents. The penetration of the reference substance into the brain is so rapid that it is determined by the rate of blood flow. Thus, if the reference substance is with ^3H and the test substance with ^{14}C labeled, the BUI is given by the following:

$$\text{Brain uptake index} = \text{BUI} = \frac{\text{brain } ^{14}\text{C}/\text{brain } ^3\text{H} \times 100}{\text{Injected } ^{14}\text{C}/\text{injected } ^3\text{H}}$$

If all the reference substance is extracted during a single pass of blood through the brain, BUI can be related to cerebrovascular PS by the Renkin-Crone equation (22).

Multiple-Pass Uptake

With the brain perfusion technique and the intravenous administration technique, solute uptake into the brain can be extended beyond that of a single-pass technique. Thus, these methods are more sensitive in determining the permeability for poor penetrating compounds. With the intravenous administration technique (23–25), a solute is injected or infused intravenously and the plasma concentration is monitored until a specific time (from 10 s to several hours) at which the brain content is determined. The cerebrovascular permeability—surface area product (PS) can be obtained from:

$$PS = -vF \ln(1 - K_{in}/vF)$$

where K_{in} is the unidirectional transfer coefficient for influx (ml/s per g), F is regional cerebral blood-flow (ml/s per g), P is capillary permeability (cm/s), S is the surface area of perfused capillaries (cm^2/g) and v is the fractional distribution volume of tracer in blood (ml/ml).

With the brain perfusion technique (26), the right cerebral hemisphere of an anesthetized rat is perfused by retrograde infusion of fluid into the right external carotid artery. After perfusion for up to 5 min with blood or a saline, the animal is decapitated and the ipsilateral brain removed. The calculation of cerebrovascular PS is equivalent to that of the intravenous administration technique with the exception that perfusion fluid concentration is used instead of arterial concentration in plasma.

Before a correlation was made with *in vitro* data, the PS products were converted into *Pe in vivo* values ($Pe_{in vivo}$) by dividing PS by an estimated value of the surface area (S) of perfused capillaries equal to $100 \text{ cm}^2/\text{g}$ of brain (27).

RESULTS AND DISCUSSION

Figure 1 illustrates the typical phenotype of confluent bovine endothelial cells co-cultured for 12 days with astrocytes as described earlier. It can be seen that the cells form a confluent monolayer of nonoverlapping and contact inhibited

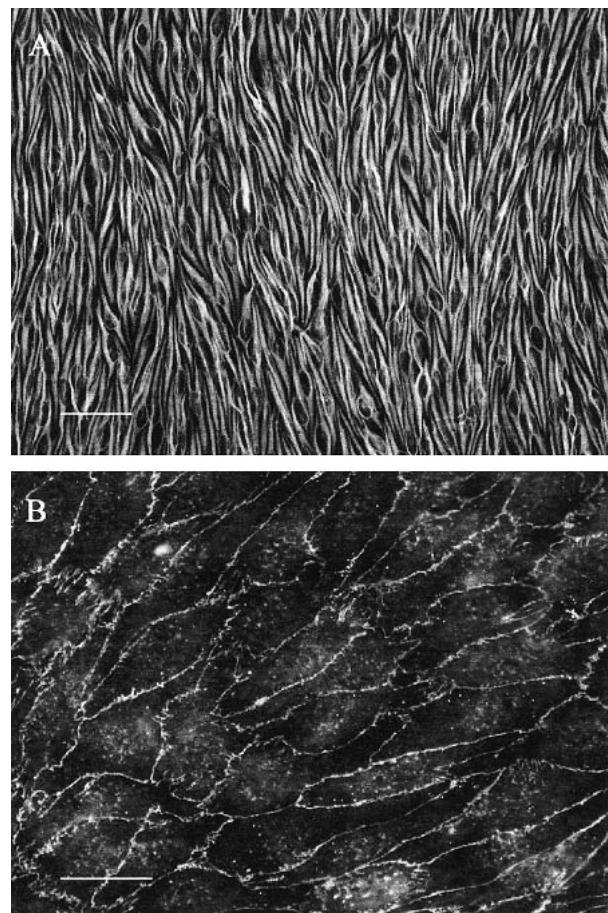


Fig. 1. Confluent monolayer of bovine brain capillary endothelial cells demonstrates homogeneity in phenotype (A). Bar, 50 μm . Bovine brain capillary ECs grown on filters were fixed and stained for tight junction protein claudin-1 (B). Bar, 100 μm .

Table I. Permeability Values Obtained *in Vivo* (BUI; PS) and on *in Vitro* Models (BBB model : Pe; Caco-2 model : Papp). Each BUI or PS value is referred

Compound	MW	$P_e \times 10^{-6}$ (cm/s)	BUI (%)	PS (ml/g/min)	$P_{app} \times 10^{-6}$ (cm/s)
Acetylsalicylic acid	180	9.0	1.8 (17)		9.09
Antipyrin	188	179.2	68 (17)	1.0 (23)	
Caffeine	194	229.7	90 (17)	1.2 (24)	30.8
Codeine	299	171.7	26 (17)	0.2 (24)	
Dexamethasone	392	19.2			12.2
Diazepam	285	227.7		1.2 (21)	33.4
Dopamine	153	22.5	3.85 (18)		9.33
Hydrocortisone	362	29.7	1.4 (19)	0.0084 (19)	14.0
Lidocaine	234	293.3		0.76 (26)	
Mannitol	182	13.0	1.94 (18)	0.00125 (25)	0.38
Morphine	285	33.3	2.6 (17)	0.03 (24)	
Nicotine	162	289.3	131 (17)		19.4
Phenytoin	252	106.7	31 (17)	0.3 (24)	26.7
Pindolol	248	44.0			16.7
Pirenzepine	351	11.8			0.44
Propranolol	259	294.3	75 (20)	0.67 (24)	21.8
Sucrose	342	8.3	1.4 (18)	0.0003 (25)	1.71
Terbutaline	225	10.7			0.47
Urea	60	63.5	2.4 (18)	0.004 (25)	4.56
Vincristine	825	8.0		0.006 (24)	

cells with no contaminating pericytes present (Fig.1A). Immunofluorescent staining of the tight-junction integral protein claudin-1, shows preferential cortical membrane localization (Fig.1B). The continuous network of labeled claudin-1 demonstrates that the tight junctions are complex and well developed. This together with previously published results (11,12,14) show that this is a highly differentiated BBB model which possesses many of the characteristics of the *in vivo* BBB.

The Caco-2 cell data were obtained from one single study (16) since culturing conditions, passage number and days in culture can influence the permeability and metabolism characteristics of the cell line as demonstrated by Artursson *et al.* (28).

The permeability (P_e) of the *in vitro* BBB to 20 compounds with molecular weights between 60 and 825 have been measured and is presented in Table I. To evaluate the relationship between *in vitro* and *in vivo* BBB permeability, all available BUI or P_e *in vivo* values in this study were correlated with the corresponding *in vitro* BBB data and as can be seen in Fig. 2A and 2B, excellent relationships were obtained ($r = 0.93$ and $r = 0.95$, respectively). To compare the predictive powers of the two *in vitro* models and to exclude the possibility of protein binding influencing the results, permeability data (P_e and P_{app} in respective model) from a set of 10 compounds were correlated with corresponding BUI values only (Fig.3A and 3B). As evidenced by Fig. 3, it can be seen that the data generated by the *in vitro* BBB model shows a superior relationship with *in vivo* BBB drug transport compared to the results obtained with the Caco-2 model. The correlation between BUI and *in vitro* BBB data was $r = 0.93$ but only $r = 0.68$ between BUI and Caco-2 data for the same set of 10 compounds. Finally, a correlation including 15 compounds was made between the two *in vitro* models. As shown in Fig.4 the correlation between the two models is relatively poor ($r = 0.74$). It can also be noted that the compounds have

different rankorder in the two *in vitro* models. Furthermore, it is interesting to note that a number of compounds such as dexamethasone, hydrocortisone, pindolol and acetylsalicylic acid show high permeability in the Caco-2 model. Also, in

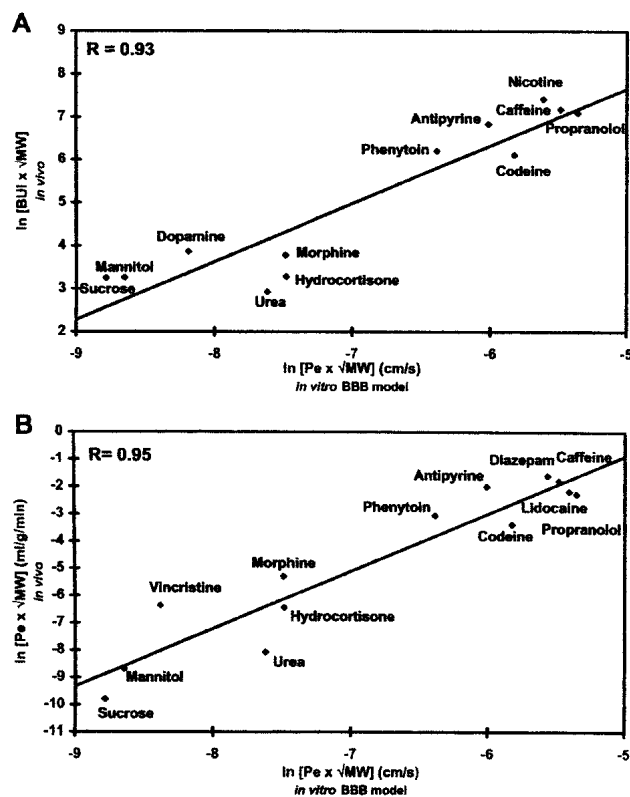


Fig. 2. *In vitro* BBB permeability as a function of BUI (brain uptake index) (A) and *in vivo* BBB permeability (B). All parameters were normalized for molecular weight.

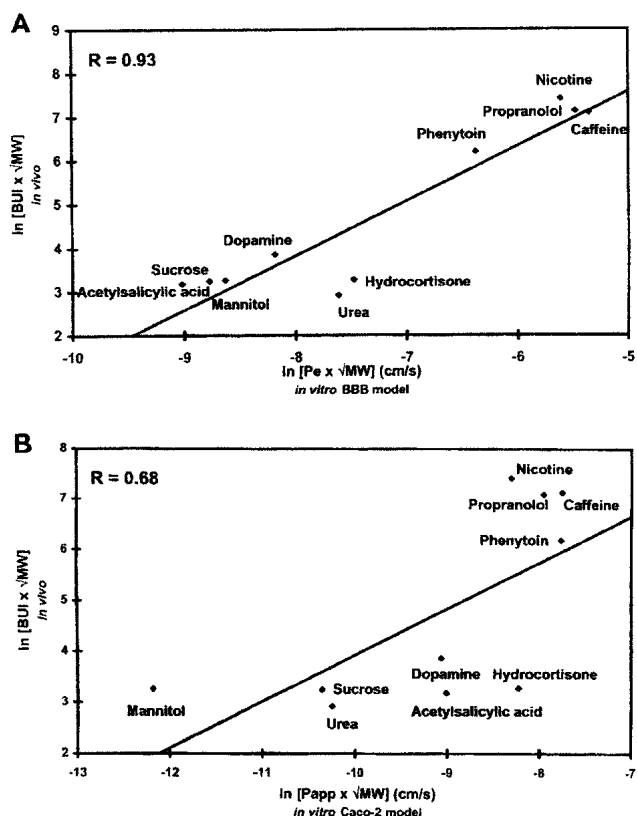


Fig. 3. A correlation between *in vitro* BBB permeability (A) or *in vitro* intestinal permeability (Caco-2) (B) and BUI (brain uptake index) of 10 compounds. All parameters were normalized for molecular weight.

most cases complete absorption in humans after oral administration; however, despite this, these compounds show only low or moderate BBB permeability *in vitro* and *in vivo*. The reason for the very low permeability of e.g. dexamethasone through the BBB *in vitro* and *in vivo* has been suggested to be due to the fact that it is a substrate for P-glycoprotein (29). In contrast, dexamethasone show high permeability in the Caco-2 cell model as well as complete absorption in humans after oral administration despite the fact, that P-glycoprotein is considered to be present both in the epithelial cells *in vivo* and *in vitro*. This might reflect the possibility of P-

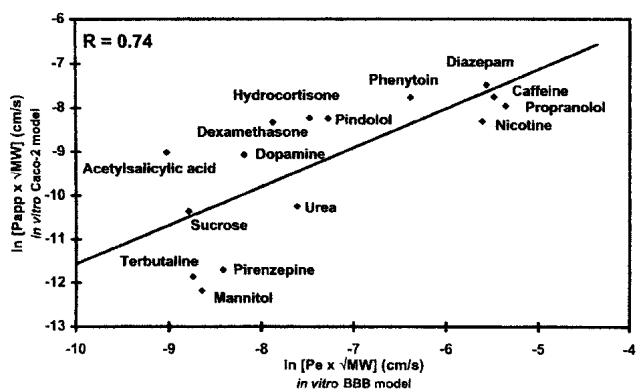


Fig. 4. Correlation between the *in vitro* BBB permeability and *in vitro* intestinal permeability. Allowance for variations in molecular weight was made.

glycoprotein being differently expressed at these two barriers, or that saturation of this efflux mechanism takes place at the level of the intestinal epithelium. Since the metabolic properties of the brain endothelial cells and Caco-2 cells also differ, this could serve as an alternative explanation for the difference in transport.

CONCLUSION

The results in this study emphasize the fact that the blood-brain barrier and the intestinal mucosa are two fundamentally different biologic barriers. To accurately predict *in vivo* CNS penetration of potential drug candidates, an *in vitro* model should possess the main characteristics of the BBB *in vivo*. This is evidenced by the present study that illustrates the limitations of the Caco-2 cell model to predict *in vivo* BBB permeability of compounds. Strong correlations ($r = 0.93$ – 0.98) were however, found between the results generated by the *in vitro* model of the BBB and two different methodologies to measure permeability across the BBB *in vivo*.

ACKNOWLEDGMENTS

This work was supported by Conseil Régional du Nord-Pas-de Calais.

REFERENCES

1. A. L. Betz and G. W. Goldstein. Specialized properties and solute transport in brain capillaries. *Annu. Rev. Physiol.* **48**:241–250 (1986).
2. S. Méresse, M. P. Dehouck, P. Delorme, M. Bensaid, J. P. Tauber, C. Delbart, J. C. Fruchart, and R. Cecchelli. Bovine Brain Endothelial Cells Express Tight Junctions and Monoamine Oxidase Activity in Long-Term Culture. *J. Neurochem.* **53**:1363–1371 (1989).
3. A. Minn, J. F. Gherzi-Egea, R. Perrin, B. Leininger, and G. Siest. Drug metabolising enzymes in the brain and cerebral microvessels. *Brain Res. Rev.* **16**:65–82 (1991).
4. B. Solhonne, C. Gros, H. Pollard, and J. C. Schwartz. Major localisation of aminopeptidase M in rat brain microvessels. *Neuroscience* **22**:225–232 (1987).
5. L. Song, E. Wilk, S. Wilk, and D. P. Healy. Localization of immunoreactive glutamyl aminotransferase in rat brain. I. Association with cerebral microvessels. *Brain. Res.* **606**:286–294 (1993).
6. T. Tatsuta, M. Naito, T. Oh-hara, I. Sugawara, and T. Tsuruo. Functional involvement of P-glycoprotein in blood-brain barrier. *J. Biol. Chem.* **267**:20383–20391 (1992).
7. A. Tsuji, I. Tamai, A. Sakata, Y. Tenda, and T. Terasaki. Restricted transport of ciclosporin A across the blood-brain barrier by a multidrug transporter, P-glycoprotein. *Biochem. Pharmacol.* **46**:1096–1099 (1993).
8. A. H. Schinkel, J. J. M. Smit, O. van Tellingen, J. H. Beijnen, E. Wagenaar, L. van Deemter, C. A. A. M. Mol, M. A. van der Valk, E. C. Robanus-Maandag, H. P. J. te Riele, A. J. M. Berns, and P. Borst. Disruption of mouse mdr1a P-glycoprotein gene leads to deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**:491–502 (1994).
9. E. M. Cornford, L. D. Braun, and W. H. Oldendorf. Carrier-mediated blood-brain barrier of choline and certain choline analogs. *J. Neurochem.* **30**:299–308 (1978).
10. K. L. Audus, R.L. Bartel, I.J. Hidalgo, and R.T Borchardt. The use of cultured epithelial and endothelial cells for drug transport and metabolism studies. *Pharm. Res.* **7**:435–451 (1990).
11. M. P. Dehouck, S. Méresse, P. Delorme, J. C. Fruchart, and R. Cecchelli. An easier, Reproducible and Mass-production Method to study the Blood-Brain Barrier In Vitro. *J. Neurochem.* **54**: 1798–1801 (1990).
12. R. Cecchelli, B. Dehouck, L. Descamps, L. Fenart, V. Bue-Scherrer, C. Duhem, S. Lundquist, M. Rentfel, G. Torpier, and

- M. P. Dehouck. In vitro model for evaluating drug transport across the blood-brain barrier. *Adv. Drug. Deliv. Res.* **5**:165–178 (1999).
13. J. Booher and M. Sensenbrenner. Growth and Cultivation of Dissociated Neurons and Glial Cells from Embryonic Chick, Rat and Human Brain in Flask Cultures. *Neurobiology* **2**:97–105 (1972).
14. L. Fenart, V. Buée-Scherrer, L. Descamps, C. Duhem, M. G. Poullain, R. Cecchelli, and M. P. Dehouck. Inhibition of P-glycoprotein: Rapid assessment of its implication in blood-brain barrier integrity and drug transport to the brain by an *in vitro* model of the blood-brain barrier. *Pharm. Res.* **15**:993–1000 (1998).
15. A. Siflinger-Birnboim, P. C. Del Becchio, J. A. Cooper, J. N. Blumenstock, J. N. Shepard, and A. B. Malik. Molecular Sieving Characteristics of the Cultured Endothelial Monolayer. *J. Cell. Physiol.* **132**:111–117 (1987).
16. M. Yazdaniyan, S. L. Glynn, J. L. Wright, and A. Hawi. Correlating Partitioning and Caco-2 Cell Permeability of Structurally Diverse Small Molecular Weight Compounds. *Pharm. Res.* **15**:1490–1494 (1998).
17. W. H. Oldendorf. Blood-brain barrier, In H. Davson and M. B. Segal (eds), *Physiology of the CSF and Blood-Brain Barriers*, CRC Press, USA, 1996 pp. 77–78.
18. W. H. Oldendorf. Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection. *Am. J. Physiol.* **221**: 1629–1639 (1971).
19. W. M. Pardridge and L. J. Mietus. Transport of Steroid Hormones through the Rat Blood-Brain Barrier. *J. Clin. Invest.* **64**: 145–154 (1979).
20. W. M. Pardridge and R. Sakiyama. and G. Fierer. Blood-brain barrier transport and brain sequestration of propranolol and lidocaine. *Am. J. Physiol.* **247**:R582–R588 (1984).
21. E. M. Cornford. The Blood-Brain Barrier, A Dynamic Regulatory Interface. *Physiol.* **7**:219–259 (1985).
22. C. Crone and D. G. Levitt. Capillary permeability to small solutes, In E. M. Renkin, and C. C. Michel (eds), *Handbook of Physiology. Section 2: The Cardiovascular system. Vol. 4: Microcirculation. Part 1.* American Physiological Society, Maryland, 1984 pp. 411–466.
23. J. D. Fenstermacher and S. Rapoport. Blood-brain barrier, In Rencim, E. M. and Michel, C. C. (eds.), *Handbook of physiology* Vol. 4, Section 2, American Physiological Society, Bethesda, 1986, pp. 969–1000.
24. J. D. Fenstermacher. Pharmacology of the Blood-Brain Barrier, In E. A. Neuwelt (ed), *Implications of the Blood-Brain Barrier and Its Manipulation. Vol.1*, Plenum Medical Book Company, New York, 1989 pp.137–155.
25. J. D. Fenstermacher. Drug transfer across the blood-brain barrier. In D. D. Breimer and P. Speiser (eds), *Topics in Pharmaceutical Sciences*. Elsevier, Amsterdam, 1983 pp.143–154.
26. W. M. Pardridge, D. Triguero, J. Yang, and P. A. Cancilla. Comparison of *in vitro* and *in vivo* models of drug transcytosis through the blood-brain barrier. *J. Pharmacol. Exp. Ther.* **253**:884–891 (1990).
27. M. Bradbury. The concept of a Blood-Brain Barrier, Wiley, New York, (1979).
28. P. Artursson and R. T Borchardt. Intestinal drug absorption and metabolism in cell cultures: Caco-2 and beyond. *Pharm. Res.* **14**: 1655–1658 (1997).
29. O. C. Meijer, E. C. de Lange, D. D. Breimer. A. G. de Boer J. O. Workel, and E. R. de Kloet. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr 1A P-glycoprotein knockout mice. *Endocrinology* **139**:1789–1793 (1998).