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PRECLINICAL PHARMACOKINETIC PROPERTIES OF THE P-GLYCOPROTEIN INHIBITOR GF120918A IN THE MOUSE, RAT, DOG, AND MONKEY

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Nonstandard Abbreviations: BCRP, breast cancer resistance protein; CYP3A, cytochrome P450 3A; DNAUC, dose-normalized AUC; HPMC, hydroxypropylmethylcellulose; PEG-300, polyethylene glycol-300; Pgp, P-glycoprotein;

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ABSTRACT

GF120918A has been used both *in vitro* and *in vivo* as a tool inhibitor of P-glycoprotein (Pgp) to investigate the role of transporters in the disposition of various test molecules. However, to date, a detailed description of the preclinical pharmacokinetic properties of GF120918A has not been published. This investigation was performed to evaluate *in vitro* and *in vivo* pharmacokinetic properties of GF120918A in the mouse, rat, dog, and monkey, and to evaluate the *in vivo* efficacy of GF120918A in modulating absorption and systemic exposure in the monkey. GF120918A demonstrated reasonable absorption and systemic exposure in each of the species studied, however, in rodents, administration of 300 mg/kg afforded a substantially less than linear increase in systemic exposure compared to 30 mg/kg. In accord with its intestinal and hepatic exposure and potency against P-glycoprotein, GF120918A demonstrated marked modulation of erythromycin systemic exposure in the monkey, with no effect on propranolol, a negative control molecule. *In vitro,* GF120918A demonstrated high plasma protein binding across species, although a definitive protein binding evaluation was precluded by poor recovery, particularly in buffer and in mouse, rat, and dog plasma. GF120918A did not demonstrate potent inhibition of several human cytochrome P450 enzymes evaluated *in vitro*, with IC50 values well above concentrations anticipated to be achieved *in vivo*. Together, these data confirm the utility of GF120918A as a tool P-glycoprotein inhibitor in preclinical species, and offer additional guidance on preclinical dose regimens likely to produce P-glycoprotein-mediated effects.

The role of P-glycoprotein (Pgp) and cytochrome P450 3A (CYP3A) as determinants of the pharmacokinetics and oral bioavailability of many classes of molecules has been well-established (Wacher et al., 2001). Experimentally, *in vivo* modulation of Pgp and/or CYP3A often can be a useful means for evaluating the functional significance of these proteins in limiting oral absorption and/or first-pass hepatic extraction of a given molecule (Bardelmeijer et al., 2000). However, Pgp and CYP3A often operate in concert, and also often appear to be susceptible to inhibition by similar pharmacophores (Cummins et al., 2002; Wandel et al., 1999). This phenomenon renders the discernment of the relative roles of Pgp and CYP3A in the disposition of a given compound difficult.

Substantial effort has been expended to identify specific modulators of either Pgp or CYP3A, with varying degrees of success. Given the potentially important role of transporters such as Pgp in mediating multidrug resistance to oncology treatments, a number of transporter inhibitors have been discovered and progressed through development as adjuncts in cancer chemotherapy (Robert and Jarry, 2003). Among these is GF120918A, initially described as an inhibitor of multidrug resistance (Hyafil et al., 1993). In the ensuing decade, GF120918A has been widely used as a Pgp inhibitor both *in vivo* and *in vitro*, and has been found to be reasonably selective for Pgp and breast cancer resistance protein (BCRP) over other transporters (Wallstab et al., 1999; Evers et al., 2000; Jonker et al., 2000), although CYP3A inhibition data for GF120918A have not been published. Additionally, despite its extensive use, very little preclinical pharmacokinetic data are available for GF120918A, and experimental dosing protocols for *in vivo* modulation of Pgp using

GF120918A in preclinical models vary dramatically between investigators. Consequently, a presentation of the pharmacokinetic properties of GF120918A in key preclinical species should be a useful tool in the design of future studies with this tool modulator. The objectives of this series of studies were to characterize the *in vivo* pharmacokinetics of GF120918A in the mouse, rat, dog, and monkey, to investigate the *in vivo* pharmacodynamic properties of GF120918A in the monkey, and to evaluate key *in vitro* pharmacokinetic parameters, including plasma protein binding and stability and CYP450 inhibition in preclinical species and in humans.

METHODS

Materials. GF120918A (the HCl salt of GF120918, 9,10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl] phenyl]-4-acridine-carboxamide) was synthesized at GlaxoSmithKline (Research Triangle Park, NC). Cavitron (hydroxypropyl-β-cyclodextrin) was purchased from Cerestar (Hammond, IN) and hydroxypropylmethylcellulose (HPMC; Grade E15) was purchased from Dow Chemical Company (Midland, MI). All other materials were purchased from standard vendors and were of the highest available purity.

Animals. Female inbred mice (C57BL/6NCrlBR; Charles River, Raleigh, NC) weighing 19 to 21 g, male outbred rats (Crl:CD(SD)IGS BR; Charles River, Raleigh, NC) weighing 260 to 440 g, male purebred Beagle dogs (Marshall Research, North Rose, NY) weighing 8.6 to 13.8 kg, and male cynomolgus monkeys (*Macaca fascicularis;* Charles River Primate Labs, Houston, TX) weighing 5.6 to 6.2 kg were used. The dogs and monkeys were equipped with indwelling hepatic portal access ports; a subset of rats also was purchased from the vendor with indwelling hepatic portal vein catheters. All animals were housed according to the Institute of Laboratory Animal Resources, National Research Council's *Guide for the Care and Use of Laboratory Animals* in individual cages in unidirectional airflow rooms with controlled temperature (22 ± 2°C) and relative humidity (50 ± 10%) and 12-h light/dark cycles. Filtered tap water was available *ad libitum*. Animals were fed a standard animal diet as appropriate for each species and were not fasted prior to treatment, to more closely match previously published pharmacodynamic experiments with GF120918A. All

studies were reviewed and approved by the Institutional Animal Care and Use Committee, and all surgical procedures were conducted using aseptic techniques in special-purpose operating suites.

In Vivo Pharmacokinetic Studies. Pharmacokinetic studies were conducted essentially as described previously (Ward et al., 2001a). Dosages were administered either as solutions in 10% aqueous polyethylene glycol-300 (PEG-300) or 6% Cavitron with 1% dimethyl sulfoxide, or as well-triturated suspensions in 0.5% aqueous HPMC containing 1% Tween-80. Particle size analysis was performed on all suspensions to ensure effective and uniform particle size reduction. A complete blood chemistry panel was performed on all non-rodents prior to each study day to obtain baseline values and ensure hematological recovery. Blood samples were collected at various times up to 48 h after drug administration; plasma (50 μL) was isolated by centrifugation and stored at -70°C until analysis. For mice, to facilitate sequential sampling while minimizing the total volume of blood withdrawn, 25 μL of whole blood was collected and mixed with 25 μL of analytical-grade water; samples were stored frozen until analysis. All doses are nominal doses expressed as mg free base equivalent/kg; actual doses varied less than 1% from nominal and were used in all calculations. All sampling times described below are nominal times; actual sampling times were recorded and were used in all calculations.

Mice. Four groups of animals (n=4 each) received GF120918A by oral gavage (10 mL/kg dose volume). Three groups received GF120918A as a suspension at 3, 30, or 300 mg/kg, and the fourth group received GF120918A as a solution in Cavitron at 3 mg/kg. Blood sampling in

mice was performed via a tail vein; mice were held in restrainers up to 1 h post-dose to facilitate sampling. Blood samples were obtained at 0.5, 1, 2, 4, 8, 24, and 32 h post-dose.

Rats. A total of seven groups of animals (n=3 to 8 rats each) received GF120918A by oral gavage (10 mL/kg dose volume). Three groups received GF120918A as a suspension at 3, 30, or 300 mg/kg, and a fourth and fifth group each received GF120918A as a solution in Cavitron or PEG-300, respectively, at 3 mg/kg. Finally, a sixth and seventh group of rats with indwelling hepatic portal vein catheters received GF120918A by oral gavage (10 mL/kg dose volume) as a suspension at 3 or 30 mg/kg, respectively. Blood sampling in rats was performed via a lateral tail vein; samples also were obtained from the hepatic portal vein catheter where applicable. Rats were held in restrainers for up to 1 h post-dose to facilitate sampling. Blood samples were obtained before dosing and at 5, 15, 30, and 45 min, and 1, 1.5, 2, 3, 4, 6, 8, 10, 24, and 32 h post-dose.

Dogs. Dogs were restrained in slings to facilitate drug administration and blood sampling for up to 2 h before being returned to their cages. The same dogs (n=3) received GF120918A by oral gavage (4 mL/kg dose volume) on three separate occasions at least one week apart; dosages were 3 and 30 mg/kg as a suspension and 3 mg/kg as a solution in Cavitron. Blood samples were obtained from a cephalic vein and from the hepatic portal vein catheter. Blood samples were obtained before dosing and at 5, 15, 30, and 45 min, and 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 32, and 48 h post-dose.

Monkeys. The monkeys were placed in restraining chairs for the first 4 h of the study to facilitate drug administration and blood sampling. The same monkeys (n=3) received GF120918A by oral

gavage (8 mL/kg dose volume) on three separate occasions at least one week apart; dosages were 3 and 30 mg/kg as a suspension and 3 mg/kg as a solution in Cavitron. Blood samples were obtained from a femoral vein via an indwelling catheter and from the hepatic portal vascular access port. Blood samples were obtained before dosing and at 5, 15, and 30 min, and 1, 1.5, 2, 4, 6, 8, 10, 24, 32, and 48 h post-dose.

In Vivo Efficacy Studies. To evaluate the *in vivo* preclinical efficacy of GF120918A at increasing absorption and/or decreasing hepatic extraction of a Pgp substrate, GF120918A was evaluated in a previously validated monkey model (Ward et al., 2004). Briefly, erythromycin was used as a tool P-glycoprotein/CYP3A substrate, and propranolol was used as a negative control molecule. Erythromycin was administered as an intraduodenal bolus at a dosage of 5 mg/kg (formulated in PEG-300, 0.5 mL/kg dose volume), and propranolol was administered as an intraduodenal bolus at a dosage of 10 mg/kg (formulated in water, 0.5 mL/kg dose volume). Three hours after the initial administration of erythromycin or propranolol, GF120918A (3 mg/kg) was administered as a 10-min intraduodenal infusion, as a solution in Cavitron. A second bolus of erythromycin or propranolol was then administered 10 min after the completion of the GF120918A infusion. Blood samples were collected simultaneously from femoral and hepatic portal vein catheters starting 5 min after the first bolus of test compound, and continuing until three hours after the second bolus of test compound, with plasma isolated from each blood sample by centrifugation.

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In Vitro Plasma Stability and Plasma Protein Binding. Plasma protein binding of GF120918A was investigated in each of the species in which *in vivo* pharmacokinetic parameters were determined, as well as in human plasma, by equilibrium dialysis as described previously (Ward et al., 2001a). Briefly, heparinized, pooled-gender plasma was purchased and stored frozen (Biological Specialty Corporation, Colmar, PA). Plasma protein binding was determined using Spectra/Por®4 dialysis membranes (Spectrum Laboratories, Rancho Dominguez, CA; molecular weight cutoff = 12-14 kDa) at final concentrations of 100, 1000 and 10,000 ng/mL. Equilibrium dialysis cells were rotated at approximately 15 rpm for 14 h at 37°C. Plasma stability of GF120918 also was investigated in the species in which *in vitro* protein binding measurements were performed. For these studies, GF120918 was incubated for up to 24 h in buffer and plasma treated with either heparin, EDTA, or citrate at a concentration of 1000 ng/ml. Samples were obtained periodically and analyzed for concentrations of GF120918.

In Vitro CYP450 Inhibition. The ability of GF120918A to inhibit several major human cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) was evaluated using a fluorimetric substrate assay with standard methodologies (Miller *et al.*, 2000). GF120918A was dissolved in dimethyl sulfoxide (2% final solvent content in incubation), and the following substrates (synthesized at GlaxoSmithKline) were used to measure CYP450 activity: ethoxyresorufin (CYP1A2); 7-methoxy-4-trifluoromethylcoumarin-3-acetic acid (CYP2C9); 3-butyryl-7-methoxycoumarin (CYP2C19); 4-methylaminomethyl-7-methoxycoumarin (CYP2D6); 7-[3-(4-phenylpiperazin-1-ylmethyl)benzyl] resorufin (CYP3A4).

Analytical Procedure. GF120918A, erythromycin, and propranolol were isolated from samples by precipitation with acetonitrile and quantified by LC/MS/MS. Erythromycin and propranolol were quantified as previously described (Ward et al., 2004). For GF120918, LC/MS/MS coupled with an atmospheric pressure chemical ionization interface (475°C) was used. Briefly, internal standard (300 μL in acetonitrile/10 mM ammonium formate pH 3.0; 95/5 v/v) was added to 50 μL of sample and the mixture was vortexed followed by centrifugation for 30 minutes at 4000 rpm. The supernatant (0.5 - 2 μL) was injected onto the LC/MS/MS system using an HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland) coupled to an Aria TX2 high-throughput liquid chromatographic system utilizing turbulent flow technology (Cohesive Technologies, Franklin, MA) in focus mode. The mobile phase consisted of a mixture of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The turbulent flow column was a 0.5 x 50 mm Cyclone P column (Cohesive Technologies, Franklin, MA) in series to a 2 x 20 mm, 4 μ Polar RP (Phenomenex, Torrance, CA) analytical column. Positive-ion multiple reaction monitoring was employed for the detection of GF120918 and internal standard. The selected precursor and product ions for GF120918 were m/z 564 and 252, respectively. Using a (1/x) weighted linear regression analysis of the calibration curve, linear responses in analyte/internal standard peak area ratios were observed for GF120918 concentrations ranging from 2.00 to 10,000 ng/mL.

Pharmacokinetic Data Analysis. Concentration versus time profiles were obtained for each analyte in individual animals and noncompartmental analysis was performed using WinNonlin Professional Version 3.3 (Pharsight, Mountain View, CA) to recover area under the curve (AUC) and other parameters. AUC was estimated as both extrapolated (0 to ∞) and truncated (0 to t) values; no differences were observed in the conclusions, and truncated AUC values are reported here. Dose-normalized AUC (DNAUC; min•kg/L) was determined by dividing AUC (min•μg/mL) by dose (mg/kg) and multiplying by 1000. For studies for which both portal and systemic data were available, absorption and first-pass hepatic extraction were estimated as described previously (Ward et al., 2001b). Briefly, the hepatic portal DNAUC values were compared to theoretical maximum values for each species, derived based on average hepatic blood flow values, to estimate absorption. Hepatic extraction was estimated based on the difference between the systemic AUC and the portal AUC (corrected for contamination from returning systemic concentrations). In the pharmacodynamic experiments with erythromycin and propranolol, all calculations were adjusted appropriately for residual drug concentrations at the time of the second dose. Where appropriate, data for each pharmacokinetic parameter from individual animals were averaged and reported as mean ± standard deviation. A two-tailed Student's *t*-test or a one-way ANOVA were used for statistical evaluation of the observed data, as appropriate. In all cases, a probability level of $p \leq 0.05$ was predetermined as the criterion of significance.

RESULTS

The oral exposure data for GF120918A in the rodent are displayed in Table 1. Most of the experiments were conducted using suspension formulations, for consistency with currently established dosing protocols for GF120918A in the literature. However, because the use of solution formulations is sometimes desirable, a solution approach also was investigated. In the rat, two different solution formulations were evaluated; formulation of GF120918A in 6% aqueous Cavitron afforded significantly greater systemic exposure than a formulation in PEG-300 (p = 0.0019), consequently, the Cavitron-based solution was used for all future solution work. In the rat, the exposure achieved with the 3-mg/kg Cavitron-based solution formulation was also significantly greater than that obtained from the 3-mg/kg suspension formulation. However, in the mouse, exposure between the solution and suspension formulations was not different (p = 0.053). In both the rat and the mouse, exposure increased approximately 10- to 15-fold for the 10-fold increase in dose between 3 and 30 mg/kg. However, in the mouse, exposure only increased 3.6-fold for the 10-fold increase in dose between 30 and 300 mg/kg, and in the rat, exposure did not significantly increase between these two dose groups. Overall, exposure in the mouse tended to be greater for a given dose than that achieved in the rat, with a shorter Tmax.

In both non-rodent species, as was observed in the mouse, administration of 3-mg/kg solution and suspension formulations of GF120918A resulted in similar (not significantly different) systemic exposure (Table 2). In the dog, only a two-fold increase in systemic exposure was

observed for the 10-fold increase in dose between 3 and 30 mg/kg, and in the monkey the same dose increase resulted in only a 4.6-fold increase. Exposure overall was similar between the dog and the monkey, as was Tmax, although somewhat greater variability was observed in the dog.

A number of experiments were conducted in which samples were obtained from both the hepatic portal and systemic circulation following oral administration of GF120918A (Table 3). In the rat, absorption was low, with moderate apparent first-pass hepatic extraction. In the dog, absorption also was low, with low apparent hepatic extraction, whereas in the monkey, absorption was improved but was coupled with higher apparent hepatic extraction. In all species, a substantial decrease in absorption was observed as the dose increased from 3 to 30 mg/kg, suggesting dissolution-mediated absorption. In all species, the Cmax achieved in the hepatic portal vein ranged from approximately 0.1 to 2 μM.

After evaluating the absorption and systemic exposure of GF120918A, this information was used to design an *in vivo* pharmacodynamic study in the monkey using erythromycin and propranolol as tool positive and negative controls for Pgp substrates, respectively. Administration of a 3-mg/kg intraduodenal solution dose of GF120918A resulted in hepatic portal and systemic exposure of GF120918A similar to that observed in the previous pharmacokinetic studies (data not shown). GF120918A produced a significant increase in both absorption and systemic exposure of erythromycin (Figure 1A), with a 11- and 9-fold increase in hepatic portal and systemic Cmax, respectively. In the presence of GF120918A, the absorption of erythromycin improved from 5.8 ±

4.9% to 42.3 ± 29.4%; hepatic extraction was essentially unchanged (89.7 ± 1.4% versus 87.8 ± 2.0% in the presence of GF120918A). In contrast, GF120918A did not significantly alter either the absorption or hepatic extraction of propranolol in the monkey (Figure 1B).

In addition to the *in vivo* pharmacokinetic studies, the *in vitro* plasma protein binding of GF120918A also was investigated. No significant differences were observed between 100, 1000, and 10,000 ng/mL, therefore, average data across all concentrations were used. Plasma protein binding was high in all species (Table 4), with concentrations on the receiver side of the equilibrium dialysis device near or below the lower limit of quantification (2.00 ng/mL). However, an accurate cross-species comparison of plasma protein binding was precluded by interspecies differences in recovery from the donor side of the equilibrium dialysis device (Table 4). In monkey and human plasma, recovery was acceptable, at 78% and 83%, respectively. In rat and dog, recovery was substantially lower, at 54% and 52%, respectively. The lowest recovery was observed in mouse plasma (18%). These recovery values suggested the possibility of instability of GF120918A in plasma over the 14 h of the equilibrium dialysis experiment. To further investigate this possibility, a separate plasma stability experiment was performed by incubating GF120918A in buffer and in heparinized mouse, rat, dog, monkey, and human plasma. Consistent with the protein binding data, GF120918A demonstrated reasonable stability in monkey and human plasma (Figure 2). Stability in rat plasma was somewhat greater than anticipated based on the plasma protein binding recovery data, and stability in dog and mouse plasma was relatively poor, as anticipated. Separate experiments also were conducted using dog and mouse serum, rather than plasma, and plasma

treated with EDTA or sodium citrate rather than heparin as an anticoagulent. None of these alternate methods improved stability in mouse plasma, although stability in dog serum and EDTA-treated plasma was improved (data not shown). Additionally, recovery from pH 7.4 phosphate buffered saline also decreased substantially over time (Figure 2), suggesting instability, insolubility, or nonspecific binding issues. Consequently, a definitive estimate of plasma protein binding could not be performed using equilibrium dialysis.

Finally, fluorimetric probe substrates for several major human CYP450 isozymes were used to estimate the CYP450-inhibitory activity of GF120918A (Table 5). The lowest observed IC50 for GF120918A was against CYP3A4, at 11 μM, with the remainder of the CYP450 isozymes evaluated demonstrating substantially greater IC50 values (19 to 50 μM).

DISCUSSION

Elucidation of the factors limiting oral bioavailability, particularly CYP3A and/or Pgp, is often an important component of the preclinical lead optimization process for a given chemical series, as well as a useful adjunct to the data package for new chemical entities progressing into preclinical development. Although previous experimental protocols have been defined using non-selective inhibitors such as ketoconazole for the determination of whether either CYP3A or Pgp limit oral bioavailability (Zhang et al., 1998; Humphreys et al., 2003; Ward et al., 2004), experimental tools to support the discrimination of the two components have not previously been available. From the experiments described herein, GF120918A appears to offer a means for clarification of the role of Pgp/BCRP in the absorption and systemic exposure of test molecules. GF120918A is not a potent inhibitor of any CYP enzymes, including CYP3A, in expressed human systems *in vitro*. Furthermore, at dosages associated with significant perturbation in erythromycin pharmacokinetics in the monkey, maximal concentrations of GF120918A in both the systemic circulation (0.1 μM) and hepatic portal vein (0.7 μM) are well below those that would be anticipated to inhibit CYP3A. Together these observations demonstrate the preclinical utility of GF120918A to probe the relative role of Pgp/BCRP and CYP3A in limiting oral bioavailability.

In the present investigation, co-administration of GF120918A resulted in an approximately 8- to 11-fold increase (based on either AUC or Cmax, respectively) in the absorption of erythromycin, a known substrate of human Pgp and CYP3A (Wacher et al., 1995). Previously

(Ward et al., 2004), a similar study with ketoconazole resulted in an approximately 4-fold increase in absorption of erythromycin. Although a full dose-ranging study has not been performed with either GF120918A or ketoconazole in this monkey model, these results showing a similar order of magnitude effect with both a selective Pgp/BCRP inhibitor and a dual Pgp/CYP3A inhibitor suggest that in the monkey, Pgp/BCRP, rather than CYP3A, may be the primary factor limiting the absorption of erythromycin. Interestingly, erythromycin demonstrates substantially lower oral bioavailability in the monkey (~2%; Girard et al., 1987 and unpublished GSK data) than in humans (~30-65%; Mather et al., 1981). Although beyond the scope of this work, it would be intriguing to compare the *in vitro* permeability and transporter profile of erythromycin side-by-side in both human and non-human primate intestinal tissue, to ascertain whether differences in transporter activity may contribute to the observed species differences in pharmacokinetics.

Another key set of observations from the present work is the rodent exposure data for GF120918A. Various investigators have used GF120918A to inhibit Pgp *in vivo* in both the mouse (Chen and Pollack, 1999; Polli et al., 1999) and rat (Letrent et al., 1998; Letrent et al., 1999) at dosages up to 500 mg/kg, with pre-treatment regimens of up to 4 days duration. The present data allow an understanding of the systemic exposure generated with such dosing protocols, and suggest that the same systemic exposure of GF120918A may have been achievable with substantially lower dosages, which have previously been shown to be efficacious *in vivo* (Hyafil et al., 1993). This nonlinear increase in exposure with dose may be related to the intestinal dissolution profile of the suspension formulations used in this investigation; the solubility of GF120918A is pH-dependent,

with improved solubility at acidic pH (internal GSK data). Additionally, although overall absorption and systemic exposure appear dissolution-rate limited, the actual concentration of drug in solution in the intestinal lumen across species is not currently well understood, and higher dosages of GF120918A could theoretically raise the possibility of confounding non-Pgp-mediated effects of GF120918A, including inhibition of CYP450. Although these considerations would not impact the outcome of the studies cited above, which primarily evaluated the effect of Pgp modulation on central nervous system disposition and effects, the present data have important implications for the design of any future studies focussing on intestinal absorption processes. Finally, it should be noted that the exposure data in the present study were generated in the fed, rather than fasted condition, to most closely match the previously published pharmacodynamic experiments. Certainly feeding status can influence exposure, particularly across species with different feeding habits; this represents another factor to be considered in future experimental design with GF120918A.

Despite its promising *in vivo* pharmacokinetic and pharmacodynamic characteristics, GF120918A also displayed decreasing concentrations over time in a simple *in vitro* incubation system designed to interrogate the plasma stability of GF120918A as an adjunct to the plasma protein binding experiments. The observations around decreasing concentrations in the buffer mixture are consistent with previous reports that neat aqueous solutions of GF120918A may demonstrate nonspecific binding to glass or plastic (Hyafil et al., 1993). The recovery issues encountered in mouse and dog plasma may also be reflective of this; the interspecies differences in

plasma recovery may reflect differences in protein binding, and thus differences in the relative ability of plasma proteins to "protect" GF120918A from binding to the test device. Regardless of the specific mechanism involved, the observation presented here has implications for *in vitro* pharmacology experiments with GF120918A. GF120918A has often been incubated *in vitro* at pH 7.4 at 37°C for various times up to 4 days by different investigators (Beck et al., 1998; de Bruin et al., 1999; Elgie et al., 1999; Zhou et al., 1997). As shown in Figure 2, concentrations of GF120918A even over a short period *in vitro* may not be constant; interpretation of the *in vitro* pharmacodynamic effects of GF120918A must take this concentration-time profile into account.

In summary, the present investigation has set forth the preclinical pharmacokinetic profile of GF120918A in the mouse, rat, dog, and monkey, and the pharmacodynamic profile of GF120918A in the nonhuman primate, and the observations from this set of experiments have important implications for interpretation of published and future pharmacology experiments with this tool molecule. Our laboratory is continuing to profile GF120918A in various preclinical models, and future work will include additional pharmacodynamic studies in rodents and in the dog, and additional dose-ranging studies in the nonhuman primate to evaluate whether lower GF120918A dosages may be equally efficacious.

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FOOTNOTE

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LEGENDS FOR FIGURES

Figure 1. Portal (●) and systemic (○) exposure of erythromycin (A) and propranolol (B) before and after a concomitant 3-mg/kg dose of GF120918A in the monkey.

Figure 2. Concentrations of GF120918A during an incubation at 37°C for 24 h in buffer and plasma from mouse, rat, dog, monkey, and human.

Table 1. Systemic pharmacokinetic parameters of GF120918A following oral administration in rodents.

	Cmax (ng/mL)	**Tmax (min)**	**AUC (min•μg/mL)**	**DNAUC (min•kg/L)**
Mouse				
3 mg/kg solution	64.1 ± 7.9	120 (120-240)	19.9 ± 1.3	6.34 ± 0.37
3 mg/kg suspension	86.0 ± 24.7	120 (120-240)	26.7 ± 4.2	7.99 ± 1.74
30 mg/kg suspension	586 ± 25	240 (120-240)	319 ± 81	11.3 ± 2.8
300 mg/kg suspension	962 ± 81	489 (240-1460)	1151 ± 77	3.84 ± 0.26
Rat				
3 mg/kg PEG solution	15.1 ± 3.6	490 (360-603)	6.37 ± 1.54	2.07 ± 0.51
3 mg/kg Cavitron solution	42.7 ± 6.6	240 (240-360)	28.6 ± 5.1	8.70 ± 1.20
3 mg/kg suspension	18.0 ± 3.8	240 (240-240)	6.65 ± 1.52	2.20 ± 0.48
30 mg/kg suspension	133 ± 29	360 (360-480)	99.9 ± 28.1	3.31 ± 0.94
300 mg/kg suspension	98.9 ± 51.0	480 (360-480)	107 ± 46	0.364 ± 0.148

Data provided as mean ± SD except Tmax, which is median (range).

Table 2. Systemic pharmacokinetic parameters of GF120918A following oral administration in non-rodents.

	Cmax (ng/mL)	**Tmax (min)**	**AUC (min•μg/mL)**	**DNAUC (min•kg/L)**
Dog				
3 mg/kg solution	117 ± 34	360 (180-360)	79.4 ± 33	26.6 ± 10.8
3 mg/kg suspension	125 ± 30	360 (180-480)	97.0 ± 40.5	32.7 ± 13.6
30 mg/kg suspension	219 ± 50	360 (242-480)	202 ± 78	7.19 ± 2.77
Monkey				
3 mg/kg solution	105 ± 43	360 (240-490)	62.3 ± 9.3	19.6 ± 2.9
3 mg/kg suspension	85.5 ± 37.0	240 (240-240)	40.4 ± 11.1	13.3 ± 3.6
30 mg/kg suspension	179 ± 100	361 (240-600)	187 ± 72	6.30 ± 2.44

Data provided as mean ± SD except Tmax, which is median (range).

Table 3. Hepatic portal pharmacokinetic parameters of GF120918A following oral administration.

	Portal Cmax (ng/mL)	**Corrected Portal DNAUC (min•kg/L)**	**Hepatic Extraction*a*** **(%)**	**Absorption*b*** **(%)**
Rat 3 mg/kg suspension	62.4 ± 15.5	4.19 ± 1.02	57.0 ± 2.1	8.87 ± 2.16
Rat 30 mg/kg suspension	346 ± 84	2.24 ± 1.29	40.5 ± 8.5	5.16 ± 2.39
Dog 3 mg/kg suspension	416 ± 121	10.1 ± 4.3	24.1 ± 9.3	12.6 ± 5.3
Dog 30 mg/kg suspension	512 ± 150	1.96 ± 1.24	23.4 ± 17.9	2.44 ± 1.54
Monkey 3 mg/kg suspension	532 ± 226	42.0 ± 7.1	76.2 ± 3.5	81.4 ± 13.8
Monkey 30 mg/kg suspension	985 ± 315	19.8 ± 3.3	76.4 ± 3.9	38.3 ± 6.4

Data provided as mean ± SD.

a First-pass hepatic extraction estimated from the portal-systemic concentration difference using the method of Ward et al., 2001b.

b Absorption estimated based on the theoretical maximum DNAUC approach of Ward et al., 2001b.

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Table 4. Estimated plasma protein binding and recovery from the equilibrium dialysis device for GF120918A.

Incubation	**Estimated Plasma Protein Binding (%)**	**Recovery from Donor Side of Equilibrium Device (%)**
Mouse	>98.5	18.1 ± 16.5
Rat	99.0 ± 0.6	53.9 ± 18.1
Dog	99.8 ± 0.1	52.2 ± 7.8
Monkey	99.9 ± 0.01	78.2 ± 5.7
Human	>99.9	82.5 ± 9.7

Data provided as mean ± SD where possible.

Table 5. *In vitro* IC50 values for the inhibition of human CYP450 enzymes by GF120918A.

Enzyme	**IC50 (μM)**
CYP1A2	22.4 ± 1.7
CYP2C9	18.5 ± 2.1
CYP2C19	38.5 ± 4.4
CYP2D6	49.9 ± 17.5
CYP3A4	10.5 ± 0.4

Data provided as mean ± SD.

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Figure 1

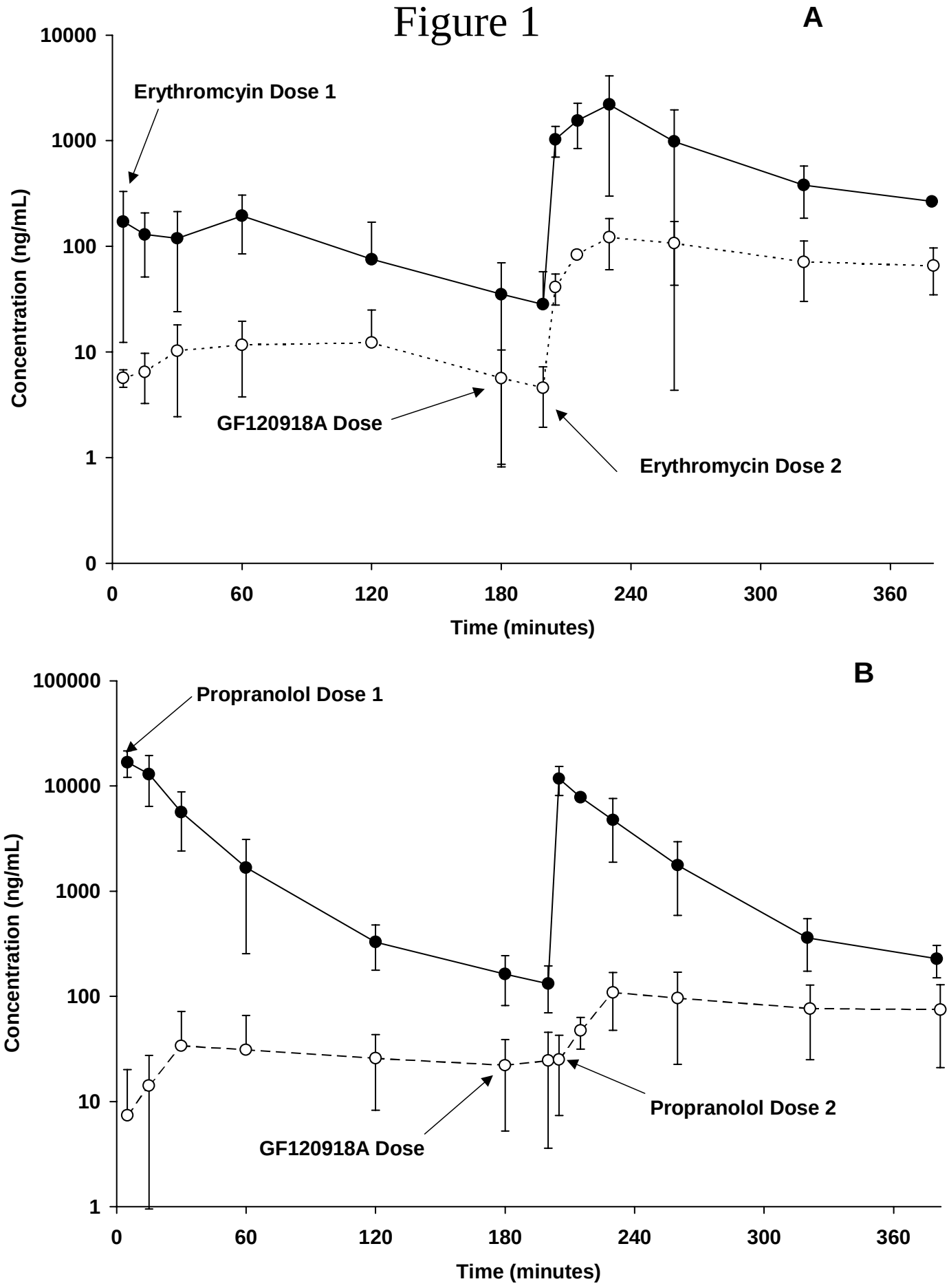

Figure 2