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P-Glycoprotein-Mediated Transport of Morphine in Brain Capillary Endothelial Cells

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ABSTRACT. Cell accumulation, transendothelial permeability, and efflux studies were conducted in bovine brain capillary endothelial cells (BBCECs) to assess the role of P-glycoprotein (P-gp) in the blood–brain barrier (BBB) transport of morphine in the presence and absence of P-gp inhibitors. Cellular accumulation of morphine and rhodamine 123 was enhanced by the addition of the P-gp inhibitors *N*-{4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918), verapamil, and cyclosporin A. Positive (rhodamine 123) and negative (sucrose and propranolol) controls for P-gp transport also were assessed. Morphine glucuronidation was not detected, and no alterations in the accumulation of propranolol or sucrose were observed. Transendothelial permeability studies of morphine and rhodamine 123 demonstrated vectorial transport. The basolateral to apical (B:A) fluxes of morphine (50 μM) and rhodamine (1 μM) were approximately 50 and 100% higher than the fluxes from the apical to the basolateral direction (A:B), respectively. Decreasing the extracellular concentration of morphine to 0.1 μM resulted in a 120% difference between the B:A and A:B permeabilities. The addition of GF120918 abolished any significant directionality in transport rates across the endothelial cells. Efflux studies showed that the loss of morphine from BBCECs was temperature- and energy-dependent and was reduced in the presence of P-gp inhibitors. These observations indicate that morphine is transported by P-gp out of the brain capillary endothelium and that the BBB permeability of morphine may be altered in the presence of P-gp inhibitors. BIOCHEM PHARMACOL **58**;6:951–957, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. morphine; P-glycoprotein; blood–brain barrier; transport; rhodamine; cell culture

P-gp‖ is a member of the ATP-Binding Cassette (ABC) transporter superfamily and utilizes a functional transport unit comprised of twelve membrane spanning domains and two ATP binding cytoplasmic domains [1]. Identification of P-gp as a member of the ABC superfamily of transport proteins, and the decreased accumulation of cytotoxic agents in MDR cell lines that overexpress P-gp, suggested a direct role of this protein in the efflux of chemotherapeutic agents out of cells [2]. The expression of P-gp has been observed in specialized epithelial and endothelial cells with either secretory or excretory functions [3]. Agents such as verapamil, the cyclosporins, the *Vinca* alkaloids, and *N*-{4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918) can reverse or modulate P-gp-mediated MDR [2, 4–6].

P-gp located in brain capillary endothelial cell membranes has been thought to function as a component of the blood–brain barrier [7–10]. Disruption of P-gp in brain capillary endothelial cells would, in theory, result in enhanced accumulation of P-gp substrates. In cultured mouse brain capillary endothelial cells, CyA is transported preferentially from the basolateral to the apical side, and the addition of verapamil increases accumulation of CyA in these cells [11]. Sakata *et al.* [7] also demonstrated ATP-dependent efflux of CyA, and decreased CyA efflux from rat brain when quinidine, a P-gp inhibitor, was delivered into the hippocampus of rats. Recent *in vitro* and *in vivo* evidence has implicated morphine as a P-gp substrate [12–14].

We previously reported that morphine antinociception in rats was enhanced in the presence of the P-gp inhibitor GF120918, and that alterations in the systemic disposition of morphine could not explain the alteration in the

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‖ *Abbreviations*: A, apical; B, basolateral; BBB, blood–brain barrier; BBCEC, bovine brain capillary endothelial cell; CyA, cyclosporin A; DPBS, Dulbecco's phosphate-buffered saline; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; MDR, multidrug resistance; MEM/F12, minimal essential medium/Ham's F-12 Nutrient Mixture; P-gp, P-glycoprotein; and TEER, transendothelial electrical resistance.

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pharmacologic effect of morphine [14]. Given the presence of P-gp in brain endothelial cells *in vivo* and the evidence that morphine is a P-gp substrate, inhibition of P-gp-mediated efflux from brain capillary endothelial cells could explain the *in vivo* finding of elevated morphine antionociception in the presence of P-gp inhibition. The objective of this study was to evaluate P-gp-mediated transport of morphine across the BBB, using the BBCEC culture model. The BBCEC model can be employed to examine a variety of processes ranging from simple diffusion, carrier-mediated transport, and metabolism, to factors modulating BBB permeability [15]. Several investigators have utilized this model system to evaluate P-gp-mediated efflux of vincristine, CyA, verapamil, and doxorubicin [7, 8, 11, 16]. To date, no results have been published evaluating P-gp-mediated efflux of morphine using models of the BBB.

MATERIALS AND METHODS

Materials

Morphine sulfate, verapamil hydrochloride, CyA, and sodium azide were purchased from the Sigma Chemical Co. Rhodamine 123 was purchased from Molecular Probes. GF120918 was donated by Glaxo Wellcome, Inc.

[^{3}H]Morphine (84.5 Ci/mmol) was purchased from New England Nuclear Life Sciences Products and was > 98.5% pure as determined by HPLC. [^{3}H]Propranolol (15 Ci/mmol) and [^{14}C]sucrose (0.442 Ci/mmol) were purchased from Amersham International plc. All drugs were used without further purification.

All culture media were obtained from Gibco BRL. Rat tail collagen (Type I) was purchased from Collaborative Research, Inc. Collagenase/dispase was obtained from Boehringer Mannheim. Horse serum, amphotericin B, polymyxin B, gentamicin, sodium heparin, bovine plasma fibronectin, dispase, dextran, and Percoll were purchased from Sigma.

BBCEC Cultures

BBCECs were isolated according to the method of Audus and Borchardt [17]. The isolated endothelial cells were combined with approximately 50 mL of a culture medium (MEM/F12) supplemented with 10% horse serum and centrifuged for 10 min at 1000 *g*. The pellet from this centrifugation was resuspended in culture medium supplemented with 20% horse serum and 10% DMSO. The cells were cryopreserved in liquid nitrogen until plating (< 3 months). Following a 10-fold dilution with culture medium, endothelial cells were counted with a hemocytometer. Cell viability was assessed by the exclusion of 0.4% Trypan blue dye. Surfaces for culture growth were coated (0.1 mL/cm^2) with rat-tail collagen (3 mg/mL in aqueous acetic acid). After 5 min, the excess collagen was removed, and the growing surfaces were exposed to ammonia fumes for 20–30 min to promote cross-linking of the collagen. The plates then were exposed to UV light for 60 min. Fibronectin (bovine; 0.04 mg/mL; Sigma) was added in excess (0.1 mL/cm^2) to the collagen-coated growing surfaces. After 30 min, the excess fibronectin was removed.

Isolated BBCECs were thawed quickly and washed with culture medium prior to seeding on the growth surface. The cells were applied at a density of 100,000 cells/cm^2 and incubated in an atmosphere of 95% air and 5% CO_2 at 37°. For the first 3 days, the BBCECs were cultured in MEM/F12 supplemented with 10% horse serum, 50 μg/mL of polymyxin B, and 100 μg/mL of heparin sulfate. After Day 3, polymyxin B was omitted. The culture medium was changed every other day until the BBCECs became a confluent monolayer (~14 days). Cultured cells were identified as endothelial cells by immunostaining using Factor-VIII related antigen and by evaluating enrichment in alkaline phosphatase and γ-glutamyl-transpeptidase activities.

Cellular Accumulation

Cells were grown in collagen/fibronectin-coated 24-well cell culture plates (Corning Costar), and uptake assays were performed in confluent monolayers at Day 14. The monolayer was preincubated for 60 min with transport buffer (DPBS containing glucose) with or without P-gp inhibitor. Immediately after the preincubation, the solution was removed and the cells were incubated at 37° with incubation medium containing 1 μM morphine and 1 μCi/well of [^{3}H]morphine with or without the P-gp inhibitor/vehicle (0.5 μM GF120918, 10 μM verapamil, 2 μM CyA, 10 mM sodium azide). The final concentration of the vehicle, DMSO, in the medium did not exceed 0.1%. At 5, 10, 20, 30, 60, or 120 min, the monolayer was washed twice with 1 mL of ice-cold PBS and lysed with 0.5 mL of 1 N NaOH. Cell lysates were neutralized with 0.5 mL of 1 N HCl, and cell-associated morphine was assessed by liquid scintillation spectrometry. The experiment was replicated for 1 μM propranolol and [^{3}H]propranolol (high permeability drug), 1 μM rhodamine 123 (P-gp substrate), and 1 μM sucrose and [^{14}C]sucrose (extracellular fluid marker) with or without the P-gp inhibitors (N = 3 per treatment). All radiolabeled tracers were added to achieve 1 μCi/well. The amount of [^{3}H]morphine or rhodamine 123 in the cell lysates was quantitated with liquid scintillation spectrometry and fluorescence spectrophotometry, respectively.

Transendothelial Transport

The TEER of cell monolayers was measured on the day of study utilizing an EndOhm-12™ tissue resistance measurement chamber (World Precision Instruments). Cell monolayers grown on Corning Costar Transwell™ inserts (3 μm pore, 11 mm diameter) were rinsed three times with DPBS at 37°. The donor side was filled with pre-warmed transport buffer containing 50 μM morphine and 1 μCi [^{3}H]morphine or 1 μM rhodamine 123 and 1 μCi [^{14}C]sucrose. The receiver chamber was filled with blank pre-warmed trans-

port buffer, and the Transwell™ was incubated for 120 min at 37° on a rotating shaker (~100 rpm). The amount of [^{3}H]morphine and [^{14}C]sucrose transported to the receiver side was quantitated with liquid scintillation spectrometry. Rhodamine 123 flux was quantitated with fluorescence spectrophotometry. Studies were repeated for morphine and rhodamine to determine flux in each direction (N = 3 per direction). Additional experiments were performed to examine the transendothelial cell flux of morphine at a concentration of 0.1 μM, and the flux of 50 μM morphine in the presence of 0.5 μM GF120918.

Cellular Efflux

Cells were grown in collagen/fibronectin-coated 24-well cell culture plates, and efflux assays were performed on confluent monolayers at Day 14. Cultured BBCECs were incubated at 37° for 30 min in transport buffer. Immediately after the preincubation, the solution was aspirated gently, and the cells were incubated for 2 hr at 37° with transport buffer containing 1 μM morphine and 1 μCi [^{3}H]morphine to load the cells. During the last 10 min of the loading period, a P-gp inhibitor/vehicle or vehicle was added. Immediately after the loading period, the medium was removed, and the cells were incubated at 37° with or without the P-gp inhibitor/vehicle or vehicle. The final concentration of DMSO in the medium did not exceed 0.1%. At 10, 30, 60, 90, 120, or 180 sec the medium was removed and quantitated for [^{3}H]morphine with liquid scintillation spectrometry. The monolayer was washed twice with 1 mL of ice-cold PBS and lysed with 0.5 mL of 1 N NaOH. Cell lysates were neutralized with 0.5 mL of 1 N HCl, and cell-associated [^{3}H]morphine was assessed. Efflux was estimated from the amount of [^{3}H]morphine recovered in the medium.

Morphine Metabolism

To assess for possible metabolism of morphine during the 120-min incubation experiments, intracellular concentrations of morphine, M3G, and M6G from the accumulation studies were determined by HPLC. Samples were mixed with internal standard (nalorphine) and mobile phase (10% acetonitrile in 0.1% trifluoroacetic acid) and injected onto the HPLC system. Chromatographic separation was achieved with a 250 × 4.6 mm C_6 5-μm column (Phase Separations, Inc.) and constant-flow gradient elution. Fluorescence of the column effluent was monitored at an excitation wavelength of 220 nm and an emission cutoff of 350 nm. M3G, M6G, morphine, and internal standard retention times were 8, 14, 17, and 21 min, respectively. Standard curves, blank samples, and quality control samples for the determination of lysate morphine, M3G, and M6G concentrations were performed each day. The quality control samples were placed every seventh sample in a concentration-randomized fashion. If the quality control samples differed by > 10% from the expected calibration, then the system was re-calibrated. Only samples injected prior to valid quality control samples were utilized for data analyses. The analytical method had a limit of quantification of 25 ng/mL when 100 μL of cell lysate was injected. Standard curves were linear up to 5000 ng/mL, with intra- and interday coefficients of variation for morphine and M3G < 15%.

Rhodamine 123 Assay

Rhodamine 123 was quantitated in samples with a cuvette-based fluorometric assay. Cell lysate (100 μL) was added to 900 μL of water and mixed by vortex for approximately 3 sec. Fluorescence was monitored at an excitation wavelength of 507 nm and an emission cutoff of 529 nm with a model A1010 fluorometer (Photon Technology Int.). Standard curves, blank samples, and quality control samples were performed each day. The quality control samples were placed every seventh sample in a concentration-randomized fashion. If the quality control samples differed by >10% from the expected calibration, then the system was re-calibrated. Only samples measured prior to valid quality control samples were utilized for data analysis.

Data and Statistical Analyses

Net uptake of the test solute was expressed as the percentage of the extracellular concentration taken up by the cells. Total recovery of drug also was calculated. Passage of solute across the BBCEC monolayer was expressed in the form of an apparent permeability coefficient by means of the following equation:

$$Pe = \frac{dX}{dt} \cdot \frac{1}{C_0 \cdot A}$$

where Pe (cm/min) is the apparent permeability coefficient, dX/dt (pmol/min) is the rate of translocation, A (cm^2) is the surface area of the polycarbonate membrane, and C_0 (pmol/cm^3) is the initial concentration of the drug in the donor chamber.

Morphine efflux was expressed as a percentage of the initial intracellular morphine that was effluxed into the extracellular medium over the specified time period. Intracellular morphine was calculated as the total amount of morphine in the cell lysate plus that recovered in the efflux medium.

Descriptive statistics for the transport parameters were calculated. ANOVA and unpaired Student's *t*-test were utilized to identify significant differences in transport parameters between treatment groups where appropriate. In all cases, the tests were two-tailed and the criterion for statistical significance was $P < 0.05$.

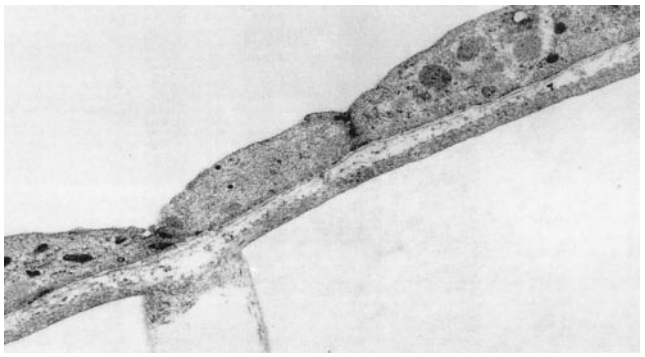

FIG. 1. Electron micrograph of BBCEC monolayer grown on Transwell™ membrane for 14 days. Cross section. Magnification: 24,000x.

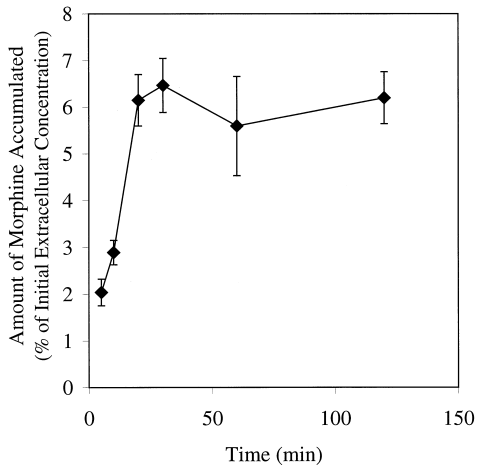

FIG. 2. Accumulation of morphine in BBCEC cultures. Data are expressed as percentages of the initial extracellular concentration of morphine that accumulated during incubation for the designated times (symbols represent mean ± SD of 3 independent experiments). The incubation medium contained 1 μM morphine and 1 μCi/well of [^{3}H]morphine. The line is included to emphasize temporal relationships in the data and does not represent the fit of a model to the data.

RESULTS

Characterization of Cell Culture Model

Bovine brain capillary endothelial cells were isolated and cultured successfully. Cells grew to a confluent monolayer within 14 days and were used for cellular accumulation, transendothelial permeability, and efflux studies on days 14–15. An electron micrograph of the BBCEC monolayer grown on the Transwell™ polycarbonate membrane demonstrates the presence of a monolayer of cells and junctional complexes between the endothelial cells (Fig. 1). The cells did not appear to migrate through the Transwell™ pores. The endothelial cell specific marker Factor VIII was expressed by > 98% of the cells. Monolayer homogenates were enriched in alkaline phosphatase and γ-glutamyltranspeptidase activity, two enzymes enriched in brain capillary endothelial cells.

Cellular Accumulation

Cellular morphine accumulation was time-dependent, and steady state was attained by 30 min with 6.21 ± 0.44% of the initial extracellular amount entering the cell (Fig. 2). The extent of rhodamine 123 accumulation (7.10 ± 0.45%) was similar to that of morphine. The effects of various P-gp inhibitors on the 120-min accumulation of morphine, rhodamine 123, propranolol, and sucrose were determined (Fig. 3). Verapamil, GF120918, CyA, and sodium azide all significantly increased (up to 100% increase) the amount of morphine and rhodamine 123 associated with the treated cells as compared with the control cells. Propranolol, a highly permeable drug, accumulated to a much higher extent (15.9 ± 1.1%) than either morphine or rhodamine 123. Sucrose accumulation was less than 1% of the initial extracellular concentration. The accumulation of propranolol and sucrose were unaffected by the P-gp inhibitor verapamil.

Transendothelial Transport

Transendothelial permeabilities for morphine and rhodamine 123 are listed in Table 1. The basolateral to apical (B:A) fluxes of morphine (50 μM) and rhodamine (1 μM) were approximately 50 and 100% higher than the fluxes from the apical to the basolateral direction (A:B), respectively. Decreasing the concentration of morphine to 0.1 μM resulted in a 120% difference between the B:A and A:B permeabilities. The addition of GF120918 abolished any significant directionality in transport rates across the endothelial cells. No significant differences were noted in sucrose permeabilities across treatment or flux direction.

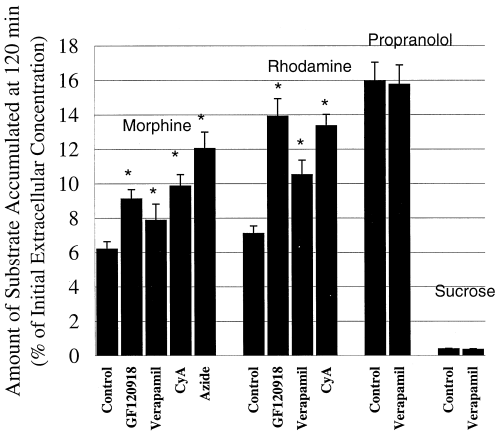

FIG. 3. Effect of P-gp inhibitors on BBCEC accumulation of substrates. Data are expressed as percentages of the initial extracellular concentration of morphine, rhodamine 123, propranolol, and sucrose that accumulated in BBCECs at 120 min with and without various P-gp inhibitors (mean ± SD of 3 independent experiments). Key: (*) $P < 0.02$, post-hoc unpaired *t*-test. The incubation medium contained 1 μM morphine and [^{3}H]morphine, 1 μM rhodamine 123, 1 μM propranolol and [^{3}H]propranolol, and 1 μM sucrose and [^{14}C]sucrose as indicated. All radiolabeled tracers were added to the incubation medium to achieve 1 μCi/well.

TABLE 1. Transendothelial permeabilities (cm/min · 10^{-3})

	A:B Pe (lumen:brain)	B:A Pe (brain:lumen)	% Difference
Morphine			
50 μM	0.96 ± 0.02	1.43 ± 0.02	49*
0.1 μM	0.53 ± 0.11	1.17 ± 0.21	120*
50 μM + GF120918	0.89 ± 0.06	0.83 ± 0.11	−7
Rhodamine			
1 μM	0.96 ± 0.06	1.92 ± 0.17	101*

Data are mean ± SD of 3 independent experiments.
*Significant difference in Pe between flux direction. ($P < 0.01$, unpaired *t*-test).

TEER values for each monolayer ranged from 72 to 184 Ω · cm^2.

Cellular Efflux

The time course of morphine efflux from BBCECs is shown in Fig. 4. The 30-sec time point was chosen for subsequent efflux studies because it was in the initial linear region of flux. The effects of temperature and various P-gp and metabolic inhibitors on morphine efflux are shown in Fig. 5. All treatments resulted in significant decreases in the amount of morphine transported from the cells. Cells maintained at 4° exhibited the largest decrease in morphine efflux (~50% decrease) followed by P-gp and metabolic inhibitors, which reduced morphine efflux by approximately 30% compared with control cells.

Morphine Metabolism

No M3G, M6G, or other unidentified peaks were observed in the cell lysate samples. The amount of morphine taken up by the cells, as determined by total radioactivity, was not significantly different from the amount of morphine taken up by the cells as determined with the specific HPLC method.

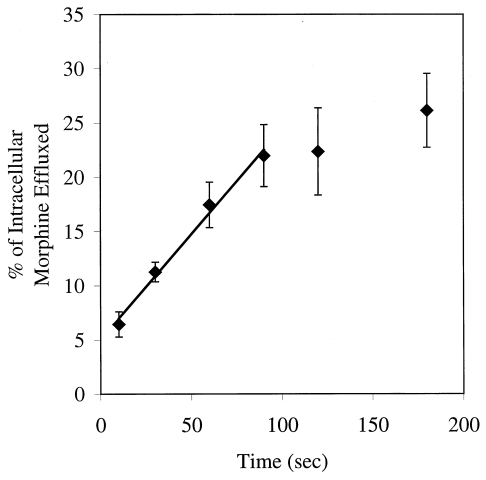

FIG. 4. Kinetics of morphine efflux from BBCEC cultures. Data are expressed as the percentage of the initial amount of intracellular morphine effluxed from the BBCEC monolayer (symbols represent mean ± SD of 3 independent experiments). Efflux was linear over the first 90 sec. The line represents linear regression of the 10, 30, 60, and 90 sec efflux data (y = 0.194x + 5.07, r^2 = 0.991, $P < 0.0001$).

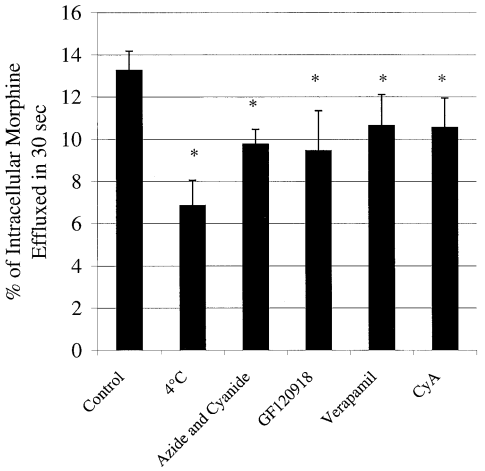

FIG. 5. Effects of temperature and P-gp inhibitors on BBCEC efflux of morphine. Data are expressed as the percentage of the initial amount of intracellular morphine effluxed from BBCEC monolayers in 30 sec with and without various P-gp inhibitors (mean ± SD of 3 independent experiments). Key: (*) $P < 0.05$, post-hoc unpaired *t*-test.

DISCUSSION

Brain capillary endothelial cells are the primary physical barrier between brain extracellular fluid and blood. Passage of morphine through the BBB is well recognized, but compared with many drugs the penetration is limited. Early studies have demonstrated that the brain uptake of morphine in rats after carotid injection is very low [18, 19]. Observed morphine brain-to-plasma concentration ratios of less than unity provide additional evidence of the limited BBB permeability of morphine [20–24]. P-gp located in brain capillary endothelial cells may be one mechanism limiting the brain exposure to morphine.

In the studies presented here, the BBCEC model was utilized to evaluate directly the role of P-gp in transporting morphine across the BBB. Morphine accumulation in the BBCEC model was time- and energy-dependent. The steady-state uptake of morphine by BBCECs was increased by P-gp inhibition. Transendothelial transport experiments with morphine demonstrated a net B:A flux process similar to that observed with rhodamine 123, a well-established P-gp substrate. The net efflux of morphine across the endothelial monolayer in the Transwell™ studies was negated in the presence of GF120918. Morphine efflux from preloaded BBCEC monolayers was reduced in the presence of various P-gp inhibitors or by metabolic energy depletion. Rhodamine 123 served as a model substrate to demonstrate the presence of functional P-gp in the BBCECs. Rhodamine 123, a cationic and lipophilic fluorescent dye that is more photostable than fluorescein, is pH insensitive under

physiologic conditions and membrane permeable [25]. This compound has been used widely as a probe for P-gp activity in a variety of cell models, including BBCEC cultures [26–29]. Rhodamine 123 accumulation was enhanced in the presence of various P-gp inhibitors, and the transendothelial transport experiments demonstrated the existence of a net efflux process for rhodamine 123. A membrane impermeant marker, sucrose, was employed in the present studies to assess the leakiness of the system and to control for any treatment-associated changes in apparent cell permeability unrelated to P-gp inhibition. Sucrose accumulation and transcellular flux were similar between all treatments. Collectively, these results indicate that P-gp activity was present in the model system and that P-gp is involved in the BBB transport of morphine.

Callaghan and Riordan [12] provided the first evidence that morphine may be a substrate for the drug efflux protein P-gp. These investigators used a Chinese hamster ovary cell line (B30) that overexpresses P-gp in comparison with its drug-sensitive counterpart (B1). Cell accumulation studies demonstrated progressive accumulation of morphine by the wild-type B1 cells that reached steady state in 20 min. In contrast, uptake of morphine by drug-resistant B30 cells was barely discernible, with the amount accumulated after 60 min more than 3-fold less relative to sensitive cells. Additional studies in *mdr1a(−/−)* mice demonstrated significant elevation of brain morphine concentrations as compared with wild-type mice [13].

Three groups of investigators indirectly provided further evidence that morphine is a substrate for P-gp at the level of the BBB [30–32]. Loh *et al.* [24] demonstrated a 2-fold increase in the brain uptake of morphine in mice that received dactinomycin. No clear mechanism for the alteration of the BBB permeability of morphine by dactinomycin could be established; however, at the time of this study, the inhibitory effect of dactinomycin on P-gp was unknown. In addition, other investigators observed that rifampin and tripelennamine, which now are known to modulate P-gp, increase brain levels of morphine without alteration of plasma concentrations of morphine [30, 31]. These results support the hypothesis that inhibition of P-gp at the BBB can increase morphine brain exposure.

Although the effect of P-gp inhibitors on the transport parameters of morphine in the BBCEC *in vitro* model were modest, previous experience with *in vitro–in vivo* correlations utilizing this model suggests that the permeability differences may be important *in vivo*. Wang *et al.* [32] used *in vivo* microdialysis to evaluate the rat brain distribution of rhodamine 123 in the presence and absence of CyA. As predicted by the BBCEC model system, CyA increased the brain distribution of rhodamine 123 approximately 3-fold. Similar results have been reported for doxorubicin, a chemotherapeutic agent that also is a P-gp substrate. During inhibition of P-gp, a 2-fold increase in doxorubicin uptake was observed using the *in vitro* BBCEC model as compared with a 17-fold increase seen *in vivo* [16]. These studies suggest that the BBCEC model can be used to assess the role P-gp may have in determining the BBB permeability of compounds; however, this system may underestimate the full extent of the effect of P-gp inhibition on the disposition of a molecule *in vivo*. Explanations for the underestimation of *in vivo* P-gp activity by the BBCEC model system may include: (a) non-physiologic conditions (lack of sink conditions, blood flow, and protein binding); (b) dysfunctional or low P-gp expression secondary to isolation and culture procedures; and (c) poor junctional complex formation resulting in an incomplete barrier during transendothelial flux experiments. Therefore, the demonstration of P-gp-mediated efflux of morphine *in vitro* may translate to a significant increase in morphine brain concentrations during inhibition of P-gp *in vivo*.

Previous studies completed by our group evaluating the effect of P-gp inhibition on the systemic disposition and CNS activity of morphine in rats are consistent with the *in vitro* data presented here and support the hypothesis that inhibition of P-gp alters the brain disposition of morphine *in vivo* [14]. Morphine antinociception was increased several fold in GF120918-treated rats as assessed by the hot-lamp tail-flick assay. This increase could not be explained by any changes in the systemic disposition of morphine in the presence of GF120918. Pharmacokinetic/pharmacodynamic modeling indicated that GF120918 treatment significantly reduced the effect offset rate constant without altering the plasma EC_{50} or the Hill coefficient. This observation was consistent with reduction of morphine egress from the site of action without significant alteration in receptor binding or activation. These findings are clearly supported by the *in vitro* studies presented here.

In summary, the P-gp inhibitors GF120918, verapamil, and CyA enhanced accumulation of morphine and rhodamine 123 in BBCECs, reducing morphine efflux via P-gp. The transendothelial cell permeabilities of morphine and rhodamine 123 were consistent with a net efflux process. These findings demonstrated that morphine is transported by P-gp in brain capillary endothelium and that the BBB permeability of morphine may be altered in the presence of P-gp inhibitors.

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