P-Glycoprotein Inhibition Leads to Enhanced Disruptive Effects by Anti-Microtubule Cytostatics at the *In Vitro* Blood-Brain Barrier

Inez C. J. van der Sandt,[1,2] Pieter J. Gaillard,[1] Heleen H. Voorwinden,[1] Albertus G. de Boer,[1] and Douwe D. Breimer[1]

Received January 8, 2001; accepted February 12, 2001

Purpose. To investigate whether P-glycoprotein (Pgp) protects the *in vitro* BBB against the cytotoxic effects of anti-tumour drugs.

Methods. In an *in vitro* BBB coculture model the influence of the anti-microtubule drugs vinblastine, colchicine, paclitaxel and the non-antimicrotubule drugs doxorubicin, fluorouracil and etoposide in the absence or presence of Pgp modulators on the trans-endothelial electrical resistance (TEER), which is an indicator for the integrity, was investigated.

Results. In the absence of Pgp modulators vinblastine, colchicine and paclitaxel dose dependently decreased TEER values to less than 20% of control. Non-anti-microtubule drugs did not affect TEER values. Following competitive inhibition of Pgp by various Pgp modulators and substrates, even low concentrations of vinblastine, colchicine and paclitaxel substantially decreased TEER. IC_{50} values of LY 335979, SDZ-PSC 833, cyclosporin A, and verapamil were 0.03, 0.25, 0.46, and 13.7 μM, respectively.

Conclusions. These results indicate that Pgp normally protects the *in vitro* BBB against the disruptive effects of anti-microtubule drugs, but its integrity is lost when anti-microtubule drugs are used in combination with potent Pgp modulators. In addition, this procedure offers the possibility to characterize Pgp modulators and substrates with respect to their efficacy and to elucidate drug interactions at the level of Pgp.

KEY WORDS: P-glycoprotein; blood-brain barrier; anti-microtubule drugs; *in vitro*; Pgp modulators.

INTRODUCTION

The main function of the blood-brain barrier (BBB) is to protect the central nervous system (CNS) against the fluctuating blood environment and to maintain homeostasis in the CNS (1). This is caused by the dynamic properties of the BBB, presented by the endothelial cells, which are influenced by astrocytes, pericytes, and neurons. With respect to its large surface area, the BBB is considered to be the most important barrier for drug transport to the CNS (2). However, certain conditions, e.g., inflammation or AIDS related dementia, can increase or decrease the transport of drugs across the BBB (3). Another important reason for the limited penetration of compounds into the CNS is the presence of various active transporters, like the multidrug efflux transporter P-glycoprotein (Pgp) (4). Pgp actively extrudes its substrates into the blood stream, thereby limiting its substrates to pass the BBB and enter the CNS.

Since Pgp is present in various other tissues (4) and multidrug resistant (MDR) tumors (5), it is conceivable that Pgp protects the BBB against damaging effects of toxic compounds and limits pharmacological effects of drugs. In case of MDR tumor cells this is a disadvantage since the efflux of anti-cancer drugs by Pgp results in low intracellular concentrations of anti-tumour drugs, thereby decreasing the effectiveness of chemotherapy.

Several studies have shown that Pgp modulators can inhibit Pgp, leading to a higher intracellular accumulation of anti-tumor drugs and thus an increased anti-tumor effect (6). However, in non-tumor cells expressing Pgp, this may give rise to unwanted side effects. It was clearly shown that the lack of the multidrug resistance related protein 1 (MRP1) gene in mice led to tissue damage induced by the anticancer drug etoposide (7).

In the current study it was investigated if Pgp protects the BBB against the pharmacological effects of various anti-tumor drugs. Previously, BBB damage was demonstrated by vinblastine when given in combination with the Pgp modulator SDZ-PSC 833 (8). In this study the inhibition of and the competition for Pgp was characterized in terms of potency by using the *in vitro* BBB coculture model comprising bovine brain capillary endothelial cells (BCEC) and rat astrocytes. Integrity assessment took place by measuring trans-endothelial electrical resistance (TEER), which particularly is a measure for paracellular permeability (9).

MATERIALS AND METHODS

Materials

SDZ-PSC 833, LY 335979, ritonavir and amprenavir were provided by Sandoz Pharma (Basel, Switzerland), Eli Lilly Company (Indianapolis, IN, USA), Abbott Laboratories (Abbott Park, IL, USA) and Glaxo Wellcome (Greenford, Great Britain), respectively. Vinblastine, colchicine, cyclosporin A, doxorubicin, etoposide, fluorouracil, and verapamil were obtained from Sigma Chemical Co. (Zwijndrecht, The Netherlands). Other chemicals were all of analytical grade. Paclitaxel was obtained in the commercial form Taxol (6 mg/ml paclitaxel in Cremophor and dehydrated ethanol). A stock solution of 1 mM SDZ-PSC 833 was made in polyethylene glycol (PEG 200, Fluka Chemie, Zwijndrecht, The Netherlands)/ethanol/5% glucose [(v/v/v) 55/5/10]. A stock solution of 2 mg/ml ritonavir and amprenavir was made in ethanol. A stock solution of 10 mM vinblastine, colchicine, and cyclosporin A was made in ethanol. Stock solutions of other drugs were made in millipore water (MilliQ). Complete medium consisted of Dulbecco's modified Eagle's Medium (DMEM) formulated with $NaHCO_3$ (3.7 g/l), Hepes (25 mM), and D-glucose (4.5 g/l) ans supplemented with extra MEM nonessential amino acids, penicillin (100.000 U/l), streptomycin (100 mg/l), L-glutamine (2 mM), (all from BioWhittaker,

[1] Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, Leiden University, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

[2] To whom correspondence should be addressed. (e-mail: I.sandt@lacdr.leidenuniv.nl)

ABBREVIATIONS: Pgp, P-glycoprotein BBB, blood-brain barrier BCEC, brain capillary endothelial cells TEER, trans-endothelial electrical resistance.

Verviers, Belgium), and 10% (v/v) heat inactivated fetal calf serum.

In Vitro BBB Model

The *in vitro* BBB model was prepared as described by Gaillard (10). Briefly, calf brains were obtained at the slaughterhouse (Molendijk B.V., Nieuwerkerk a/d IJssel, The Netherlands). Brain capillaries were isolated from cortices of the brains. After homogenization of the cortices, trapping on nylon meshes and enzymatic digestion, brain capillaries were obtained. Astrocytes were isolated from cortices of brains of newborn Wistar rats (Harlan B.V., Zeist, The Netherlands) and used to prepare astrocyte-conditioned medium and co-cultures. BCEC were cultured from brain capillaries on collagen and fibronectin coated culture flasks in 50% astrocyte-conditioned medium. $125*10^3$ astrocytes per cm^2 were seeded on the bottom of collagen coated Transwell polycarbonate filters [0.4 μM pore size, surface area: 0.33 cm^2 (filter type A) or 1 cm^2 (filter type B) Transwell™, Corning Costar, Schiphol-Rijk, The Netherlands] in complete medium. After 2 or 3 days $30*10^3$ BCEC were seeded on the top of the filters in complete medium supplemented with 125 μg/ml heparin. Tight monolayers were obtained 4–5 days after seeding the BCEC. At the day of the experiment TEER values of the *in vitro* BBB ranged between 240 and 300 Ω $*cm^2$ with a mean of 283 Ω $*cm^2$.

TEER Studies

First, the time-dependent effect (at t = 0–6 h) of various concentrations of the anti-tumor drugs vinblastine, paclitaxel, colchicine, doxorubicin, etoposide, and fluorouracil on TEER was investigated. Next, various concentrations of the Pgp modulators LY 335979, SDZ-PSC 833, verapamil, cyclosporine A, and the Pgp substrates ritonavir and amprenavir were administered in the presence of 10 nM vinblastine, 0.3 μM paclitaxel, and 0.1 nM colchicine and subsequently TEER was measured for 6 h. TEER was measured with a current-passing and voltage-measuring electrode (Millicell-ERS, Millipore Corporation, Etten-Leur, The Netherlands). The mean TEER of blank filters (n = 4) were subtracted, while a correction was made for the surface area of the filter.

Data Analysis

TEER values were expressed as a percentage of the initial value (t = 0 h) which was set at 100%. All measurements were performed at least on 8 filters. All results were expressed as mean ± S.D.

TEER values at t = 6 h were used to obtain effect parameters of the Pgp modulators with respect to Pgp inhibitory potency. The data were fitted with a sigmoidal E_{max} model using WinNonlin version 1.5 (Pharsight, Mountain View, CA, USA) to characterise Pgp inhibition:

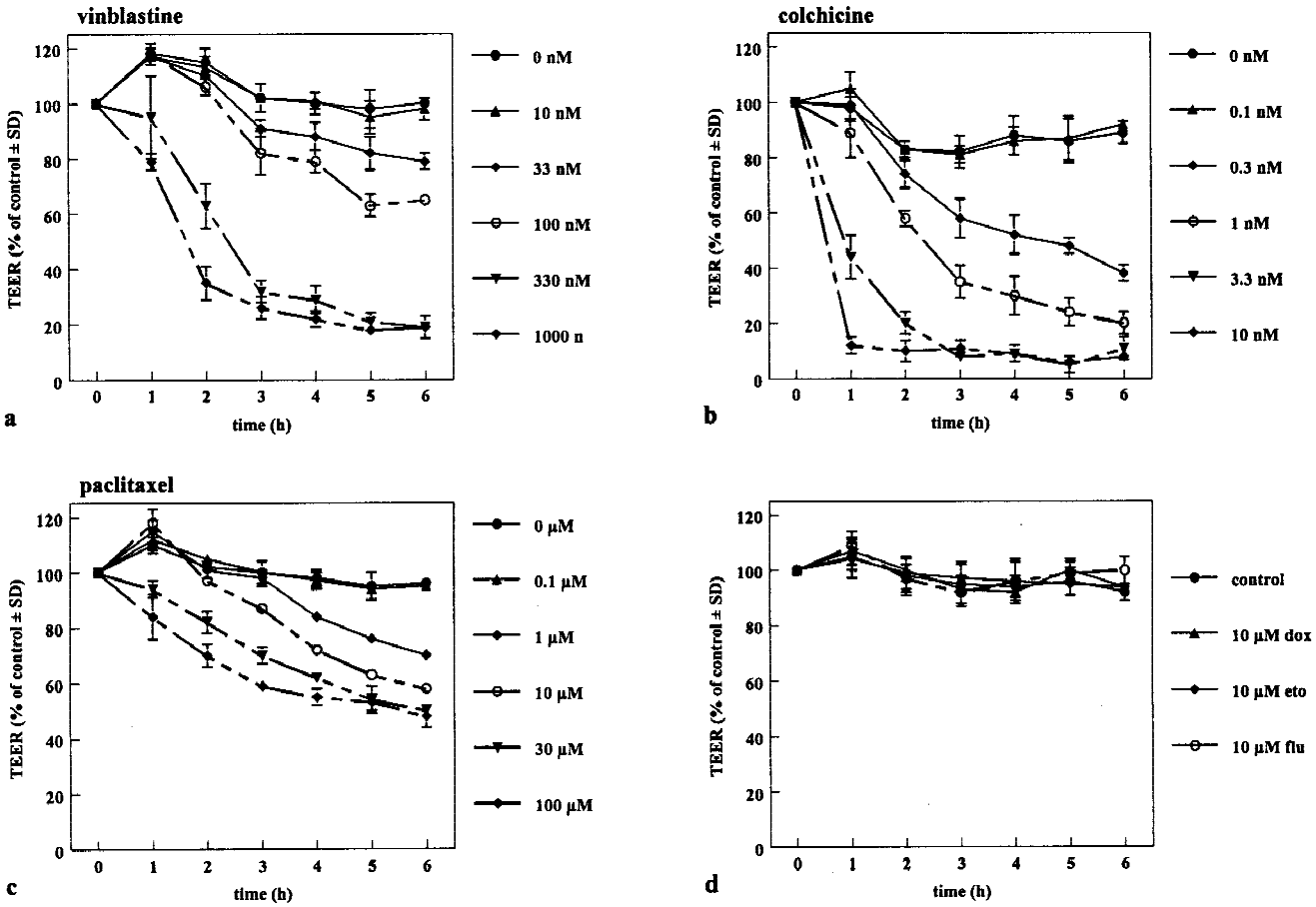

Fig. 1. Concentration dependent effect of vinblastine (a), colchicine (b), paclitaxel (c), and doxorubicin (dox), etoposide (eto) and fluorouracil (flu) (d) on TEER in the *in vitro* BBB coculture model. At t = 0 h drugs were applied and TEER was set to 100%. Data are expressed in mean ± S.D.

$$\text{TEER} = \frac{E_{max} * C}{IC_{50} + C} \quad (1)$$

where E_{max} is the maximal decrease of TEER (percentage), IC_{50} is the concentration at half maximal decrease, and C is the concentration of the inhibitor.

Statistical difference ($p < 0.05$) of TEER values at t = 6 h was calculated by an unpaired Student's t-test (two-tailed).

RESULTS

First, the effect of various anti-tumor drugs on the integrity of the *in vitro* coculture BBB model, as measured by TEER, was investigated for 6 h (Fig. 1a–c). Apart from small fluctuations, TEER was relatively constant over time in the absence of these compounds (control). The anti-tumor drugs etoposide, doxorubicin, and fluorouracil did not change the TEER values in comparison to the control situation in a concentration range from 0.1 nM to 10 μM (Fig. 1d). Although in the presence of 10 nM vinblastine or 0.1 nM colchicine no difference in TEER in comparison to the control situation was found, higher concentrations of vinblastine or colchicine significantly decreased TEER (Fig. 1a,b). TEER decreased to less than 20% in the presence of 330 nM or more for vinblastine and 3.3 nM or more for colchicine, meaning that the BBB had lost its integrity. In the presence of paclitaxel TEER decreased at concentrations higher than 0.3 μM, but the maximal reduction did not exceed 50% (Fig. 1c).

Since 10 nM vinblastine, 0.1 nM colchicine, and 0.1 μM paclitaxel did not affect TEER values, i.e., BBB integrity, these concentrations were used to investigate if Pgp modulators would enhance the effect of these drugs after inhibition of Pgp. The presence of 10 nM vinblastine together with 0.01 μM cyclosporin A did not change TEER, but higher concentrations of cyclosporin A decreased the TEER to less than 20% (Fig. 2a). Similar results were obtained with 0.1 nM colchicine and 0.1 μM paclitaxel although the maximal decrease of TEER by paclitaxel was about 50% (Fig. 2b–c). Cyclosporin A itself did not affect TEER at any of the concentrations applied (data not shown). Since the effect of vinblastine, colchicine, and paclitaxel was clearly concentration-dependently enhanced in the presence of cyclosporin A, it was explored if this effect could also be used to quantitatively estimate E_{max} and IC_{50} values of the Pgp modulators cyclosporin A, LY 335979, verapamil, and SDZ-PSC 833. The percentage decrease in TEER after 6 h was used as the experimental parameter and depicted as a concentration-effect re-

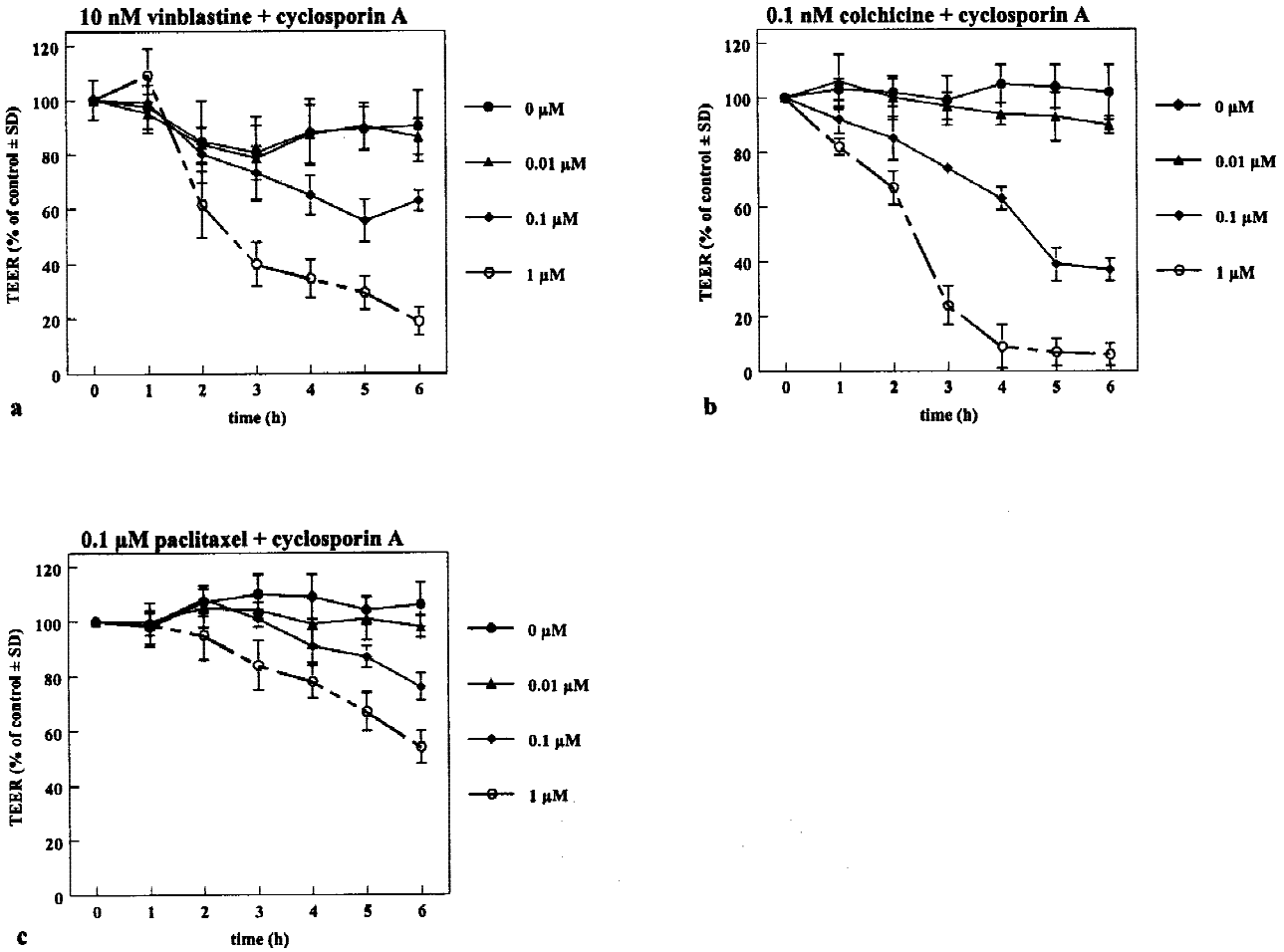

Fig. 2. Concentration dependent effect of cyclosporin A in the presence of 10 nM vinblastine (a), 0.1 nM colchicine (b), and 0.1 μM paclitaxel (c) on TEER in the *in vitro* BBB coculture model. Cyclosporin A was applied at t = −1 h and the anti-tumor drugs were applied at t = 0 h. TEER was set to 100% at t = 0 h. In control situation only 10 nM vinblastine, 0.1 nM colchicine or 0.1 μM paclitaxel was applied. Data are expressed in mean ± S.D.

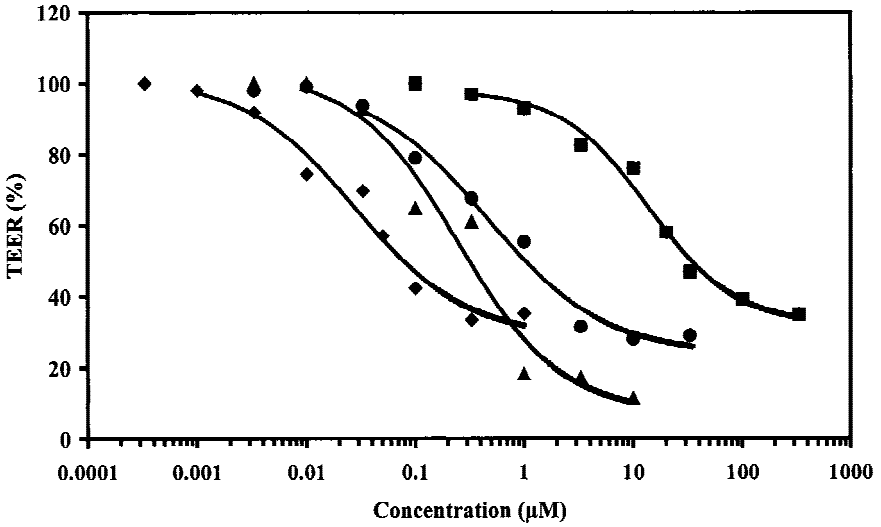

Fig. 3. Concentration dependent effect of Pgp modulators on TEER in the presence of 10 nM vinblastine in the *in vitro* BBB coculture model. The curve is fitted through the mean ($n = 8$) of the TEER values at t = 6 h according to Eq. 1 (see Table 1 for parameter estimates). ◆ LY 335979; ▲ cyclosporin A; ● SDZ-PSC 833; ■ verapamil.

lationship in Fig. 3. Using the sigmoidal E_{max} model it was possible to estimate E_{max} and IC_{50} of the different Pgp modulators used (Table I). The IC_{50} of LY 335979 was the lowest of all Pgp modulators tested, followed by cyclosporin A, SDZ-PSC 833, while the IC_{50} of verapamil was the highest. In addition, this method was used to characterise other Pgp substrates as well. This was shown by using the HIV protease inhibitors ritonavir and amprenavir. In the presence 10 nM vinblastine together with various concentrations of ritonavir or amprenavir, TEER decreased concentration-dependently as well (Fig. 4). At all concentrations used, Pgp modulators or HIV protease inhibitors alone did not affect TEER (data not shown).

DISCUSSION

Pgp limits the entry of Pgp substrates into the brain and therefore brain concentrations of Pgp substrates will be relatively low under normal conditions. This is consistent with the view that Pgp protects the brain from penetration by potentially toxic compounds. Indeed, brain concentrations of Pgp substrates in mdr1a Pgp knockout mice are much higher in comparison to wild-type mice (11). The limitation of Pgp substrates to enter the brain has also pharmacodynamic consequences. Mdr1a/mdr1b double knockout mice were at least eight-fold more sensitive to the sedative effect of asimadoline in comparison to wild-type mice (12). Furthermore, inhibition of Pgp led to alteration of morphine antinociception in rats (13).

Etoposide, doxorubicin, and fluorouracil (at concentrations until 10 μM) did not influence TEER values in comparison to the control situation. Their pharmacological action is at the level of DNA synthesis, which apparently does not have direct effects on the integrity of this BBB model. In contrast, vinblastine, colchicine, and paclitaxel exhibited a concentration dependent decrease of TEER. Since TEER values represent the permeability of the BBB (9), this implies increased paracellular permeability caused by the pharmacodynamic effects on microtubuli of these compounds, which resulted in reduced BBB integrity. Vinblastine, colchicine, and paclitaxel are anti-microtubule drugs. Vinblastine and colchicine destabilise the polymerisation of microtubules in cells, while paclitaxel has a stabilising effect (14–15). After binding of these compounds to free tubulin or assembled microtubules, cell cycle progression is delayed and a dramatic increase in apoptosis occurs (16). Concentrations of such drugs necessary for the anti-proliferative effect do not affect the overall architecture of the microtubules (17–18), but at higher concentrations they induce progressive nonspecific cell death (19).

In vivo, it has been reported that 10 mg/kg vinblastine disrupted the BBB in rats as was concluded from increased uptake of Evans blue dye in the brain (20). This is most likely

Table I. Calculated E_{max} and IC_{50} of Various Pgp Modulators Using the Sigmoidal E_{max} Model. IC_{50}'s Obtained in this Study Are Comparable with IC_{50}'s Found in Other Studies (Ford, 1995 (26); Fish *et al.*, 1995 (27))

Pgp substrate	E_{max}	IC_{50} in this study	IC_{50} (25)	IC_{50} (26)
Verapamil	66%	13.7 ± 0.1 μM	6–10 μM	6–10 μM
SDZ-PSC 833	77%	0.25 ± 0.03 μM	0.1 μM	0.5–2 μM
Cyclosporin A	91%	0.46 ± 0.05 μM	0.8–2 μM	2–4 μM
LY 335979	72%	0.03 ± 0.001 μM	Not reported	Not reported

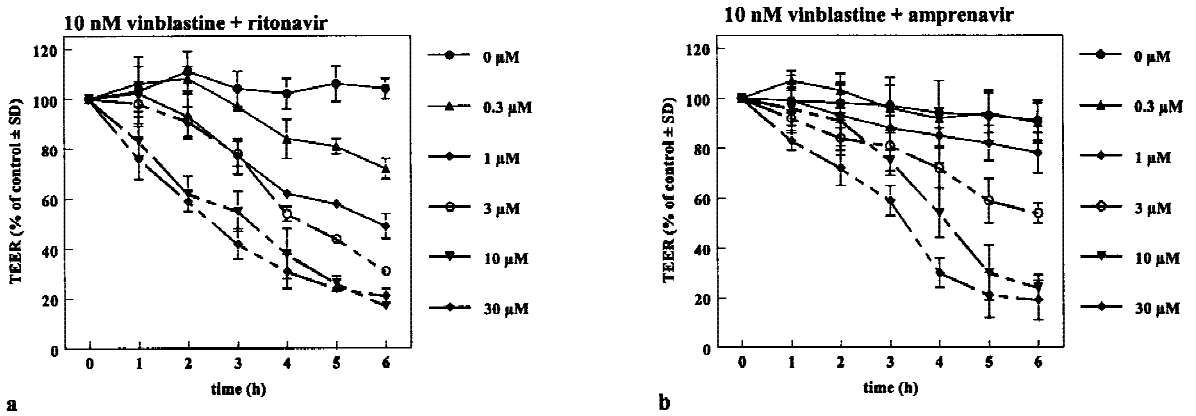

Fig. 4. Concentration dependent effect of various concentrations of ritonavir (a) and amprenavir (b) in the presence of 10 nM vinblastine on TEER in the *in vitro* BBB coculture model. Ritonavir or amprenavir was applied at t = −1 h and vinblastine was applied at t = 0 h. TEER was set to 100% at t = 0 h. In control situation only 10 nM vinblastine was applied. Data are expressed in mean ± S.D.

caused by increased paracellular transport through the tight junctions next to increased pinocytotic transport. Furthermore, Nag *et al.* (21) demonstrated that in the presence of an actin disrupting agent (colchicine and cytochalasin B) BBB permeability was increased *in vivo*. Apparently the BBB is likely to be opened both *in vivo* and *in vitro* following application of anti-microtubule drugs above certain treshold concentrations.

Concentrations of vinblastine, colchicine, and paclitaxel that did not alter TEER, were used to demonstrate that Pgp, expressed on the endothelial cells of the BBB, has a protective function. These concentrations are comparible with plasma concentrations reported *in vivo* (22–24). The results clearly showed that after inhibition of Pgp, the disruptive effect of the anti-microtubule drugs on BBB integrity was strongly enhanced. TEER decreased in the presence of a Pgp modulator, similar to the decrease in TEER after application of a higher concentration of the anti-microtubule drug (Fig. 2). This indicates that after inhibition of Pgp the pharmacodynamic effects of these drugs are increased, most probably by increased intracellular concentration in the endothelial cells of the BBB. Subsequently, the increased pharmacodynamic effects in the presence of Pgp modulators have led to a loss in integrity of the BBB at considerably lower concentrations than in the control experiments. The protective function of the Pgp at the BBB was consideraby impaired by Pgp inhibition, and the microtubuli destabilising Pgp substrates (colchicine and vinblastine) were more effective than the stabilizing one (paclitaxel).

The influence of anti-microtubule drugs on the permeability of the BBB was used to quantitatively evaluate the Pgp modulators, LY 335979, SDZ-PSC 833, verapamil, and cyclosporin A with respect to their Pgp inhibitory capacity. Since the effect of the anti-microtubule drugs on TEER was concentration-dependently enhanced in the presence of the Pgp modulators, this phenomenon was used to quantify their inhibitory effect parameters. Using the sigmoid model for TEER inhibition, E_{max} and IC_{50} values could be estimated (Fig. 3). The IC_{50} values of the Pgp modulators to inhibit Pgp in this study are in the same range with those observed in other *in vitro* studies (Table I; 25–26). In those studies intracellular accumulation of a Pgp substrate was measured in the presence and absence of Pgp modulators.

Furthermore, the present data show that it is possible to demonstrate similar effects of compounds that interact with Pgp (Fig. 4). Ritonavir and amprenavir, which have shown to be Pgp substrates interfered also in our *in vitro* coculture BBB model (27,28), with the vinblastine efflux by Pgp as indicated by the reduction in TEER. In conclusion, this study demonstrates that the disruptive effects of anti-microtubule drugs on the BBB are enhanced after inhibition of Pgp. This implies that the function of Pgp is not only to limit compounds from entering the brain, and thereby to protect the brain, but also to protect the endothelial compartment of the BBB against pharmacodynamic effects of Pgp substrates. Furthermore, the assay as proposed can be used as a model to quantitatively assess Pgp modulators and demonstrate interactions of Pgp substrates with Pgp.

ACKNOWLEDGMENTS

The authors thank Prof. M. Danhof and J.M.K. van Fessem for critically reading this manuscript.

REFERENCES

1. M. Bradbury. *The concept of the blood-brain barrier,* John Wiley and Sons, Chichester, 1979.
2. W. M. Pardridge. *Knocking on the cerebral door; a challenge for CNS drug development,* Odessey **1**:46–51 (1995).
3. H. E. de Vries, J. Kuiper, A. G. de Boer, T. J. van Berkel, and D. D. Breimer. The blood-brain barrier in neuroinflammatory diseases. *Pharmacol. Rev.* **49**:143–155 (1997).
4. C. Cordon-Cardo, J. P. O'Brien, D. Casals, L. Rittman-Grauer, J. L. Biedler, M. R. Melamed, and J. R. Bertino. Multidrug resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc. Natl. Acad. Sci. USA* **86**:695–698 (1989).
5. N. Kartner, J. R. Riordan, and V. Ling. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cells. *Science* **221**:1285–1288 (1983).
6. J. Robert. Approaches to multidrug resistance reversal. *Exp. Opin. Invest. Drugs* **7**:929–939 (1998).
7. J. Wijnholds, G. L. Scheffer, M. van der Valk, P. van der Valk, J. H. Beijnen, R. J. Scheper, and P. Borst. Multidrug resistance protein 1 protects the oropharyngeal mucosal layer and the testicular tubules against drug-induced damage. *J. Exp. Med.* **188**: 797–808 (1998)
8. P. J. Gaillard, I. C. van der Sandt, L. H. Voorwinden, D. Vu, J. Nielsen, A. G. de Boer, and D. D. Breimer. Astrocytes increase

the functional expression of P-glycoprotein in an vitro model of the blood-brain barrier. *Pharm. Res.* **17**:1198–1205 (2000).
9. P. J. Gaillard, A. G. de Boer, and D. D. Breimer. Relationship between permeability status of the blood-brain barrier and in vitro permeability coefficient of a drug. *Pharm. Res.***12**:95–102 (1995).
10. P. J. Gaillard, L. H. Voorwinden, J. Nielsen, A. Ivanov, R. Atsumi, H. Engman, C. Ringbom, A. G. de Boer, and D. D. Breimer. Establishment and functional characterization of an in vitro model of the blood-brain barrier, comprising a coculture of brain capillary endothelial cells and astrocytes. *Eur. J. Pharm. Sci.* **12**:215–222 (2001).
11. A. H. Schinkel, E. Wagenaar, L. van Deemter, C. A. Mol, and P. Borst. Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J. Clin. Invest.* **96**:1698–705 (1995).
12. J. W. Jonker, E. Wagenaar, L. van Deemter, R. Gottschlich, H. M. Bender, J. Dasenbrock, and A. H. Schinkel. Role of blood-brain barrier P-glycoprotein in limiting brain accumulation and sedative side-effects of asimadoline, a peripherally acting analgesic drug. *Br. J. Pharm.* **127**:43–50 (1999).
13. S. P. Letrent, G. M. Pollack, K. R. Brouwer, and K. L. Brouwer. Effects of a potent and specific P-glycoprotein inhibitor on the blood-brain barrier distribution and antinociceptive effect of morphine in the rat. *Drug Metab. Dispos.* **27**:827–834 (1999).
14. L. Wilson and M. A. Jordan. Microtubule dynamics: taking aim at a moving target. *Chem. Biol.* **2**:569–573 (1995).
15. D. T. Hung, T. F. Jamison, and S. L. Schreiber. Understanding and controlling the cell cycle with natural products. *Chem. Biol.* **3**:623–639 (1996).
16. P. K. Sorger, M. Dobles, R. Tournebize, and A. A. Hyman. Coupling cell division and cell death to microtubule dynamics. *Current Opinion in Cell Biology.* **9**:807–814 (1997).
17. M. A. Jordan, D. Thrower, and L. Wilson. Mechanism of inhibition of cell proliferation by Vinca alkaloids. *Cancer Res.* **51**:2212–2222 (1991).
18. M. A. Jordan, R. J. Toso, D. Thrower, and L. Wilson. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc. Natl. Acad. Sci. USA.* **90**:9952–9556 (1993).
19. J. F. Diaz and J. M. Andreu. Kinetics of dissociation of the tubulin-colchicine complex. *J. Biol. Chem.* **266**:2890–2896 (1991).
20. C. Mouchard-Delmas, B. Gourdier, R. Vistelle, and M. Wiczewski. Modification of the blood-brain barrier permeability following intracarotid infusion of vinorelbine. *Anticancer Res.* **15**: 2593–2596 (1995).
21. S. Nag. Role of the endothelial cytoskeleton in blood-brain-barrier permeability to protein. *Acta Neuropathol.* **90**:454–460 (1995).
22. G. M. Ferron, M. Rochdi, W. J. Jusko, and J. M. Scherrmann. Oral absorption characteristics and pharmacokinetics of colchicine in healthy volunteers after single and multiple doses. *J. Clin. Pharmacol.* **36**:874–883 (1996).
23. M. T. Huizing, J. B. Vermorken, H. Rosing, W. W. ten Bokkel-Huinink, I. Mandjes, H. M. Pinedo, and J. H. Beijnen. Pharmacokinetics of paclitaxel and three major metabolites in patients with advanced breast carcinoma refractory to anthracycline therapy treated with a 3-hour paclitaxel infusion: A European Cancer Centre (ECC) trial. *Ann. Oncol.* **6**:699–704 (1995).
24. R. Rahmani, J. P. Kleisbauer, J. P. Cano, M. Martin, and J. Barbet. Clinical pharmacokinetics of vindesine infusion. *Cancer Treat. Rep.* **69**:839–844 (1985).
25. J. M. Ford. Modulators of multidrug resistance, *Drug Res. Clin. Oncol. Hematol.* **9**:337–361 (1995).
26. G. A. Fisher, B. L. Lum, and B. I. Sikic. The reversal of multidrug resistance. *Cancer. Treat. Res.* **78**:45–70 (1995).
27. R. B. Kim, M. F. Fromm, C. Wandel, B. Leake, A. J. Wood, D. M. Roden, and G. R. Wilkinson. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J. Clin. Invest.* **101**:289–294 (1998).
28. I. C. van der Sandt, C. M. Vos, L. Nabulsi, M. C. Blom-Roosemalen, H. H. Voorwinden, A. G. de Boer, and D. D. Breimer. Assessment of active transport of HIV protease inhibitors in various cell lines and the in vitro blood-brain barrier. *AIDS* **15**:483–491 (2001).