Journal of Neuroscience Research 49:80–88 (1997)

Role of P-170 Glycoprotein in Colchicine Brain Uptake

N. Drion, P. Risede, N. Cholet, C. Chanez, and J.M. Scherrmann*
INSERM U26, Hôpital Fernand Widal, Paris, France

To study the role of P-glycoprotein (P-gp) in the delivery of colchicine from blood to brain, the pharmacokinetics of colchicine in plasma and brain was studied in the rat by an in vivo method and by the in situ brain perfusion technique. Colchicine was administered intravenously at three doses (1, 2.5, and 5 mg/kg) with or without an inhibitor of P-gp, verapamil (0.5 mg/kg IV); blood and brain samples were taken at $t = 1$, 2, and 3 hr. Areas under the colchicine curve at doses from 2.5 to 5 mg/kg were proportional to dose for plasma but not for brain. At a colchicine dose of 5 mg/kg, verapamil co-treated rats showed a 1.65-fold enhancement of the colchicine concentration in plasma but a 4.5-fold enhancement in brain. During short experimental times (in situ brain perfusion technique), a comparable enhancement was found (4.26-fold): mean distribution volumes of colchicine were enhanced from 0.23 ± 0.17 to 0.98 ± 0.19 μl/g for the eight gray areas, and no effect was observed in the choroid plexus, which do not express P-gp. These results clearly show that P-gp, present at the luminal surface of the capillary endothelial cells, is responsible for the weak penetration of colchicine into the brain. J. Neurosci. Res. 49:80–88, 1997. © 1997 Wiley-Liss, Inc.

Key words: blood-brain barrier; colchicine; P-glycoprotein; pharmacokinetics; verapamil

INTRODUCTION

Cells that express the P-170 glycoprotein (P-gp), a transmembrane ATP-dependent protein, specifically prevent the cellular penetration of several drugs. This energy-dependent efflux pump, a product of the multidrug resistance (MDR) gene, was first described as one of the main supports of the multidrug resistance phenomenon observed with a large group of cytotoxic compounds, such as anthracyclines, vinca alkaloids, the epipodophyllotoxins, and paclitaxel, in cancer chemotherapy (Gottesman and Pastan, 1993).

P-gp is present in cells that acquire resistance to cytotoxic drugs after previous exposure and also in several normal types of epithelial and endothelial tissues that have secretory and excretory fonctions. P-gp is maximally expressed at the luminal surface of the endothelium forming the blood-brain barrier (BBB; Cordon-Cardo et al., 1989; Tsuji et al., 1992) and may be partially responsible for the limited brain uptake of cytotoxic drugs. This limited brain penetration could also concern colchicine, a natural alkaloid that is a potent inhibitor of cellular mitosis and that is neurocytotoxic (when given intracerebrally, since the low passage across the BBB is not sufficient to cause cytotoxicity; Desrayaud et al., 1996).

Earlier studies showed that colchicine interacts with P-gp in different cellular models, including immortalized cell line of rat brain endothelial cells (RBE4, e.g., Begley et al., 1996), and a few in vivo studies have reported low brain colchicine levels in different species in spite of its highly lipophilic nature (Bennett et al., 1981; Begley and Evans, 1992).

To study the role of P-gp as one of the possible barriers to the brain uptake of colchicine, we measured the colchicine concentration at variable times and doses in plasma and total brain homogenate with an in vivo technique and with the in situ brain perfusion technique the colchicine uptake in several cerebral regions following IV administration. Similar experiments were performed both in rats receiving colchicine alone and in others receiving colchicine and verapamil simultaneously. Verapamil is a calcium channel blocker that has been found to antagonize multidrug resistance in a variety of cell lines and in in vivo tumor models (Ford and Hait, 1990). Verapamil was co-administered with colchicine, which crosses the BBB poorly to investigate the role of P-gp in the limitation of colchicine transport into the brain.

MATERIALS AND METHODS

Chemicals

^{3}H-colchicine (ring C; methoxy-^{3}H, 39 Ci/mmol) and [U-^{14}C]sucrose (0.442 Ci/mmol) were obtained from

This study is dedicated to Nicolas Drion, who, at age 27 years, died on July 8, 1996, in a mountaineering accident. He was the inspiration for this work that we are continuing in his memory.

*Correspondence to: J.M. Scherrmann, INSERM U26, Hôpital Fernand Widal, 200, rue du Faubourg Saint Denis, 75475 Paris cedex 10, France.

Received 5 November 1996; Revised 11 February 1997; Accepted 26 February 1997

Du Pont-New England Nuclear (Les Ulis, France) and were used as tracer. Colchicine and verapamil hydrochloride were obtained from Sigma (St-Quentin Fallavier, France). Stock solutions (colchicine 5 mg/ml, verapamil 0.5 mg/ml) in 0.9% NaCl were prepared before each experiment. All other chemicals were of pure grade.

Animals

Adult male rats (Sherman, 250–350 g), received standard chow (Iffa-Credo) ad libitum under isothermal conditions with a 12-hour light-dark cycle.

Brain and Plasma Colchicine Pharmacokinetics

In vivo method. Three to five rats were used for each time point. Animals were anesthetized with pentobarbital (50 mg/kg). The first group received colchicine solution (1 ml) by slow IV injection in the femoral vein at three different doses of 1, 2.5, or 5 mg/kg. The second group received colchicine solution at the same concentration and simultaneously verapamil at 0.5 mg/kg; this dose was chosen because of the long experimental time and the cardiac toxicity of verapamil. Rats were decapitated 60, 120, and 180 min after injection, and blood was immediately collected in heparinized tubes. The samples were centrifuged at 3,000× *g* for 10 min. Plasma was separated from cellular components and stored at −30°C until analysis. Brains were quickly removed and homogenized in PBS (50% w/v) by using a Potter-Elvejhem homogenizer at 1,000× *g*. The brain tissue homogenates were centrifuged at 20,000× *g* in a Sorvall SW 34. The pellet was discarded, and the nonsedimentable aqueous soluble fraction was removed and frozen at −30°C.

Colchicine concentrations in plasma and brain tissues were determined by radioimmunoassay as previously described (Scherrmann et al., 1980). Several colchicine metabolites were assayed for cross-reactivity at concentrations in the range of the calibration curve from 0.15 to 10 ng/ml, i.e., 2-demethylcolchicine, 3-demethylcolchicine, lumicolchicine, and *N*-desacetylcolchicine. They yielded apparent colchicine concentrations of less than 0.15 ng/ml, which is the lower limit of the assay. The lowest metabolite concentration able to yield apparent colchicine (0.15 ng/ml) is observed at 50 ng/ml for 2-demethylcolchicine (Chappey et al., 1993).

The area under the plasma or brain concentration curves was calculated by linear interpolation from time 1 to 3 hr by applying the trapezoidal rule (Yeh and Kwan, 1978).

Results are means ± SEM. Values were compared by using Fisher's *f*-test, followed by the Mann-Whitney nonparametric test or Student's *t*-test.

In situ brain perfusion technique. We used the in situ brain perfusion technique of Takasato et al., 1984, as modified by Smith et al., 1990. Rats were deeply anesthetized with pentobarbital (50 mg/kg). The right pterygopalatine and occipital arteries were electrocoagulated and the right external carotid was ligated. A tight ligature was placed on the heart side of the right common carotid artery, which was fitted with a polyethylene catheter (internal diameter of 0.58 mm; Biotrol, France) filled with heparinized 0.9% sodium chloride solution (2 mg/ml of heparin; Prolabo, France).

The perfusion fluid was a HCO_3^- buffered saline solution filtered through a 0.22-µm acetate cellulose filter and containing (in mM) 2.4 $NaH_2PO_4 \cdot H_2O$, 4.2 KCl, 24.0 $NaHCO_3$, 128.0 NaCl, 1.5 $CaCl_2 \cdot 2H_2O$, 0.9 $MgSO_4$, and 9 D-glucose, added prior to infusion. The solution was oxygenated for 3 min with 75% N_2, 20% O_2, 5% CO_2 (pH 7.40 ± 0.05). Tracers were added to perfusates at concentrations of 0.3 µCi/ml for ^{14}C-sucrose and 1.5 µCi/ml for ^{3}H-colchicine. Each perfusate was equilibrated at 37°C in a water bath.

Rats were divided into two groups and pretreated intravenously 5 min before perfusion as follows: one with 0.5 ml of 0.9% NaCl and one with verapamil (1 mg/kg) dissolved in 0.5 ml of 0.9% NaCl; 1 mg/kg was used because perfusion time was very short (20 sec) compared with the in vivo experiments (1–3 hr).

The thorax of the animal was opened, the heart was cut, and perfusion was started with a flow rate of 5.0 ml/min. It was terminated by decapitation of the rat at 20 sec, with a 5-sec correction for the time for the perfusion to reach the cerebral hemisphere and the brain removed from the skull. The leptomeninges and subarachnoid vessels were removed, and the brain was dissected on ice following the procedure of Ohno et al., 1978. Nine brain areas (olfactory bulb, hypothalamus, frontal, parietal and occipital cortex, striatum, hippocampus, thalamus, and choroid plexus) were dissected from the right cerebral hemisphere, placed in preweighed vials, and weighed. Samples were digested for 2 hr at 60°C in soluene-350 (Packard, Rungis, France); 10 ml of scintillation cocktail (Pico-fluor, Packard) was added, and dual label counting was performed (Beckman LS9000 liquid scintillation counter, Beckman instruments, Fullerton, CA).

Colchicine uptake was expressed as the distribution volume (V_d in µl/g) defined as the ratio between the radioactivity expressed in dpm of tracer per gram of brain (Q_{tis}), and dpm/ml in the perfusate saline or plasma (C_p):

$$\text{distribution volume} = Q_{tis}/C_p$$

The residual vascular tracer in tissue samples was substracted from all tissue samples as:

$$Q_{tis} = Q_{tot} - V_v C_p$$

where Q_{tot} is the total quantity of tracer in samples, V_v is the vascular volume, and C_p the perfusate tracer concen-

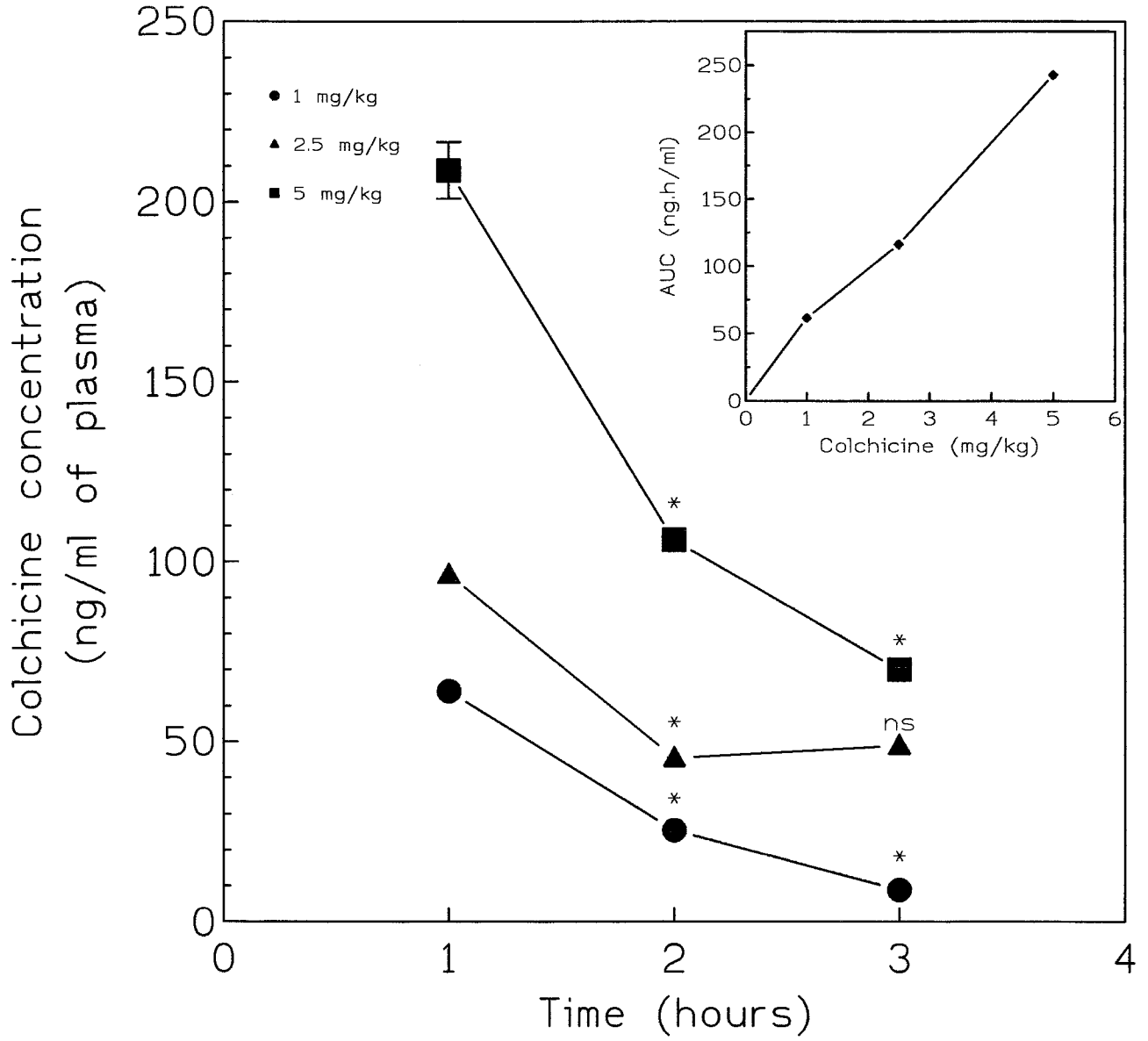

Fig. 1. Plasma concentration-time profile of colchicine after IV injection of three different doses of colchicine (1, 2.5, and 5 mg/kg). Values are means ± SEM of three to five independent experiments. *$P < 0.05$; ns, not significant difference, $P > 0.05$ compared with the plasma colchicine concentration at t − 1 hr for the same dose of injected colchicine. **Inset:** Areas under the curve (AUC_{1-3h}) of the plasma concentration-time profile, according to the three injected doses of colchicine.

tration; the vascular volume was evaluated by the sucrose space and calculated by the ratio between radioactivity of ^{14}C-sucrose (expressed in dpm of sucrose per gram of brain) and the perfusate sucrose concentration (Blasberg et al., 1983).

All experiments were performed on five rats. Data are expressed as the mean value of the eight gray areas.

All values are the means ± SEM. Values were compared by analysis of variance, followed by the Newman-Keuls procedure or Student's *t*-test.

RESULTS

Figures 1 and 2 show the plasma and brain colchicine disposition over 1–3 hr following intravenous administration. Colchicine disposition was linear in plasma within the range of the three doses as the AUC_{1-3} (area under the curve) were proportional to the dose, whereas a nonlinear increase in the brain colchicine AUC_{1-3} was observed, suggesting that brain colchicine distribution might be saturable. This hypothesis was confirmed with the

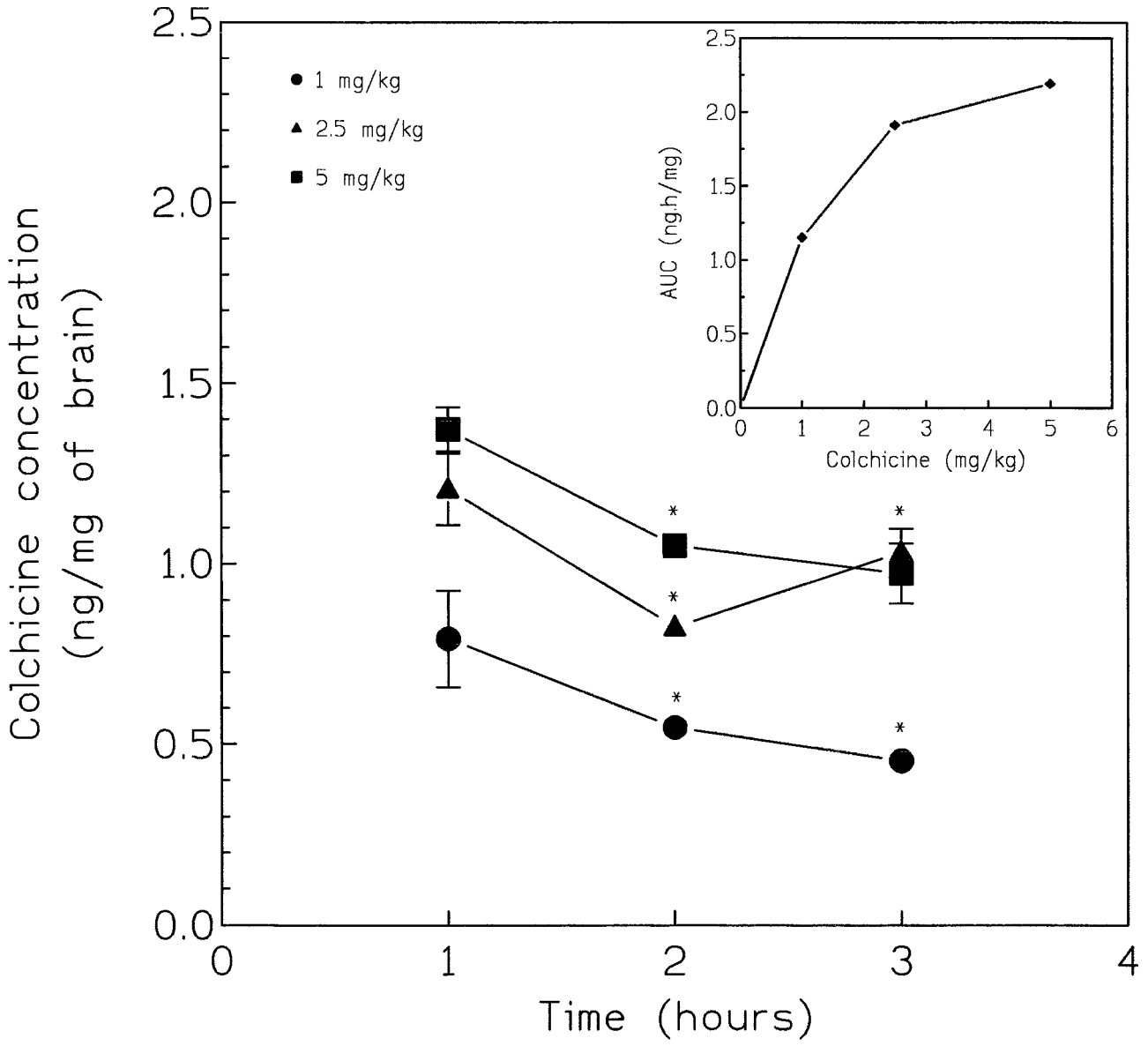

Fig. 2. Brain concentration-time profile of colchicine after IV injection of three different doses of colchicine (1, 2.5, and 5 mg/kg). Values are means ± SEM of three to five independent experiments. *$P < 0.05$ compared with the brain colchicine concentration at t − 1 h for the same dose of injected colchicine. **Inset:** Areas under the curve (AUC_{1-3h}) of the brain concentration-time profile, according to the three injected doses of colchicine.

verapamil co-treated group. Verapamil increased cerebral colchicine concentration (Fig. 3): at 2.5 mg/kg colchicine, the increase was 1.16-fold in plasma (from 96.44 ± 2.53 to 112.05 ± 0.54 ng/ml, $P < 0.05$) and 1.75-fold in brain (from 1.21 ± 0.10 to 2.11 ± 0.01 ng/mg, $P < 0.05$); at 5 mg/kg colchicine, the increase was 1.65-fold in plasma (from 208.71 ± 7.83 to 343.67 ± 19.15 ng/ml, $P < 0.05$) and 4.5-fold in brain (from 1.372 ± 0.061 to 6.179 ± 0.155 ng/mg, $P < 0.05$). The previous nonlinear brain uptake of colchicine was abolished by the coadministration of verapamil, suggesting that the inhibition of the P-gp by verapamil at the BBB interface allows the diffusion of colchicine through the endothelium.

This effect of verapamil at the BBB was confirmed with the in situ brain perfusion technique. Pretreatment with verapamil increased the distribution volume of colchicine after 20 sec of perfusion in all eight gray areas ($P < 0.01$), with mean values of 0.23 ± 0.17 µl/g for 0.9% NaCl pretreated rats and 0.98 ± 0.19 µl/g for verapamil pretreated rats (Fig. 4). Colchicine distribution

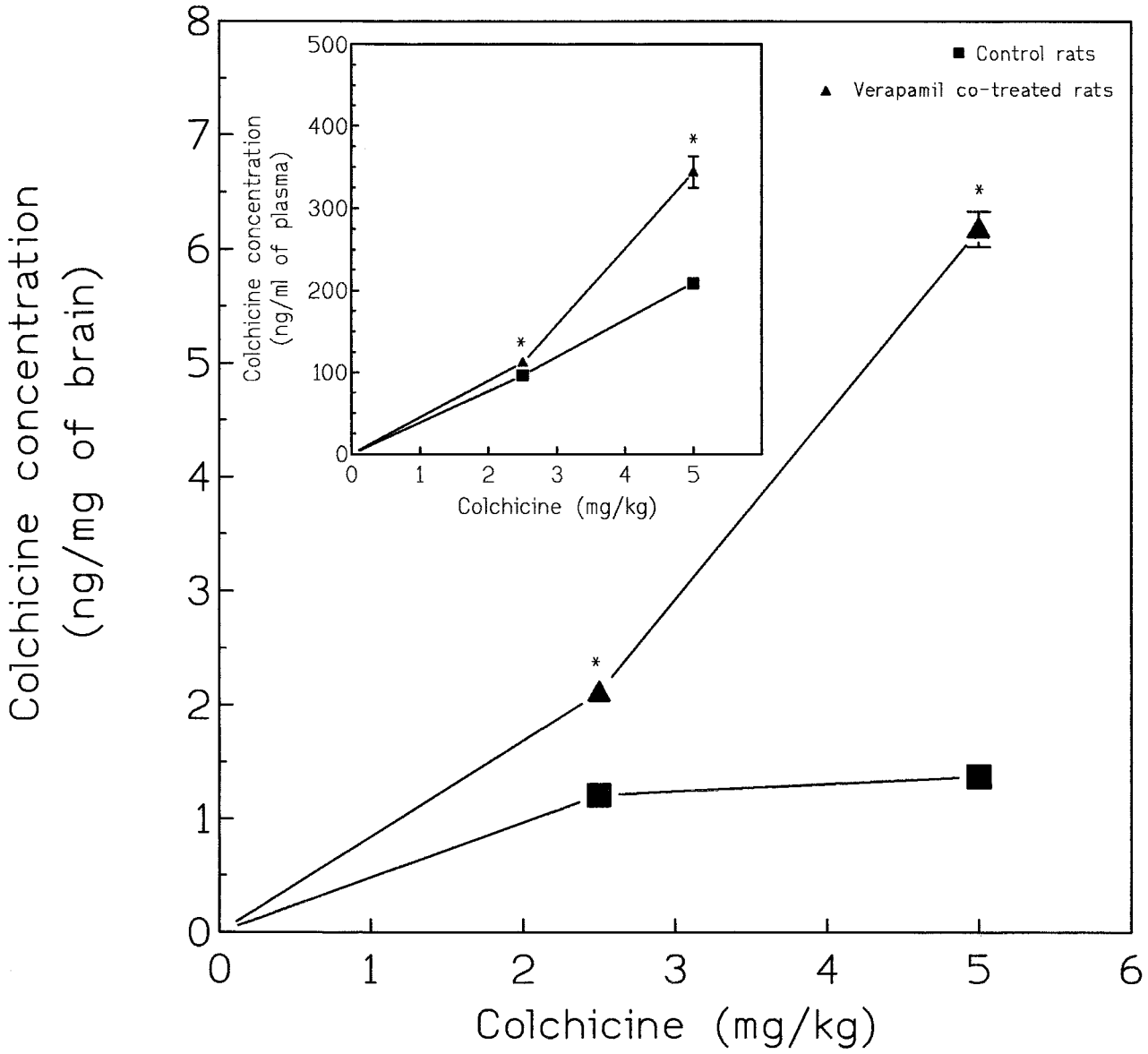

Fig. 3. Brain concentration of colchicine, according to two IV injected doses of colchicine (2.5 and 5 mg/kg), with or without coadministration of verapamil (0.5 mg/kg IV). Values are means ± SEM of three to five independent experiments. *$P <$ 0.05 compared with the brain colchicine concentration in nonverapamil co-treated rats, for the same dose of injected colchicine. **Inset:** Plasma concentration of colchicine, according to two IV injected doses of colchicine (2.5 and 5 mg/kg), with or without coadministration of verapamil (0.5 mg/kg IV). Values are means ± SEM of three to five independent experiments. *$P < 0.05$, compared with the plasma colchicine concentration in nonverapamil co-treated rats for the same dose of injected colchicine.

volume was considerably greater in the choroid plexus (325.6 ± 35.9 µl/g in control rats, 277.8 ± 24.6 µl/g in verapamil pretreated rats) than in the gray areas ($P < 0.001$) and was not influenced by the verapamil pretreatment (Fig. 4).

DISCUSSION

For many years, the multidrug resistance phenomenon in cancer chemotherapy has in great part been attributed to the presence of an ATP-dependent efflux pump in the cell membrane, which is able to reduce intracellular concentrations of several cytotoxic drugs (Kartner and Ling, 1989; Pinedo and Giaccone, 1995). This pump was termed P-170 glycoprotein and, more recently, also has been characterized in several normal epithelial cells and in capillaries of the central nervous system, testes, and papillary dermis (Cordon-Cardo et al., 1989). P-gp is expressed at the luminal surface of the

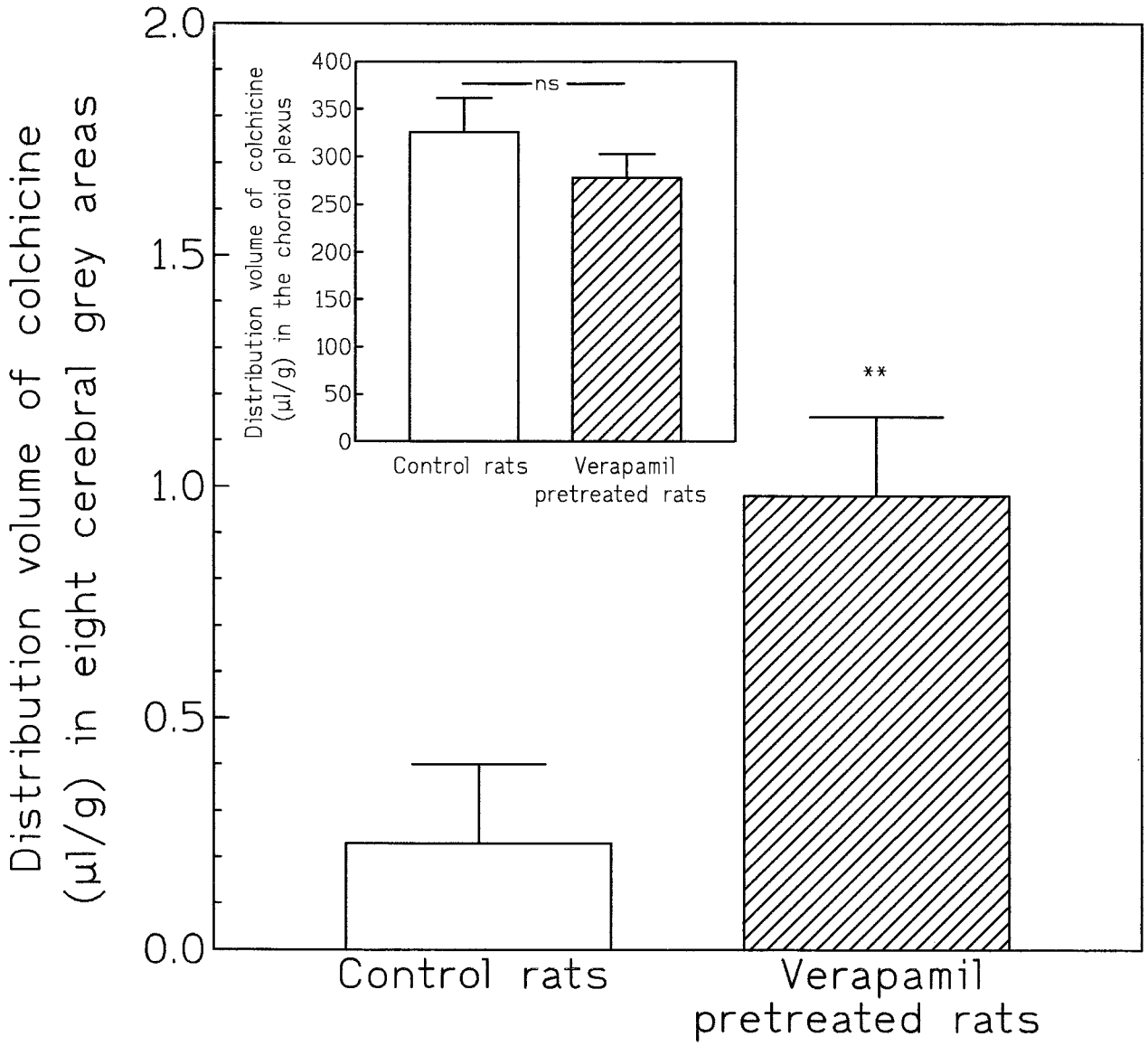

Fig. 4. Distribution volume of colchicine in eight gray areas of rat brain after 20 sec of perfusion (in situ brain perfusion technique). Rats were previously pretreated intravenously with either 0.9% NaCl (control) or verapamil (1 mg/kg). Values are means ± SEM of five independent experiments. $^{**}P < 0.01$ compared with the control group. **Inset:** Distribution volumes of colchicine in the choroid plexus of rat brain after 20 sec of perfusion. Rats were pretreated intravenously with either 0.9% NaCl (control) or verapamil (1 mg/kg). Values are means ± SEM of five independent experiments. ns, no significant difference between NaCl and verapamil-pretreated groups. Difference between the distribution volumes in choroid plexus and in gray areas is $P < 0.001$.

endothelial cells of the BBB (Cordon-Cardo, 1989; Tsuji et al., 1992; Tatsuta et al., 1992) and is also present in the liver at the biliary canalicular surface (apical plasma membrane of hepatocytes) (Thiebault et al., 1987; Kamimoto et al., 1989) and in the kidney at the brush border of proximal tubules (Fojo et al., 1987; Thiebault et al., 1987). Although the tissue expression is dependent on the gene specificity (in the rat, high expression of mdr2 mRNA in liver, lower in brain; high expression of mdr1b mRNA in liver, and lower in kidney; Silverman et al., 1991; Brown et al., 1993), the pharmacokinetic effect tends frequently toward drug detoxification processes, such as facilitation of drug elimination by active biliary excretion (Speeg et al., 1992a) or renal tubular secretion (Speeg et al., 1992b; De Lannoy et al., 1994). The P-gp function might also affect drug pharmacokinetics and

especially brain penetration of P-gp interacting drugs. Several studies have used in vitro models based on primary cultures of bovine cerebral capillary endothelial cells (Lechardeur and Scherman, 1995), RBE4 immortalized cultures (Begley et al., 1996), or isolated brain capillaries (Jette et al., 1995) and have shown that inhibition of drug penetration into the brain can be suppressed by the addition of chemical compounds, such as verapamil and cyclosporine (El Hafny et al., 1997), which antagonize the effect of P-gp. However, these in vitro techniques cannot predict what the in vivo role of P-gp might be in the reduction of the brain bioavailability of the drug. Few studies concern this domain (Begley et al., 1992; Habgood et al., 1996).

To investigate the role of P-gp, we selected colchicine, which interacts with P-gp and crosses the BBB poorly, and chose verapamil as P-gp inhibitor. Two complementary rat models were used. The first is based on a classical pharmacokinetic study of plasma and brain levels, with and without P-gp inhibitor; the second is the in situ brain perfusion technique, which specifically explores drug transfer into the brain, whereas the first integrates the effects of P-gp in each part of the body where it is expressed. Plasma and brain concentrations in the in vivo technique were measured by a specific radioimmunoassay that is not interfered with by colchicine metabolites. This permits comparison with data from the in situ technique, which used tritiated colchicine but over a very short time of 20 sec, excluding the possible interference of metabolites or tritiated water, which occurs in longer pharmacokinetic protocols. Doses of verapamil higher than 0.5 mg/kg were not used in the in vivo approach because of its cardiotoxicity in the rat and previous experiments had shown that the simultaneous administration of colchicine and verapamil was the more efficient protocol (unpublished). Finally, we found no influence of the perfusion medium (buffer or plasma) on brain uptake with the in situ brain perfusion technique (data not shown), and data were expressed as the average value of the eight gray areas because no significant differences in the uptake of colchicine with or without verapamil were observed as also previously reported (Drion et al., 1996).

The in vivo pharmacokinetic study used three doses of colchicine to evaluate the pharmacokinetic linearity of colchicine disposition in plasma and brain. In contrast to plasma, where colchicine concentrations were proportional to the dose, a nonlinear brain colchicine uptake was observed, which indicated the existence of saturable processes in the blood to brain transport of colchicine. The fact that coadministration of verapamil restored the linearity of the colchicine brain distribution suggested that P-gp was involved. The interaction between P-gp and verapamil at the BBB level was proved by using the in situ brain perfusion technique. Pretreatment with verapamil increased the colchicine brain uptake. Transport was increased in a very similar manner, using the two techniques, by a factor of 4.5 with the in vivo approach and 4.26 with the in situ brain perfusion technique. This demonstrates clearly that the effect occurs at the BBB level and that P-gp inhibits brain uptake of colchicine.

Nevertheless, our study does not completely show that verapamil effectively increases the penetration of colchicine into the brain. The increased transport observed with both techniques also might result from an increased uptake of colchicine within the endothelial cells themselves. Therefore, a more recent in vivo study with simultaneous plasma and brain microdialysis in rat shows that the blockade of P-glycoprotein by SDZ PSC-833 (an analog of cyclosporine) results in the increase of the brain extracellular concentration of colchicine (Desrayaud et al., 1997).

The distribution volume of colchicine in choroid plexus was greater than in the eight gray areas, and there was no enhancement of this distribution volume following verapamil treatment. Choroid plexus is a cerebral zone without a blood-brain barrier: there is a free movement of substances across the endothelial cells through fenestrations and intercellular spaces, in contrast to the limited diffusion of solutes across brain capillary endothelial cells characterized by tight junctions. The blood-cerebrospinal fluid barrier at the choroid plexus consists of epithelial cells that are joined together by tight junctions (Van Deurs and Koehler, 1979). Thus, passage of colchicine through the capillaries occurs in this area. Moreover, no P-gp expression has been found in the choroid plexus (Cordon-Cardo et al., 1989).

However, in spite of verapamil pretreatment, the increase in colchicine uptake in the eight gray areas still remains low compared with other compounds, such as imipramine, cocaine, and diazepam, that have comparable lipophilicity (colchicine log $P = 2.52$; Matsuyama et al., 1989) but a 100-fold greater penetrance across the BBB (Levin, 1980). We suggest that P-gp is either partially inhibited (difficulty of fully saturating P-glycoprotein, especially under in vivo conditions) or not the only barrier to colchicine.

Furthermore, interpretation of the in vivo data showed the interest of the simultaneous measurement of plasma and brain drug concentrations. For example, colchicine clearance is known to depend highly on its biliary excretion (Sabouraud et al., 1992), and the resulting effect of verapamil is also to block P-gp in other tissues of the body, e.g., in liver and kidney, which results in reduced elimination of colchicine. These effects on colchicine clearance were confirmed in the present study

by the 1.65-fold increase in colchicine plasma levels at the dose of 5 mg/kg. This increase in plasma colchicine results in a higher gradient between blood and brain and could explain why brain uptake was higher in the verapamil group. However, because brain colchicine levels were enhanced 4.5-fold in verapamil co-treated rats, the higher brain uptake of colchicine did not result only from the increase in plasma colchicine but mainly from the P-gp inhibition at the BBB. Our data clearly indicate that in vivo studies limited only to the measurement of drug brain distribution would not be sufficient to show the effect of P-gp and that the overall processes of distribution and clearance of a P-gp interacting drug must be considered before evaluating the influence of P-gp at the BBB interface. Moreover, the combination of the in vivo investigation with the in situ brain perfusion technique enables the mechanisms at the BBB to be understood and excludes the interference of P-gp from other tissues involved in drug clearance.

In conclusion, our data established a close relationship between an in vivo approach and the in situ brain perfusion technique and showed the role of P-gp in colchicine transport into the brain.

ACKNOWLEDGMENT

We thank Corinne Ragusi for her contribution to this study.

REFERENCES

Begley DJ, Evans JE (1992): Vincristine in therapeutic dosage appears to increase blood-brain barrier permeability to colchicine in the anaesthetised guinea pig. J Physiol 446–499.

Begley DJ, Lechardeur D, Chen Z-D, Rollinson C, Bardoul M, Roux F, Scherman D, Abbott NJ (1996): Functional expression of P-glycoprotein in an immortalised cell line of rat brain endothelial cells, RBE4. J Neurochem 67:988–995.

Bennett EL, Alberti MH, Flood JF (1981): Uptake of [^{3}H]colchicine into brain and liver of mouse, rat, and chick. Pharmacol Biochem Behav 14:863–869.

Blasberg RG, Fenstermacher JD, Patlak CS (1983): Transport of alpha-aminoisobutyric acid across brain capillary and cellular membranes. J Cereb Blood Flow Met 3:8–31.

Brown PC, Thorgeirsson SS, Silverman JA (1993): Cloning and regulation of the rat mdr2 gene. Nucleic Acids Res 21:3885–3891.

Chappey ON, Niel E, Wautier JL, Pham Hung P, Dervichian M, Cattan D, Scherrmann JM (1993): Colchicine disposition in human leukocytes after single and multiple oral administration. Clin Pharmacol Ther 54:360–367.

Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR (1989): Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci USA 86:695–698.

De Lannoy IAM, Mandin RS, Silverman M (1994): Renal secretion of vinblastine, vincristine and colchicine in vivo. J Pharmacol Exp Ther 263:388–395.

Desrayaud S, Boschi G, Rips R, Scherrmann JM (1996): Dose-dependent delivery of colchicine to the rat hippocampus by microdialysis. Neurosci Lett 205:9–12.

Desrayaud S, Guntz P, Scherrmann JM, Lemaire M (1997): Effect of the P-glycoprotein inhibitor, SDZ PSC 833, on the blood and brain pharmacokinetics of colchicine. Life Sci (in press).

Drion N, Lemaire M, Lefauconnier JM, Scherrmann JM (1996): Role of P-glycoprotein in the blood-brain transport of colchicine and vinblastine. J Neurochem 67:1688–1693.

El Hafny B, Cano N, Piciotti M, Regina A, Scherrmann JM, Roux F (1997): Role of P-glycoprotein in colchicine and vinblastine cellular kinetics in an immortalized rat brain microvessel endothelial cell line. Biochem Pharmacol (in press).

Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I (1987): Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci USA 84:265–269.

Ford JM, Hait WN (1990): Pharmacology of drugs that alter multidrug resistance in cancer. Pharmacol Rev 42:155–199.

Gottesman MM, Pastan I (1993): Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 62:385–427.

Habgood MD, Begley DJ, Abbott NJ (1996): Effects of vincristine on brain uptake of P-glycoprotein substrates in anaesthetized adult rats. J Physiol 491:37–38.

Jette L, Murphy GF, Leclerc JM, Beliveau R (1995): Interaction of drugs with P-glycoprotein in brain capillaries. Biochem Pharmacol 50:1701–1709.

Kamimoto Y, Gatmaitan Z, Hsu J, Arias IM (1989): The function of Gp170, the multidrug resistance gene product, in rat liver canalicular membrane vesicles. J Biol Chem 264:11693–11698.

Kartner N, Ling V (1989): Multidrug resistance in cancer. Sci Am 260:44–51.

Lechardeur D, Scherman D (1995): Functional expression of the P-glycoprotein mdr in primary cultures of bovine cerebral capillary endothelial cells. Cell Biol Toxicol 11:283–293.

Levin VA (1980): Relationship of octanol/water coefficient and molecular weight to rat brain capillary permeability. J Med Chem 23:682–684.

Matsuyama SS, Braun LD, Oldendorf WH, Fu TK, Jarvik LF (1989): Colchicine and the blood-brain barrier: Implications for Alzheimer patients. Age 12:107–108.

Ohno K, Pettigrew KD, Rapoport SI (1978): Lower limits of cerebrovascular permeability to nonelectrolytes in conscious rats. Am J Physiol 235:H299–H307.

Pinedo HM, Giaccone G (1995): P-glycoprotein—A marker of cancer-cell behavior. N Engl J Med 333:1417–1419.

Sabouraud AE, Urtizberea M, Benmoussa K, Cano NJ, Scherrmann JMG (1992): Fab-bound colchicine appears to adopt Fab fragment disposition in rats. J Pharm Pharmacol 44:1015–1019.

Scherrmann JM, Bourdet L, Pontikis R, Nguyen-Hoang-Nam, Fournier E (1980): A sensitive radioimmunoassay for colchicine. J Pharm Pharmacol 32:800–802.

Silverman JA, Raunio H, Gant TW, Thorgeirsson SS (1991): Cloning and characterization of a member of the rat multidrug resistance (mdr) gene family. Gene 106:229–236.

Smith QR, Fukui S, Robinson P, Rapoport SI (1990): Influence of cerebral blood flow on tryptophan uptake into brain. In Lubec G, Rosenthal GA (eds): ''Amino Acids: Chemistry, Biology and Medicine.'' Amsterdam: ESCOM Science Publishers, pp. 364–369.

Speeg VK, Maldonato AL, Liaci J, Muirhead D (1992a): Effect of cyclosporine on colchicine secretion by a liver canalicular transporter studied in vivo. Hepatology 15:899–903.

Speeg VK, Maldonato AL, Liaci J, Muirhead D (1992b): Effect of cyclosporine on colchicine secretion by the kidney multidrug transporter studied in vivo. J Pharmacol Exp Ther 261:50–55.

Takasato Y, Rapoport SI, Smith QR (1984): An in situ brain perfusion technique to study cerebrovascular transport in the rat. Am J Physiol 247:H484–H493.

Tatsuta T, Naito M, Oh-hara T, Sugawara I, Tsuruo T (1992): Functional involvement of P-glycoprotein in blood-brain barrier. J Biol Chem 267:20383–20391.

Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willigham MC (1987): Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 84:7735–7738.

Tsuji A, Terasaki T, Takabatake Y, Tenda Y, Tamai I, Yamashima T, Moritani S, Tsuruo T, Yamashita J (1992): P-glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. Life Sci 51:1427–1437.

Van Deurs B, Koehler JF (1979): Tight junctions in the choroid plexus epithelium. A freeze-fracture study including complementary replicas. J Cell Biol 80:662–673.

Yeh KC, Kwan KC (1978): A comparison of numerical integrating algorythms by trapezoidal, Lagrange, and spline approximations. J Pharmacokinet Biopharm 6:79–98.