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RESEARCH ARTICLE

Exploration of the African green monkey as a preclinical pharmacokinetic model: oral pharmacokinetic parameters and drug–drug interactions

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Abstract

1. African green monkeys (vervets) have been proposed as an alternate species that might allow improved access and provide high-quality pharmacokinetic results comparable with other primates. However, no oral data are available in vervets to evaluate cross-species predictive performance. Therefore, this study was conducted to evaluate the use of the vervet to predict human oral pharmacokinetics and drug interactions.
2. Oral pharmacokinetic studies were conducted in the vervet for eight compounds: phenytoin, moxifloxacin, erythromycin, lidocaine, propranolol, ciprofloxacin, metroprolol, and prednisolone. To assess drug–drug interactions, co-administration experiments were conducted with ketoconazole and either propranolol or erythromycin.
3. In general, the vervet provided similar predictivity for human oral exposure as cynomolgus or rhesus monkeys. In all non-human primates, human exposure to phenytoin would be over-predicted, and erythromycin, lidocaine, and propranolol under-predicted, with good predictivity for the other compounds studied. Furthermore, in the vervet, ketoconazole co-administration resulted in a six-fold increase in exposure to erythromycin, demonstrating proof of concept for drug–drug interaction screening.
4. These data support further exploration of the vervet as an alternate primate species for use in preclinical pharmacokinetic screening.

Keywords: *African green monkey; vervet; primate; pharmacokinetic; bioavailability*

Introduction

The minimization of attrition of drug molecules, particularly in clinical development, is a well-recognized success factor in pharmaceutical development. Marked success has been realized in reducing attrition directly attributable to poor clinical pharmacokinetics (Kola and Landis 2004), and the investment made in predictive preclinical pharmacokinetic tools appears to have contributed to this success. Although such predictive tools include both *in vitro* and *in silico* assays, *in vivo* data continue to play a major role in preclinical drug discovery. In particular, non-human primates have been shown to be particularly useful in predicting human pharmacokinetics for small-molecule therapeutics (Ward and Smith 2004; Ward et al. 2005; Evans et al. 2006; Tang et al. 2007). However, pharmacokinetic studies in non-human primates, especially cynomolgus or rhesus monkeys, can be complicated due to relatively high cost and short supply, and the identification of alternative suitable non-human primates for these studies would be highly desirable. Similarly, while substantial efforts are generally expended to minimize the use of non-human primates in non-clinical safety studies, occasionally other non-rodent species are not feasible for this use and a non-human primate is required to satisfy data needs for product registration. Identification of alternate species for this work would

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clearly represent a major advantage in the drug-development process.

One such alternate non-endangered non-human primate is the African green monkey or vervet (*Chlorocebus aethiops*, or the St. Kitts subspecies *Chlorcebus sabaeus*). Despite widespread use in other areas of biomedical research, African green monkeys have not been commonly used in drug-development studies. Recently, it has been demonstrated that for a test set of small molecules, the African green monkey has the potential to be used as a surrogate for cynomolgus or rhesus monkeys in preclinical pharmacokinetic studies, particularly for the study of clearance processes (Ward et al. 2008). However, the previous study involved only intravenous pharmacokinetic parameters, whereas extrapolation of oral exposure of new chemical entities is at least as important an endeavour in drug discovery. Additionally, non-human primates are occasionally required for use in non-clinical safety assessments, where oral administration of new chemical entities is a key component; understanding the oral pharmacokinetic behaviour of test agents in the African green monkey would help validate this species for toxicological studies. Furthermore, yet another key attribute commonly assessed during lead optimization is the potential impact of active barriers to oral absorption, such as cytochrome (CYP) 3A4 and P-glycoprotein. Very little is currently known about the pharmacokinetic behaviour of substrates of such active processes in the African green monkey, or the ability of preclinical drug–drug interaction studies in this species to predict similar clinical outcomes in humans.

Previously, the authors have developed an *in vivo* screening model in cynomolgus monkeys to allow rapid screening of new molecules in a drug-discovery setting (Ward et al. 2001), and they have further demonstrated the use of this model to characterize the role of P-glycoprotein and/or CYP3A in the oral disposition of molecules (Ward et al. 2004). The present investigation extended the application of this model to the African green monkey, with the intent of enabling the expanded use of this species in preclinical pharmacokinetic lead optimization and non-clinical safety assessment. The objectives of this investigation were to evaluate the predictive performance of the African green monkey with respect to human oral exposure, and to determine the feasibility of using a ketoconazole-based drug–drug interaction model to assess potential clinical drug interactions in this non-human primate species.

Materials and methods

Materials

Test agents were acquired from standard commercial sources and were of the highest available purity. Compounds were formulated per the manufacturers' instructions if available, or with up to 10% dimethyl sulfoxide and 22.5% hydroxypropyl-β-cyclodextrin as required to obtain solution formulations. High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol and American Chemical Society (ACS)-grade formic acid were purchased from EMD (Durham, NC, USA). HPLC-grade water was purchased from Burdick & Jackson (Muskegon, MI, USA). All other reagents and materials were purchased from standard vendors and were of the highest available purity. All compounds were administered as solutions, with dosages based on either clinical or laboratory animal experience; all dosages are expressed as mg parent molecule kg^{-1} body weight. Based on previous studies in other non-human primates (Ward et al. 2004), ketoconazole (10 mg kg^{-1}) was selected as a P-glycoprotein/cytochrome (CYP) 3A inhibitor, and its effects on absorption of erythromycin (a known substrate) and propranolol (known to have minimal clinical interaction) were evaluated.

Animals

For all studies, adult male African green monkeys (4.1–6.7 kg body weight) were used. The animals were collected and studied under the auspices of the St. Kitts Biomedical Research Foundation (St. Kitts, West Indies), and all work was conducted with the prior approval of the Institutional Animal Care and Use Committee of that facility. Monkeys were individually housed in 3 × 2.6 × 2 ft squeeze cages in free-standing enclosures exposed to ambient environmental conditions, which approximated a 12:12 h light–dark cycle with temperatures between 25 and 30°C. Monkeys were fed a standard laboratory primate chow (TekLad, Madison, WI, USA) and were fasted for 6 h before sedation with water provided *ad libitum* by a Lixit valve. Ketamine:xylazine (5:1) was administered intramuscularly (0.2 ml kg^{-1} of 100 mg ml^{-1} ketamine and 20 mg ml^{-1} xylazine) for the placement of a catheter in the saphenous vein, which permitted serial blood draws to the 60-min time point. Animals were maintained under continuous sedation for that interval; catheter patency was maintained with a 0.9% saline drip of approximately 60 ml h^{-1}. After 60 min, blood was collected by femoral venipuncture under repeated intramuscular ketamine/xylazine sedation (0.2 ml kg^{-1}). Monkeys were monitored at all times for distress or other signs of adverse drug reactions.

Oral pharmacokinetic studies

For the assessment of oral exposure, test compounds were administered at target dosages of 0.5–15 mg kg^{-1} (0.2–2.5 ml kg^{-1} dose volume; $n = 3$ animals per test agent), with doses selected based on previous oral data

in either humans or non-human primates. These studies were conducted in a cross-over design, that is, the same animals which previously had received intravenous doses of these test compounds also were used for the oral studies described herein, although different sets of test animals were used for each test compound representing data in approximately 24 different subjects. Following test article administration, timed venous blood samples were collected and centrifuged to obtain plasma. Samples were taken pre-dose, and at 15, 30, 60, 120, 240, 360, 480, 720, and 1440 min post-dose. At each phlebotomy time point, 1.0–1.5 ml of blood were collected into a heparinized syringe, transferred to a centrifuge tube, and immediately centrifuged at 3000 rpm for 15 min at 4°C. The plasma supernatant was then flash-frozen in liquid nitrogen for transport.

Ketoconazole interaction studies

For the purpose of evaluating the African green monkey in a drug-interaction setting, either erythromycin or propranolol were administered orally (5 mg kg^{-1}; $n = 3$ animals), plasma samples obtained for up to 3 h post-dose, and the animals returned to their cage overnight. Twenty-four hours later, the same animals were given an oral dose of ketoconazole (10 mg kg^{-1}), allowed to rest for 40 min, and then were re-challenged with the same dose of erythromycin or propranolol as on day 1, with samples obtained for up to 3 h post-dose.

Analytical procedures

Quantitative analysis was performed on plasma samples for each test compound using an HPLC/tandem mass spectrometric detection method optimized for each individual analyte in the appropriate biological matrix. The HPLC system utilized Shimadzu (Kyoto, Japan) LC-l0ADvp binary HPLC pumps, a Shimadzu SCL-10ADvp system controller, and a Leap Technologies HTC Pal (Carrboro, NC, USA) autosampler equipped with a chiller. The API 3000 mass spectrometer controlled by Analyst™ Software was from Applied Biosystems/MDS Sciex (Toronto, Ontario, Canada). Analyst™ version 1.4.2 was used as the data-acquisition software. The analytical column used was a 4.60 × 50 mm Gemini C-18, 5µ, from Phenomenex (Torrance, CA, USA). Standards and quality control samples were prepared from two separate stock solutions in parallel. For all samples, a fully automated sample extraction procedure was performed using protein precipitation with a CaptiVac™ (Varian) 96-well plate vacuum manifold and Captiva™ filtration kit. Extraction was achieved with a 2:1 volume of either methanol or acetonitrile containing the internal standard (carbamazepine). A total of 10–40 µl of the filtered samples was injected on-column for analysis. A generic gradient HPLC method with a flow rate of 0.5 ml min^{-1} was utilized for separation, with mobile phase A 98% and mobile phase B 2% held for 30 s after injection, then a linear ramp to 90% mobile phase B over 1.0 min, 100% mobile phase B held for 30 s, then an immediate ramp to initial conditions for 60 s for re-equilibration. Mobile phase A consisted of 0.1% formic acid in water (v/v) and mobile phase B consisted of 0.1% formic acid in neat acetonitrile (v/v). The eluent was subjected to Turbo IonSpray positive-mode ionization multiple-reaction monitoring, and each analyte was characterized by an appropriate mass spectral transition of the parent precursor ion to a product ion, generated at an optimized collision energy. Data were reported as quantitative drug concentrations as determined by standard calibration curve analysis, using linear fitting of a $1/x$-weighted plot of the analyte/internal standard peak area ratio versus analyte concentration. Using these optimized conditions, lower limits of quantitation were achieved as follows: 1 ng ml^{-1} for prednisone, quinidine, ciprofloxacin, moxifloxacin, propranolol, and lidocaine, 5 ng ml^{-1} for verapamil and erythromycin, and 10 ng ml^{-1} for metoprolol, diclofenac, and phenytoin.

Data analysis

Plasma concentration versus time profiles were generated for each animal. Standard non-compartmental pharmacokinetic analysis was performed using WinNonlin Professional version 5.0 (Pharsight Corporation, Mountain View, CA, USA) to estimate the area under the concentration versus time curve (AUC) from zero to 24 h post-dose. Absolute oral bioavailability (F; %) was calculated using the oral data from this study and the intravenous exposure data from the authors' previous study in the same individual African green monkeys (Ward et al. 2008). For the prediction of human pharmacokinetic parameters based on these data, the animal oral exposure was scaled using a liver blood flow correction factor as described previously (Ward et al. 2005). For the purpose of this investigation, it was assumed that African green monkey liver blood flow was the same as that in other Old World monkeys (approximately 45 ml min^{-1} kg^{-1}), with any potential effects of the sedation regimen or hydration status not being taken into account. Comparative data from other macaques (cynomolgus and rhesus) and from humans were obtained from the literature to evaluate the relative extrapolation performance of the African green monkey.

Results

A total of eight compounds were evaluated in the present study for which apparently reliable oral pharmacokinetic

data were identified in the literature for humans and either rhesus or cynomolgus monkeys. In the African green monkey pharmacokinetic studies in the present investigation no issues were observed with tolerability of the test agents, and all the animals successfully completed the studies. The oral exposure data (expressed as dose-normalized AUC values (DNAUC; kg·min l^{-1}) and the oral bioavailability data are presented in Table 1. Three of the eight compounds tested (erythromycin, lidocaine, and propranolol) demonstrated negligible systemic exposure following oral administration in the African green monkey. For the remaining five compounds, the concentration versus time profiles obtained are displayed in Figure 1. In general the sampling regimen employed allowed the capture of a majority of the area under the curve, with plasma concentrations of all compounds falling below the limit of quantitation (LOQ) of the assay by 24 h post-dose except for ciprofloxacin and moxifloxacin.

Based on these exposure data, comparisons were performed between the predicted DNAUC in humans from both African green monkeys and cynomolgus/rhesus monkeys to that observed in clinical studies (Figure 2). Likewise, absolute oral bioavailability values between the various test species were compared (Figure 3). In general, compounds fell into one of two categories. For phenytoin, ciprofloxacin, prednisone, metoprolol, and moxifloxacin, predictions from both the African green and the cynomolgus/rhesus monkeys generally fell within two-fold of the

Table 1. Oral pharmacokinetic parameters from African green monkeys, cynomolgus/rhesus monkeys, and humans for the eight test compounds studied in the present investigation.

	Pharmacokinetic parameter					
	Apparent *F* (%)			Dose-normalized AUC (kg·min l^{-1})		
Compound	African green monkeys	Cynomolgus/rhesus monkeys	Human	African green monkeys	Cynomolgus/rhesus monkeys	Human
Phenytoin	151	344	90	60.7	435	1568
Moxifloxacin	45	52	82	20.3	45.1	373
Erythromycin	n.c.	1.9	35	n.c.	1.32	36.8
Lidocaine	n.c.	25	35	n.c.	3.79	38.0
Propranolol	n.c.	1.8	26	n.c.	1.32	17.3
Ciprofloxacin	54	40	63	69.9	85.2	77.0
Metoprolol	31	25	38	9.08	8.33	25.3
Prednisone	38	30	80	43.3	45.4	222

Notes: African green monkey data are from the present investigation; other data are from the literature (see El Dareer et al. 1977 for prednisone; and Ward et al. 2005 for all other data). n.c., Parameter not calculated (negligible oral exposure measured); AUC, area under the curve.

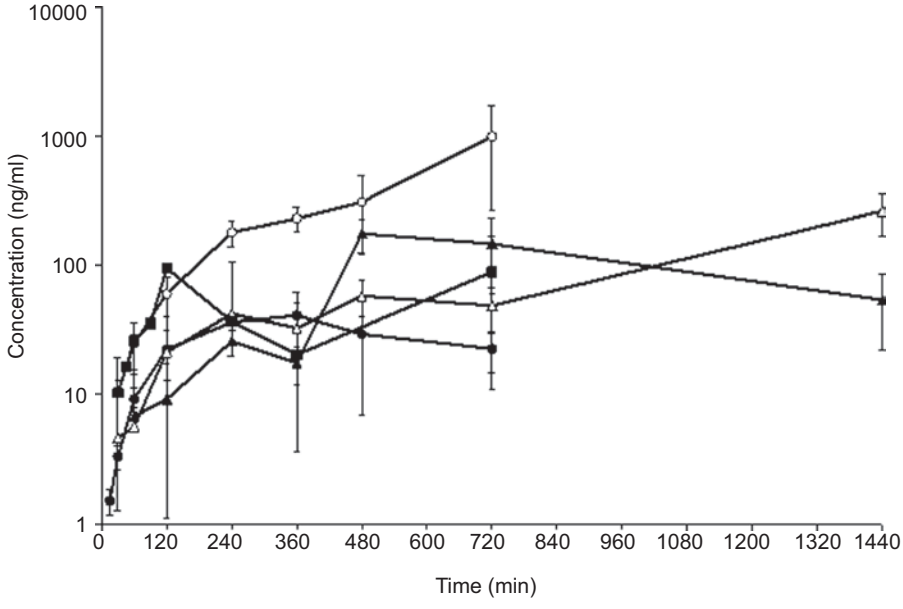

Figure 1. Concentration versus time profiles for five agents following oral administration of a solution formulation in the African green monkey: ●, prednisone; ▲, moxifloxacin; ○, phenytoin; △, ciprofloxacin; and ■, metoprolol. Symbols represent the mean ± standard deviation (SD).

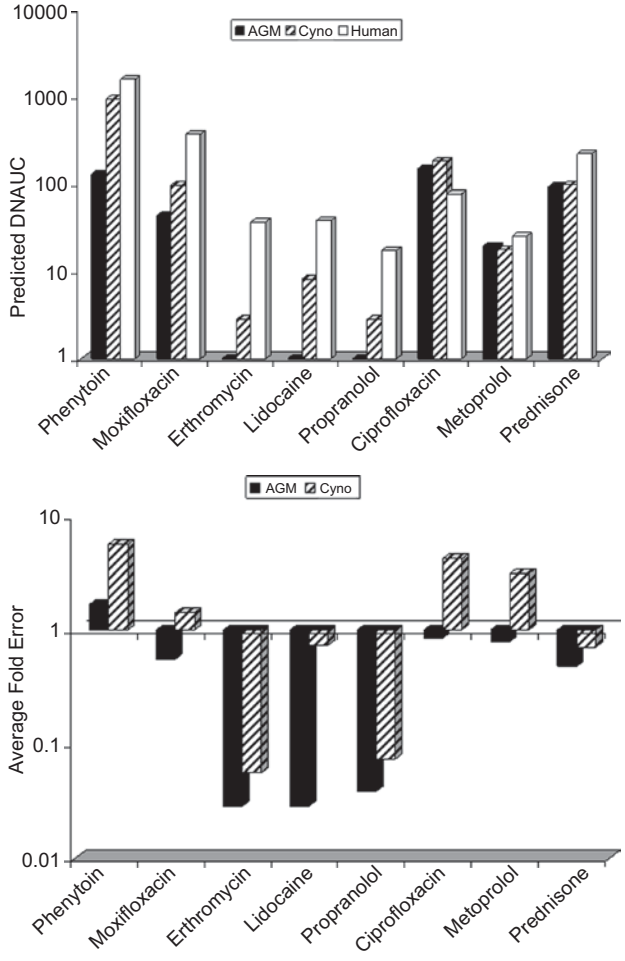

Figure 2. Comparison of human oral exposure (clear bars; dose-normalized area under the curve (DNAUC), kg·min l^{-1}) and predicted human oral exposure from either African green monkeys (solid bars) or cynomolgus/rhesus monkeys (hashed bars). The top panel presents absolute DNAUC values, while the lower panel displays fold-difference from actual values.

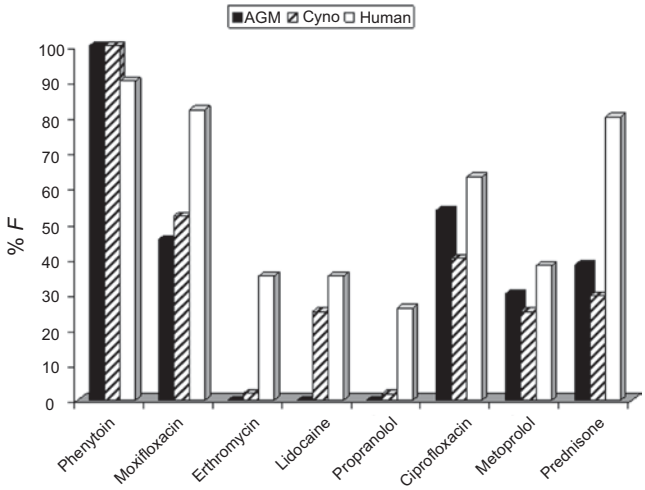

Figure 3. Comparison of human absolute oral bioavailability (clear bars; *F* (%)) and predicted human oral exposure from either African green monkeys (solid bars) or cynomolgus/rhesus monkeys (hashed bars). Apparent oral bioavailability values greater than 100% are truncated at 100% for this depiction; actual bioavailability values can be found in Table 1.

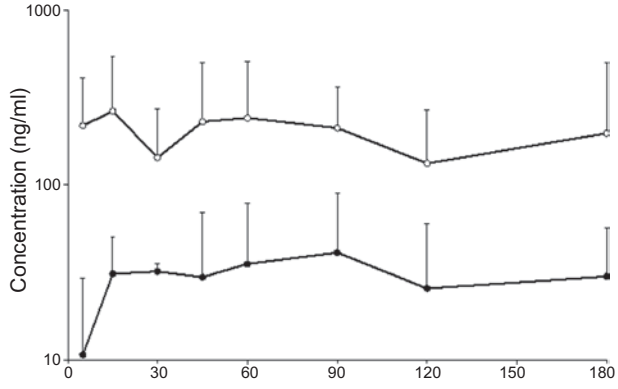

Figure 4. Systemic exposure of erythromycin in the African green monkey following oral administration in the absence (●) or presence (○) of ketoconazole (10 mg kg^{-1}). Symbols represent the mean ± standard deviation (SD).

measured human exposure and oral bioavailability values. For the second category of test compounds, including erythromycin, lidocaine, and propranolol, both African green monkeys and cynomolgus/rhesus monkeys dramatically under-predicted human oral exposure.

With respect to the ketoconazole drug–drug interaction study, the data for the erythromycin portion of the experiment are displayed in Figure 4. Co-administration of ketoconazole produced a clear (6.2-fold) increase in systemic exposure to erythromycin, with AUC values increasing from 5.5 ± 4.5 min · ng ml^{-1} to 35.0 ± 31.5 min · ng ml^{-1}. In contrast, co-administration of ketoconazole and propranolol did not increase systemic exposure to propranolol to a meaningful extent, with all concentrations in both legs of the study below the lower limit of quantitation of the assay (1 ng ml^{-1}).

Discussion

As a test species, non-human primates offer indisputable phylogenetic and pharmacokinetic advantages over other commonly available laboratory species for the conduct of preclinical lead optimization and toxicological studies. However, issues of expense and scarcity, as well as concerns about the protected status of certain non-human primates, result in practical barriers to their more widespread use in drug discovery and development. One potential means to overcome these issues is through the use of alternate non-human primates, such as the African green monkey. African green monkeys, particularly those found in the West Indies, are generally pathogen-free and non-endangered, both attributes

appealing in a laboratory animal species. Moreover, being smaller in size than either cynomolgus or rhesus monkeys, they afford the advantage of requiring less test compound, which can be particularly advantageous when supporting long-term safety studies. Therefore, initiatives are ongoing in the authors' laboratories to address the relative paucity of experimental data in these animals compared with cynomolgus or rhesus monkeys, and probe their feasibility as a preclinical model species.

With respect to oral pharmacokinetics, very little data have been previously published from African green monkeys, particularly those originating in the West Indies. In one study using alprazolam and lorazepam, St. Kitts African green monkeys appeared to demonstrate lower dose-normalized oral exposure compared with humans (Friedman et al. 1991). However, interspecies study design differences, and the lack of comparative data in a different non-human primate for these agents, complicate the interpretation of these results. In contrast, an oral study with chlorzoxazone in West Indies African green monkeys suggested similar oral systemic exposure to that observed in humans (Lee et al. 2006). Despite the lack of previous pharmacokinetic experience, from a physiologic perspective African green monkeys would appear to be a reasonable model for human oral pharmacokinetics; with the exception of a larger caecum in the African green monkey, anatomical features of this species are similar to those of human (Clemens and Maloyi 1981).

Indeed, the data from the present study bear out this hypothesis to a large extent. Oral exposure data from the African green monkey were in general quite similar to those obtained in other Old World primates. Absolute oral systemic exposure in the African green monkey tended to be somewhat lower than that in literature data from other Old World primates, but it is not entirely possible to distinguish between true interspecies differences and differences related to variability in experimental design. Importantly, for the purposes of screening new chemical entities in a drug-discovery setting, the compounds tended to rank order in the same manner among the primate species, and the overall qualitative assessment of the performance of the compounds was similar. One potential study design-related difference between most of the literature data and the present study involves the use of chair restraints in most studies involving cynomolgus and rhesus monkeys, whereas no restraints were used in this study. One of the known effects of chair restraint in non-human primates is a decrease in total intestinal transit time; one study in rhesus monkeys demonstrated a decrease in total intestinal transit time from 46 to 19 h with the use of such restraint (Rabot et al. 1997). It is possible that the lack of restraints in this study resulted in more physiologic intestinal transit times, and that the absorption of certain test compounds could be different under these conditions. Further, in the present study animals were sedated for the purpose of collecting blood samples, and as has been noted previously (Ward et al. 2008) this procedure could also contribute to any inter-study pharmacokinetic differences.

Beyond simple cross-species exposure comparisons, the data in this study suggest, at least on a preliminary basis, that the major mechanistic factors governing pharmacokinetic performance in the cynomolgus or rhesus monkey are operable in the African green monkey. For example, phenytoin is known to have markedly non-linear elimination kinetics (Ludden 1991), resulting in an apparent oral bioavailability in excess of 100% in the cynomolgus monkey; a similar phenomenon was observed in the African green monkey.

Table 2. Summary table comparing the overall pharmacokinetic performance of a series of small molecules in African green monkeys and cynomolgus/rhesus monkeys.

	Clearance (ml min^{-1} kg^{-1})		V_{dss} (l kg^{-1})		Oral F (%)	
Compound	African green monkeys	Cynomolgus/rhesus monkeys	African green monkeys	Cynomolgus/rhesus monkeys	African green monkeys	Cynomolgus/rhesus monkeys
Ciprofloxacin	7.35	4.70	2.70	1.80	54	40
Diclofenac	7.80	8.70	1.07	0.170	102	n.a.
Erythromycin	31.6	14.3	8.24	0.930	n.c.	1.9
Lidocaine	3.02	66.0	0.350	0.980	n.c.	25
Metoprolol	21.9	30.0	14.0	3.00	31	25
Moxifloxacin	21.2	11.5	7.27	4.90	45	52
Phenytoin	19.1	7.9	19.1	7.80	151	344
Prednisone	7.54	13	1.99	1.60	38	30
Propranolol	13.1	14	4.24	0.600	n.c.	1.8
Quinidine	41.1	13	3.00	0.550	44.8	n.a.
Verapamil	7.56	37.1	1.28	4.54	1.88	n.a.

Notes: All data represent mean pharmacokinetic parameters and derive either from the present investigation (African green monkeys, F (%)) or from the literature (Ward and Smith 2004; Ward et al. 2008). n.a., Not available from the literature; n.c., parameter not calculated (negligible oral exposure measured).

Most interestingly, this study tested three compounds that would be considered to demonstrate predictive 'failure' from cynomolgus monkey data: erythromycin, lidocaine, and propranolol. Each of these compounds demonstrates substantially lower oral systemic exposure in cynomolgus monkeys compared with humans, and a similar phenomenon was observed in the African green monkey. As has been noted previously (Ward et al. 2005), when oral exposure in cynomolgus or rhesus monkey differ from those in humans, exposure is almost always lower in the non-human primate. The observation of a similar trend in the African green monkey suggests that this may be an appropriate species for further mechanistic exploration of this general phenomenon. As a further verification of the mechanistic cross-primate similarities, the drug–drug interaction studies conducted as part of the current investigation revealed very similar behaviour between cynomolgus and African green monkeys, with both species serving as a reliable model of a P-glycoprotein- and/or cytochrome 3A-mediated drug–drug interaction. Co-administration of ketoconazole resulted in a 7.5-fold increase in erythromycin systemic exposure in the cynomolgus monkey (Ward et al. 2004), and a 6.2-fold increase in the African green monkey.

In summary, the present data support continued exploration of the African green monkey as an alternate non-human primate species for use in drug discovery and development. Qualitatively similar systemic oral exposure was generally observed in African green monkeys compared with other Old World monkeys, and the utility of this species in drug–drug interaction screening was demonstrated. To place the current work in perspective, Table 2 provides a summary evaluation of key intravenous and oral pharmacokinetic parameters in African green monkeys versus other Old World monkeys. As can be readily seen, African green monkeys generally display similar pharmacokinetic properties to cynomolgus/rhesus monkeys which form the basis of many non-clinical safety and pharmacokinetic assessments. These data highlight the utility of this species for non-clinical support work. Future research will include investigation of baseline physiologic parameters in the African green monkey to support more refined pharmacokinetic studies.

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