Effect of GF120918, a Potent P-glycoprotein Inhibitor, on Morphine Pharmacokinetics and Pharmacodynamics in the Rat

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Purpose. The objective of this study was to evaluate the effect of a potent P-gp inhibitor, GF120918, on the systemic pharmacokinetics and antinociceptive pharmacodynamics of a single intravenous dose of morphine in rats.

Methods. Male Sprague-Dawley rats received either 500 mg base/kg/d GF120918 or vehicle for 4 days by gavage, or no pretreatment. On day 4, morphine was administered as a 1- or 2-mg/kg i.v. bolus. Antinociception, expressed as percent of maximum possible response (%MPR), was evaluated over 300 min after morphine administration. Serial blood samples were collected and analyzed for morphine and morphine-3-glucuronide (M3G) by HPLC.

Results. Morphine clearance and distribution volume were not altered significantly by GF120918. M3G AUC in the GF120918-treated rats was approximately 2-fold higher than in vehicle-treated rats. For both morphine doses, %MPR and the area under the effect-time curve at 300 min were significantly higher in the GF120918-treated rats. A pharmacokinetic/pharmacodynamic effect model accurately described the effect-concentration data for the rats that received 1-mg/kg morphine; k_{e0} was significantly smaller for GF120918- vs. vehicle-treated and control rats (0.060 ± 0.028 vs. 0.228 ± 0.101 vs. 0.274 ± 0.026 min^{-1}, $p=0.0023$). EC_{50} and γ were similar between treatment groups.

Conclusions. Pretreatment with GF120918 enhanced morphine antinociception, as assessed by the hot-lamp tail-flick assay, and elevated systemic M3G concentrations in rats. The differential pharmacologic response to morphine in the GF120918-treated animals could not be attributed to alterations in systemic morphine pharmacokinetics.

KEY WORDS: morphine; morphine-3-glucuronide; P-glycoprotein; pharmacokinetics; pharmacodynamics; antinociception; central nervous system; analgesia.

INTRODUCTION

P-glycoprotein (P-gp) is a transmembrane glycoprotein expressed in multidrug resistant (MDR) tumor cells and several normal tissues including the liver, intestine, kidney, adrenal gland, gravid uterus, and brain capillary endothelium (1,2). This plasma membrane protein pump extrudes various chemotherapeutic agents from tumor cells and is thought to be one mechanism of tumor cell resistance (3). Substrates for P-gp exhibit few structural or functional similarities; however, P-gp substrates are usually aromatic, cationic hydrophobic molecules (4,5).

Morphine is a naturally occurring opiate with a planar 3-ringed aromatic structure and a substituted piperidine ring. Morphine is slightly lipophilic (octanol/water partition coefficient of 1.4) (6) and is ionized at physiologic pH (pKa of 9.85). Recent experimental evidence has demonstrated that morphine may be a substrate for P-gp (7,8). Schinkel *et al.* (8) investigated the tissue distribution of morphine in *mdr1a*(−/−) mice. Following a single intravenous dose of [^{3}H]morphine, various organs were harvested at four hours and analyzed for tritium by liquid scintillation counting. Two tissues, the brain and gall bladder, exhibited significantly higher radioactivity (1.7- and 2.2-fold, respectively) in *mdr1a*(−/−) compared to *mdr 1a*(+/+) mice.

Callaghan and Riordan *et al.* (7) examined morphine accumulation in Chinese hamster ovary cells which overexpress P-gp (B30) and their drug sensitive counterparts (B1). Progressive accumulation of morphine was observed in the wild type cells that reached steady-state at approximately 20 min. Uptake by the MDR cells was barely discernible; the amount of morphine that accumulated after 60 min was more than 3-fold lower than in sensitive cells. Depletion of cellular ATP significantly increased the accumulation of morphine in the P-gp overexpressing cells (B30) to a level that approached morphine accumulation in sensitive cells (B1). Verapamil and vinblastine significantly increased morphine accumulation in the B30 cells.

Agents such as verapamil, quinidine, the cyclosporins, tamoxifen, and the vinca alkaloids can reverse or modulate P-gp-mediated MDR *in vitro;* however, these agents act as competitive substrates for P-gp and require micromolar or higher concentrations to be effective. Systemic side effects and toxicities prevent effective use of these agents *in vivo.* For example, full reversion of MDR by verapamil requires an approximate 10 μM concentration in most cell culture models whereas plasma levels above 1 μM result in atrioventricular block in patients (9). In contrast, GF120918, a potent inhibitor of P-gp, achieves adequate P-gp inhibition *in vivo* without significant systemic side effects in animals (10,11).

In light of the regional tissue distribution of P-gp, inhibition of P-gp *in vivo* may have significant implications for morphine pharmacokinetics and pharmacodynamics. Mechanisms by which P-gp inhibitors could alter the systemic disposition of morphine include inhibition of P-gp-mediated biliary excretion, renal excretion, and intestinal transport. Inhibition of P-gp-mediated efflux from brain capillary endothelial cells also could have significant implications on the central nervous system (CNS) disposition and antinociceptive action of morphine. The objective of this study was to evaluate the effect of a potent P-gp inhibitor, GF120918, on the systemic pharmacokinetics and antinociceptive pharmacodynamics of a single intravenous dose of morphine in the rat.

MATERIALS AND METHODS

Materials

Morphine sulfate, morphine-3-glucuronide, and nalorphine hydrochloride were purchased from Sigma Chemical Company (St. Louis, Missouri). N-{4-[2-(1,2,3,4-tetrahydro-

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6,7-dimethoxy-2-isoquinolinyl)-ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918) was donated by Glaxo Wellcome, Inc. (Research Triangle Park, North Carolina). Heparin sodium injection was obtained from Elkins-Sinn Inc. (Cherry-Hill, New Jersey). Acetonitrile, trifluoroacetic acid, and ammonium sulfate were of analytical grade; hydroxypropylmethylcellulose (HPMC) and Tween 80 were of pharmaceutical grade.

GF120918 was suspended in a HPMC:Tween 80:water, 0.5:1.0:98.5 v/v/v formulation for oral administration. The GF120918 suspension (300 mg base/mL) was stored in a tightly sealed glass container wrapped with tin foil. Placebo for GF120918 was HPMC:Tween 80:water, 0.5:1.0:98.5 v/v/v. Morphine sulfate was dissolved in 0.9% sterile saline for injection to achieve a final morphine concentration of 1 mg/mL.

Animals

Adult male Sprague-Dawley rats (225–250 g) were purchased from Charles River Laboratories (Raleigh, North Carolina). Rats were housed in stainless steel hanging cages in a temperature controlled room (25 ± 3°C) with a 12-hr dark/12-hr light cycle. The rats had free access to food [ProLab Animal Diet—Rat, Mouse, Hamster 3000, Agway Co. (Syracuse, New York)] and water and were acclimated six days prior to experimentation. The rats were weighed daily during the experiment, monitored for any signs of distress, and handled according to the "Principles of Laboratory Animal Care" (NIH publication #85-23, rev 1985).

Experimental Method

The pharmacokinetics and pharmacodynamics of morphine following four days of pretreatment with GF120918 were examined in a controlled, parallel fashion. Twenty-four rats were randomized to GF120918, vehicle, or no pretreatment (control) groups. GF120918 (500 mg base/kg/d) or vehicle was administered on study days −3, −2, −1, and 0 at 0800 with an oral syringe. On the day prior to morphine administration, rats received the daily dose of GF120918 at 0800 hr and were anesthetized with diethyl ether at 1000 hr for placement of a jugular vein cannula. The period of anesthesia lasted less than 30 min. The animals had free access to food and water during the recovery period.

On the day of study (day 0) the rats were placed in Plexiglass restraining cages 30 min prior to morphine administration. Baseline tail-flick measurements and pre-dose blood samples for morphine and M3G were obtained after the acclimation period. Morphine sulfate was administered as either a 1- or 2-mg free base/kg i.v. rapid infusion (<10 sec) with subsequent serial duplicate tail-flick evaluations and blood sample collections. Tail-flick measurements were performed at 7, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min following the morphine dose. Immediately following each tail-flick evaluation, blood (150 to 300 μL) was withdrawn from the jugular vein cannula. The blood samples were allowed to clot in polypropylene microcentrifuge tubes, and serum was harvested following centrifugation at 2000 x g for 10 min at 25°C. The serum samples were stored in polypropylene microcentrifuge tubes at −20°C until chromatographic analysis.

Tail-flick latency was measured in duplicate utilizing a hot lamp tail-flick analgesia meter [Model 0570-001L, Columbus Instruments International Corp. (Columbus, Ohio)]. The instrument was placed in the auto-detect mode with both optical sensors active and the lamp intensity of 10. This setting elicited a tail-flick within 4 sec. A point 5 cm from the distal end of the tail was exposed to the lamp via a shutter mechanism. The time that elapsed between the shutter opening and tail-flick was recorded. A maximum response time of 15 sec was set to prevent damage to the tail during multiple evaluations.

Antinociception due only to GF120918 was evaluated separately. Nine rats were randomized to receive either GF120918 (500 mg/kg/d), vehicle, or control (water) by oral gavage for 4 days. Just prior to and 2 hr after each dose, antinociception was assessed using the tail-flick analgesia assay. The rats were placed in Plexiglass restraining cages 30 min prior to the tail-flick evaluations. Tail-flick latency was assessed in the same fashion as for the morphine exposed rats (see above).

Morphine and M3G Analyses

The concentrations of morphine and M3G in serum were determined by a slight modification of the high performance liquid chromatography (HPLC) method of Glare *et al.* (12) and Venn and Michalkiewicz (13) as published by Ouellet and Pollack (14). After internal standard (nalorphine) addition, solid-phase extraction of alkalinized serum samples was performed using C8 columns. Analytes were eluted with methanol, evaporated to dryness, and reconstituted in mobile phase (10% acetonitrile in 0.1% trifluoroacetic acid) and injected onto the HPLC system. Chromatographic separation was achieved with a C6 column 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, morphine, and internal standard retention times were 7, 15, and 20 min, respectively. Standard curves, blank samples, and quality control samples for the determination of serum morphine and M3G 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 >15% from the expected calibration, then the system was recalibrated. Only samples injected prior to valid quality control samples were utilized for study analyses. The analytical method had a limit of quantification of 25 ng/mL when 100 μL of rat serum was extracted. Standard curves were linear up to 5000 ng/mL, with intra- and inter-day coefficients of variation for morphine and M3G < 15%.

Pharmacokinetic, Pharmacodynamic, and Statistical Analyses

Antinociception was expressed as percent of maximum possible response (%MPR) according to the following equation:

$$\%MPR = \frac{\text{Test Latency} - \text{Baseline Latency}}{\text{Cutoff Time} - \text{Baseline Latency}} \bullet 100$$

Area under the concentration-time curve (AUC) and area under the effect-time curve (AUE) were calculated by the linear trapezoidal method. Extrapolation to infinite time according to the terminal rate constant was utilized for AUC approximation. Noncompartmental pharmacokinetic analysis (15) was performed to determine morphine clearance (CL), mean residence time (MRT), and volume of distribution (V_{ss}).

Combined pharmacokinetic and pharmacodynamic models were developed to fit simultaneously serum morphine concentrations and %MPR (16) by nonlinear least-squares regression (PCNONLIN version 4.2, Statistical Consultants, Cary, North Carolina) to estimate the pharmacokinetic and pharmacodynamic parameters for each rat. The relative performance of different models was assessed by residual analysis, Akaike's Information Criterion (AIC), and graphical analysis.

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

RESULTS

Effect data were obtained from a total of 24 rats of which 21 had concentration data for pharmacokinetic and combined pharmacokinetic/pharmacodynamic analysis (see Table 1 for number of rats per treatment group). Mean morphine and M3G concentration vs. time profiles for the different treatment groups are presented in Figures 1 and 2. A biexponential decline was observed in serum morphine and M3G concentrations in all rats. Morphine and M3G could be detected in serum for up to 120 and 180 min, respectively. Peak concentrations of M3G were observed at the 15- or 30-min blood collections for all rats.

The results of the noncompartmental pharmacokinetic analyses are summarized in Table 1. No significant differences in morphine AUC, CL, V_{ss}, or MRT were observed between treatment groups. Mean M3G AUC (see Table 1) was approximately 2-fold higher in GF120918-treated compared to vehicle-treated and control rats ($p = 0.0786$ and $p = 0.1472$, ANOVA, for 1- and 2-mg/kg morphine dose groups, respectively).

Mean effect vs. time profiles for the different treatment groups are presented in Figure 3. At the 2-mg/kg morphine dose, all rats were at or near 100% MPR up to 120 min. The GF120918-treated rats remained near 100% MPR through 300 min, while the effect in vehicle-treated and control rats declined toward baseline. Maximal antinociceptive response occurred only during the first few assessments for all the animals that received 1-mg/kg morphine. Antinociceptive effect declined more slowly in GF120918-treated rats than in vehicle-treated and control rats. %MPR at 300 min was higher in the GF120918-treated compared to vehicle-treated or control rats ($p = 0.0987$ and $p = 0.0429$, ANOVA, for 1- and 2-mg/kg morphine dose groups, respectively). Mean AUE (see Table 2) in the 1-mg/kg dose group was approximately 4-fold higher in the GF120918-treated compared to vehicle-treated and control rats ($p = 0.0015$, ANOVA).

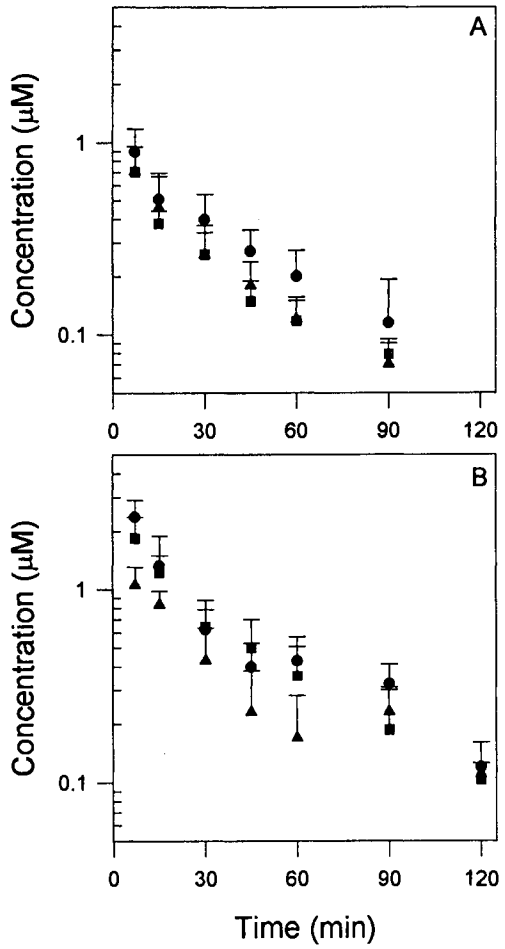

Fig. 1. Morphine serum concentration (mean ± SD) vs. time profiles in control— (▲), vehicle— (■), and GF120918— (●) treated rats that received (A) 1-mg/kg morphine or (B) 2-mg/kg morphine.

Mean effect vs. morphine serum concentration profiles for the different treatment groups are presented in Figure 4. The profiles for the 1-mg/kg morphine dose clearly displayed counter-clockwise hysteresis. An integrated pharmacokinetic/pharmacodynamic model (Figure 5) adequately characterized

Table 1. Morphine Pharmacokinetic Parameters (Mean ± SD) by Treatment Group and Morphine Dose[a]

	1 mg/kg			2 mg/kg		
Treatment	Control	Vehicle	GF120918	Control	Vehicle	GF120918
n	5	3	3	3	4	3
AUC (μM*min)	28.2 ± 9.8	24.1 ± 5.8	37.8 ± 11.3	56.2 ± 12.8	67.7 ± 19.4	86.6 ± 12.5
CL (mL/min/kg)	141 ± 66	151 ± 41	98 ± 29	129 ± 34	112 ± 39	82 ± 13
MRT(min)	22.7 ± 3.4	25.2 ± 3.4	33.6 ± 8.3	31.8 ± 13.4	27.4 ± 10.4	24.2 ± 4.2
Vss (L/kg)	3.1 ± 0.9	3.8 ± 0.9	3.1 ± 0.3	3.8 ± 0.9	2.8 ± 0.6	2.0 ± 0.6
M3G AUC (μM*min)	29.7 ± 12.7	17.9 ± 4.3	44.6 ± 16.1	53.1 ± 30.4	49.3 ± 13.3	94.9 ± 32.4

[a] Differences among treatment groups at each dose level were not statistically significant (ANOVA).

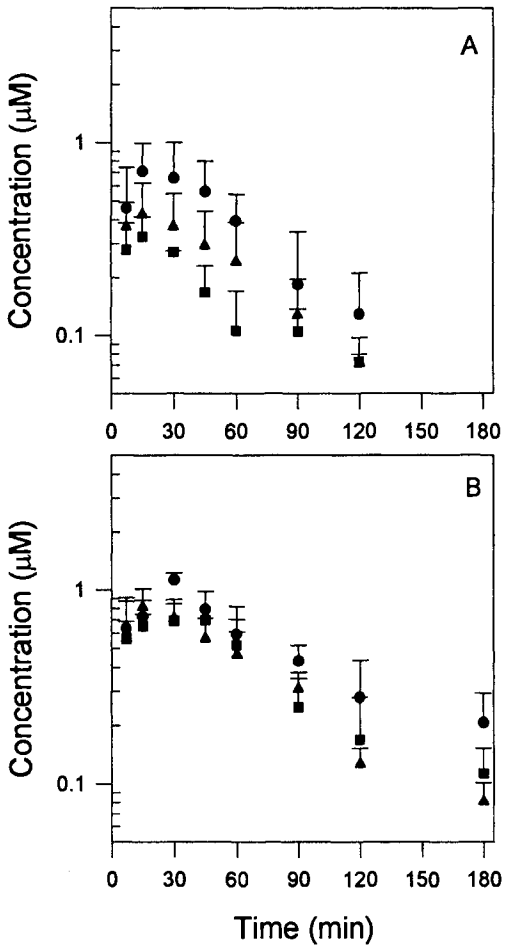

Fig. 2. M3G serum concentration (mean ± SD) vs. time profiles in control— (▲), vehicle— (■), and GF120918— (●) treated rats that received (A) 1-mg/kg morphine or (B) 2-mg/kg morphine.

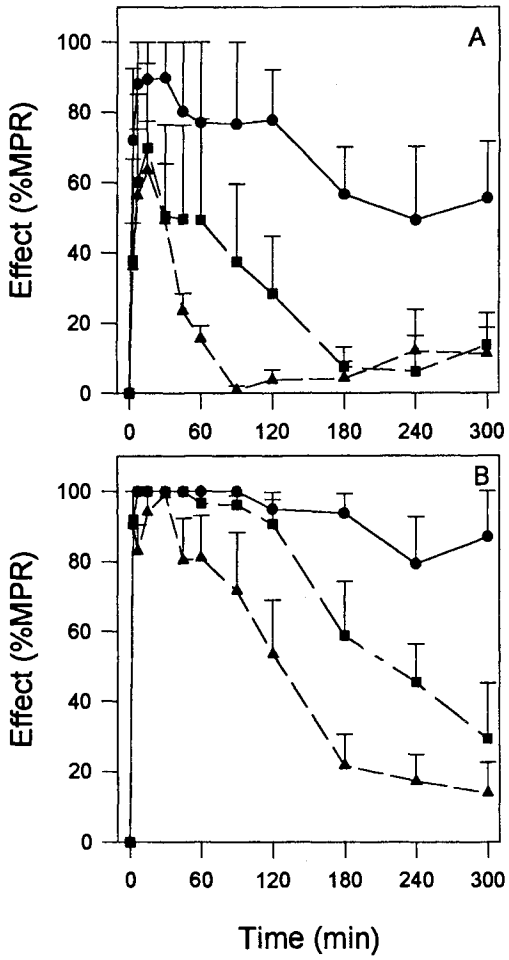

Fig. 3. Antinociceptive effect (mean ± SE) vs. time profiles in control- (▲), vehicle— (■), and GF120918— (●) treated rats that received (A) 1-mg/kg morphine or (B) 2-mg/kg morphine. Lines are included to emphasize temporal relationships in the data and do not represent the fit of a model to the data.

the profiles (Table 2, Figure 6). EC_{50} and γ were not significantly different between treatment groups; however, k_{e0} was significantly less in the GF120918-treated compared to the vehicle-treated and control rats ($p = 0.0023$, ANOVA). Due to limited concentration data during offset of antinociception, effect-concentration data from the 2-mg/kg morphine dose group could not be described adequately by the model.

In the evaluation of any inherent GF120918 antinociceptive activity, effect data were obtained from nine rats (3 rats per treatment group). No statistically significant differences in percent of baseline tail-flick latency were observed between treatment groups or day of dosing. GF120918 alone did not significantly alter tail-flick latency.

DISCUSSION

Morphine analgesia is produced by activation of opioid receptors within the CNS at both spinal and supraspinal levels (17). The concentration of morphine at any point in time at active sites in the CNS will depend on the systemic and the CNS disposition of morphine. Alteration at either of these locations may influence morphine CNS concentrations and thus the degree of antinociception.

P-gp is a transmembrane glycoprotein expressed in multidrug resistant (MDR) tumor cells and several normal tissues (1,2). The exact physiologic function of P-gp is unknown; however, strategic localization of P-gp on the apical membrane of secretory epithelia suggests a role in excretion of endogenous or exogenous toxins. P-gp located in brain capillary endothelial cell membranes may function as a component of the blood-brain barrier (18–21). Morphine may be a substrate for P-gp, as demonstrated recently with *in vitro* and knockout mice studies (7,8). Disruption of P-gp in brain capillary endothelial cells would in theory result in enhanced accumulation of morphine in the brain. Inhibition of P-gp transport in the canalicular membrane of hepatocytes may result in decreased biliary excretion of morphine and/or the glucuronide conjugates of morphine. Thus, P-gp inhibition during morphine therapy could result in clinically significant alterations in the CNS and/or systemic disposition of morphine.

Pretreatment of rats with GF120918, a potent inhibitor of P-gp, resulted in significant alterations in morphine antinociception without significant changes in the systemic clearance of morphine. The pharmacokinetic parameters obtained for all treatment

Table 2. Pharmacodynamic Parameter Estimates (Mean ± SD) by Treatment Group for Rats Receiving 1-mg/kg Morphine

Parameter	Control	Vehicle	GF120918	ANOVA p-value
n	5	3	3	NA
E_{max} (%)	100 (fixed)	100 (fixed)	100 (fixed)	NA
EC_{50} (μM)	0.340 ± 0.209	0.441 ± 0.335	0.171 ± 0.220	0.4414
γ	6.45 ± 0.86	4.83 ± 4.31	4.43 ± 0.82	0.4460
k_{e0} (min^{-1})	0.274 ± 0.026	0.228 ± 0.101	0.060 ± 0.028[a,b]	0.0023
AUE (%MPR * hr)	72.3 ± 39.2	126 ± 70	405 ± 85[a,b]	0.0015

[a] $p < 0.05$, post-hoc unpaired t-test between control- and GF120918-treated rats.
[b] $p < 0.05$, post-hoc unpaired t-test between vehicle- and GF120918-treated rats.

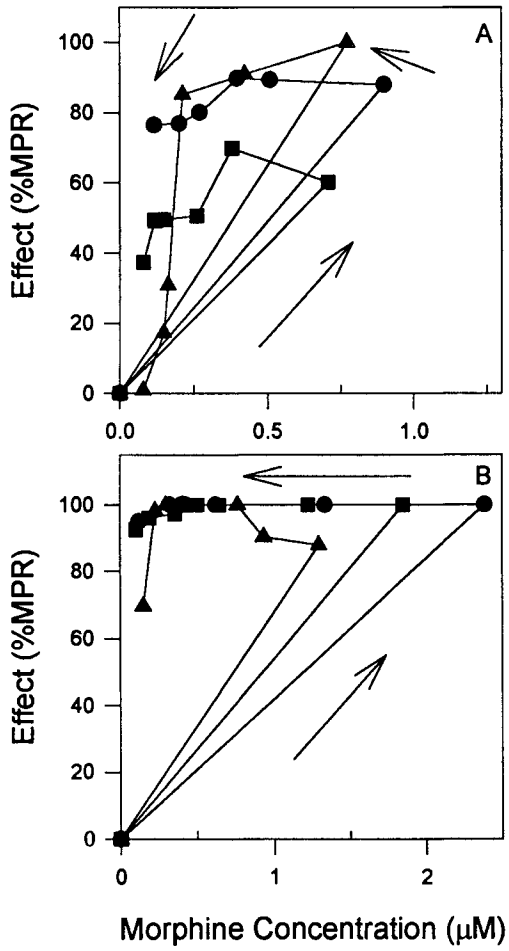

Fig. 4. Antinociceptive effect vs. morphine serum concentration profiles in control— (▲), vehicle— (■), and GF120918— (●) treated rats which received (A) 1-mg/kg morphine or (B) 2-mg/kg morphine. Symbols represent mean; lines and arrows are included to emphasize the temporal relationships in the data and do not represent the fit of a model to the data.

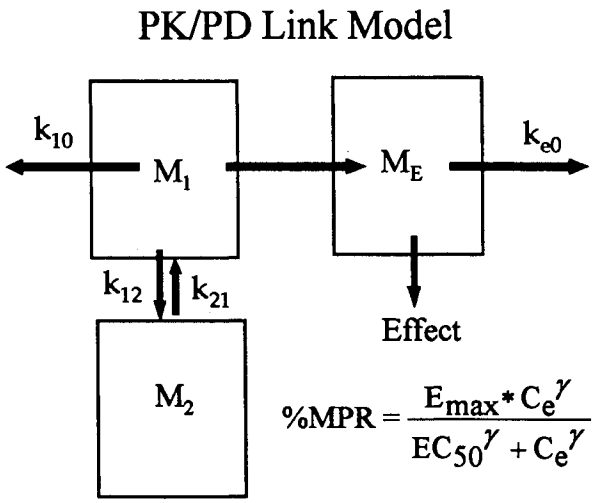

Fig. 5. Representation of the pharmacokinetic/pharmacodynamic peripheral effect model.

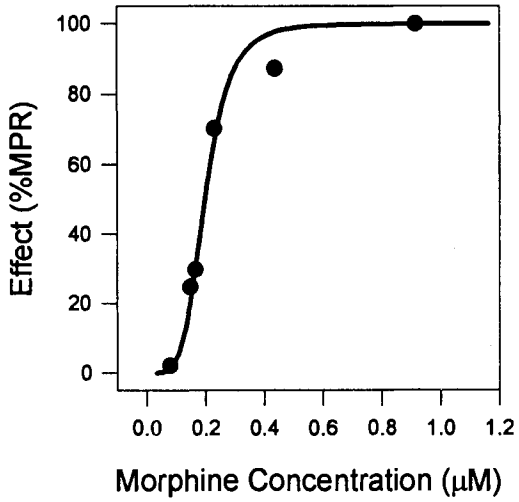

Fig. 6. Representative fit of the pharmacokinetic/pharmacodynamic model to effect vs. serum concentration data for one rat (1-mg/kg dose). Symbols represent observed data; line represents fit of the model to the data.

groups were within the range of reported values for morphine disposition in the rat (22). The lack of a significant alteration in morphine AUC by GF120918 may have been due to the limited contribution of P-gp-mediated clearance to the overall systemic clearance of morphine. Horton and Pollack (23) demonstrated that approximately 60% of the systemic clearance of morphine in rats is due to hepatic metabolism, while approximately 30% is

accounted for by renal metabolism. Less than 10% of morphine undergoes biliary excretion as unchanged parent drug. Even if canalicular P-gp was the mechanism solely responsible for biliary excretion of morphine, the alteration in systemic morphine clearance by inhibition of canalicular P-gp would be minimal.

P-gp located in hepatocyte canalicular and renal brush border membranes may influence significantly the disposition of a compound such as M3G with a large dependence on biliary and renal excretion. M3G AUC was elevated approximately 2-fold in the GF120918-treated rats. Little data have been published evaluating P-gp-mediated efflux of glucuronide conjugates. Gosland *et al.* (24) and Liu *et al.* (25) demonstrated that estradiol-17β-D-glucuronide is a substrate for P-gp located in the canalicular membrane of hepatocytes. Recently, Huwyler *et al.* (26) demonstrated that the uptake of morphine-6-glucuronide (M6G) in primary cultures of porcine brain capillary endothelial cells was enhanced approximately 2-fold in the presence of the P-gp inhibitors verapamil and vincristine. The uptake of M3G was not evaluated; however, M3G and M6G are physiochemically similar. Further investigations are necessary to confirm the role of P-gp in M3G disposition.

Morphine antinociception was elevated significantly in the GF120918-treated rats as demonstrated by the %MPR data. This elevation could not be explained by changes in the systemic pharmacokinetics of morphine in the presence of GF120918. The pharmacodynamic parameters indicated that GF120918 treatment significantly reduced the effect onset/offset rate constant (k_{e0}) without affecting EC_{50} or γ. This observation is consistent with alteration of morphine egress from the site of action without significant alteration of receptor binding or activation.

The alterations in morphine pharmacodynamics by GF120918 cannot be explained by the observed elevation of M3G concentrations in GF120918-treated rats. M3G does not possess antinociceptive activity and does not significantly antagonize morphine activity as shown by several investigators (27–29). The apparent enhancement of morphine-associated analgesia in the GF120918-treated rats might have been due to an antinociceptive property of GF120918. However, using the same pretreatment method as in the morphine antinociception study, GF120918 alone did not possess antinociceptive activity. Schinkel *et al.* (8) demonstrated that brain concentrations of morphine increased moderately (1.7-fold) following administration of morphine to mice genetically deficient in *mdr1a*. The data from this study are consistent with the hypothesis that inhibition of P-gp may reduce efflux of morphine from brain capillary endothelial cells resulting in elevated morphine concentrations at its site of action and enhanced morphine antinociception. However, further investigation of the exact mechanism(s) by which GF120918 alters morphine antinociception is warranted.

In summary, pretreatment with GF120918 enhanced morphine antinociception, as assessed by the hot-lamp tail-flick assay, and elevated systemic M3G concentrations in rats. The differential pharmacologic response to morphine in the GF120918-treated animals could not be attributed to alterations in morphine systemic pharmacokinetics as assessed by AUC, MRT, CL, and Vss.

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