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; k21 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). EC50 and γ were similar between treatment groups.

Conclusions. Pretreatment with GF120918 enhanced morphine antinociception, as assessed by the hot-plate 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 [3H]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 mdr1a(+/-) 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 cyclosporinins, tamoxifen, and the vincra 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-(4,4'-[1,2,3,4-tetrahydro-
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, trifu-
luoroacetic 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 injec-
tion to achieve a final morphine concentration of 1 mg/mL.

Animals

Adult male Sprague-Dawley rats (225–250 g) were pur-
chased from Charles River Laboratories (Raleigh, North Car-
olina). 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 acclimatized six days prior to experi-
mentation. 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 mor-
phine 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 administra-
tion, rats received the daily dose of GF120918 at 0800 hr and were
anesthetized with diethylether 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 Plexi-
glass restraining cages 30 min prior to morphine administra-
tion. 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 collec-
tions. 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 micro-
centrifuge tubes, and serum was harvested following centri-
figation 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 instru-
ment 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 sep-
arately. 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 alkalized serum samples was per-
formed 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 wave-
length 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 quali-
ty 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 concen-
tration randomized fashion. If the quality control samples dif-
fered 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:

\[
\text{%MPR} = \frac{\text{Test Latency} - \text{Baseline Latency}}{\text{Cutoff Time} - \text{Baseline Latency}} \times 100
\]

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 per-
formed to determine morphine clearance (CL), mean residence
time (MRT), and volume of distribution (Vss).