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Comparative pharmacokinetics between a microdose and therapeutic dose for clarithromycin, sumatriptan, propafenone, paracetamol (acetaminophen), and phenobarbital in human volunteers

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ABSTRACT

A clinical study was conducted to assess the ability of a microdose (100 μg) to predict the human pharmacokinetics (PK) following a therapeutic dose of clarithromycin, sumatriptan, propafenone, paracetamol (acetaminophen) and phenobarbital, both within the study and by reference to the existing literature on these compounds and to explore the source of any nonlinearity if seen. For each drug, 6 healthy male volunteers were dosed with 100 μg ^{14}C-labelled compound. For clarithromycin, sumatriptan, and propafenone this labelled dose was administered alone, i.e. as a microdose, orally and intravenously (iv) and as an iv tracer dose concomitantly with an oral non-labelled therapeutic dose, in a 3-way cross over design. The oral therapeutic doses were 250, 50, and 150 mg, respectively. Paracetamol was given as the labelled microdose orally and iv using a 2-way cross over design, whereas phenobarbital was given only as the microdose orally. Plasma concentrations of total ^{14}C and parent drug were measured using accelerator mass spectrometry (AMS) or HPLC followed by AMS. Plasma concentrations following non-^{14}C-labelled oral therapeutic doses were measured using either HPLC-electrochemical detection (clarithromycin) or HPLC–UV (sumatriptan, propafenone). For all five drugs an oral microdose predicted reasonably well the PK, including the shape of the plasma profile, following an oral therapeutic dose. For clarithromycin, sumatriptan, and propafenone, one parameter, oral bioavailability, was marginally outside of the normally acceptable 2-fold prediction interval around the mean therapeutic dose value. For clarithromycin, sumatriptan and propafenone, data obtained from an oral and iv microdose were compared within the same cohort of subjects used in the study, as well as those reported in the literature. For paracetamol (oral and iv) and phenobarbital (oral), microdose data were compared with those reported in the literature only. Where 100 μg iv ^{14}C-doses were given alone and with an oral non-labelled therapeutic dose, excellent accord between the PK parameters was observed indicating that the disposition kinetics of the drugs tested were unaffected by the presence of therapeutic concentrations. This observation implies that any deviation from linearity following the oral therapeutic doses occurs during the absorption process.

1. Introduction

Microdosing is a technique in which small doses of drug (⩽100 μg) are given to human volunteers to obtain pharmacokinetic (PK) data very early in drug development (Lewis, 2009; Garner, 2010). The low doses are associated with minimal anticipated risk of toxicity and therefore regulatory authorities require only limited preclinical safety data to allow administration to humans (ICH-M3, 2009). Microdosing is increasingly being adopted by the pharmaceutical industry as one of the means of selecting drugs with suitable PK characteristics before committing to a full clinical development programme (Zhou et al., 2009).

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Table 1
Dosing schedule for each drug.

Drug	Dose period 1	Dose period 2	Dose period 3*
Clarithromycin	^{14}C oral microdose	^{14}C iv microdose	^{14}C iv tracer dose + oral therapeutic dose (250 mg)
Sumatriptan	^{14}C oral microdose	^{14}C iv microdose	^{14}C iv tracer dose + oral therapeutic dose (50 mg)
Propafenone	^{14}C oral microdose	^{14}C iv microdose	^{14}C iv tracer dose + oral therapeutic dose (150 mg)
Paracetamol	^{14}C oral microdose	^{14}C iv microdose	None
Phenobarbital	^{14}C oral microdose	None	None

* Administration of 100 μg ^{14}C-drug intravenously and a therapeutic non-radiolabelled oral dose.

Much of the considerable debate about the utility of microdosing has focused on the ability to predict human PK at pharmacologically active doses (Bertino et al., 2007; Rowland, 2007; Ings, 2009), and although a body of such evidence is accumulating (Lappin et al., 2006a,b) some doubt still remains. Of the published microdose studies the most comprehensive has been the CREAM (Consortium for Resourcing and Evaluating AMS Microdosing) Trial (Lappin et al., 2006a,b) in which the PK of 5 drugs, mainly eliminated by CYP450 catalyzed oxidation, were chosen for study that illustrated problematic situations using animal or *in vitro* data. Since then, another larger study, with seven additional compounds, has been performed under the auspices of the European Microdosing accelerator mass spectrometry (AMS) partnership programme (EUMAPP – www.EUMAPP.com). This programme comprises three parts. (1) A comparison of observed therapeutic dose PK with linear dose-scaled predictions following microdose administration. (2) Exploration of the potential benefit of incorporating *in vitro* data in those cases where dose-dependency in PK is seen. (3) A comparison between microdosing and physiologically-based pharmacokinetic modelling, using a combination of *in vitro* and independent physiological data. Reported here are the findings associated with the first part of EUMAPP. Each compound was selected to illustrate an additional facet that raises a potential problem with existing predictive methodologies, in part due to a lack of a substantial body of *in vitro* quantitatively predictive methods for the processes involved. The compounds chosen were fexofenadine, clarithromycin, sumatriptan, propafenone, paracetamol (acetaminophen), phenobarbital, and a Servier compound, S-19812, all marketed drugs except for the last compound. Our findings with fexofenadine, a non-metabolised, low clearance drug, whose PK is heavily dependent on transporters, have been previously reported (Lappin et al., 2010). Reported here are the findings for the remaining five marketed drugs. Clarithromycin was chosen as representative of a large lipophilic drug whose PK depends on both CYP3A4 and the efflux transporter, including P-glycoprotein (PgP); sumatriptan as a compound eliminated primarily by mixed function monoamine oxidases, while propafenone represents a high clearance, low oral bioavailable, drug extensively metabolised by CYP2D6. Paracetamol is representative of drugs extensively eliminated by sulphate and glucuronide conjugation, whereas phenobarbital was chosen because it is so metabolically stable that clearance *in vitro* cannot be measured accurately.

2. Methods

2.1. Test substance and reagents

^{14}C-labelled drugs, clarithromycin (6-(4-dimethylamino-3-hydroxy-6-methyl-tetrahydropyran-2-yl)oxy-14-ethyl-12,13-dihydroxy-7-[^{14}C]-methoxy-3,5,7,9,11,13-hexamethyl-1-oxa-cyclotetradecane-2,10-dione), sumatriptan (3-(2-dimethylamino)ethyl)-*N*-methyl-2-[^{14}C]-1H-indole-5-methanesulfonamide succinate), propafenone (1-(2-(2-hydroxy-3-(1-[^{14}C]-propylamino)propoxy)phenyk)-3-phenylpropane-1-one), paracetamol (*N*-(4-hydroxy-[^{14}C]-phenyl) acetamide), and phenobarbital (5-ethyl-5-[^{14}C]-phenyl-pyrimidine-2,4,6(1H,3H,5H)-trione) were prepared by PRI, Warsaw, Poland, certified to at least 96% radio and chemical purity at a radioactive concentration of approximately 2.2–3.7 MBq/mL. Non-labelled standards were obtained from Aldrich Chemical Company, UK, with a certified purity of >98%.

AMS standard "ANU" sugar was obtained from Quaternary Dating Research Centre, Australian National University, Canberra, Australia. All reagents were of the purest grade available. Liquid paraffin, used for isotopic dilution, was obtained from Sigma Aldrich Chemical Company, UK.

2.2. Dose formulation and administration

One hundred microgram (containing 7.4 KBq (200 nCi)) of each ^{14}C-labelled compound was administered on separate occasions to 6 male volunteers per cohort as an oral microdose and, for some of the compounds, as an iv tracer alone and together with the oral therapeutic dose, to test linearity of disposition kinetics and enable estimation of absolute oral bioavailability. These were designated dose periods 1, 2, and 3, respectively (Table 1). Oral drinking and intravenous solutions were prepared from a commercially available iv formulation of each compound: Perfalgan® 10 mg/mL, Bristol–Myers Squibb, (paracetamol); Phenobarbital 50 mg/mL, Pharmachemie; Imigran® 12 mg/mL, GlaxoSmithKline, (sumatriptan succinate); Rytmonorm® solution 3.5 mg/mL, Abbott BV, (propafenone HCl); and Klaricid® solution 2 mg/mL, Abbott, (clarithromycin lactobionate) all sourced from within the Netherlands, except Klaricid®, sourced from the UK. All iv and oral 100 μg doses were administered as solutions in 10 mL 0.9% w/v NaCl. The iv doses were sterilized by filtration (0.22 μm pore size). Actual iv doses administered were determined by weight. Clarithromycin was orally administered in period 3 (Table 1) as 125 mL of a 2 mg/mL solution, sumatriptan as 4.17 mL of a 12 mg/mL solution diluted to 50 mL with water for injection, and propafenone as 42.86 mL of a 3.5 mg/mL solution. All oral doses were administered under fasting conditions with water to make the total fluid intake 200 mL. The supplemental water was used to rinse oral dosing syringes (period 1) and drinking bottles (period 3).

2.3. Nonspecific binding

Nonspecific binding of each microdose to the dosing apparatus materials was assessed before dose administration. Test dose preparations were passed through the dosing apparatus and sterilization filters and the radioactive recovery was measured by liquid scintillation counting (Packard Tri-Carb 2770 TR SL). Recoveries were greater than 95% for all compounds.

2.4. Study design

A total of 30 healthy male subjects (age range 18-55 years) with a body mass index between 18 and 30 kg/m^2, who did not smoke more than 10 cigarettes per day and abstained from smoking dur-

ing the study, were enrolled. Their test results were negative for drugs of abuse and they had not had an illness within 5 days before dosing. The clinical study was performed at PRA International (Zuidlaren, The Netherlands) and received independent ethical approval (Stichting Beoordeling Ethiek Bio-Medisch Onderzoek', P.O. Box 1004, 9400 BA Assen, The Netherlands). All subjects provided written prior consent as defined within the recommendations of the WHO, the International Conference on Harmonisation (ICH) E6 Guideline for GCP, and the EU Clinical Trial Directive (Directive 2001/20/EC).

Subjects were fasted 10 h before and 4 h following dosing. Except for phenobarbital, the study was either a 2 or 3-way crossover design (see Table 1). Following dose administration, blood samples (10 mL) were taken at preselected times *via* an indwelling Venflon® catheter or by direct venepuncture into sodium heparin-containing polypropylene or Vacutainer® tubes. Blood was centrifuged to produce plasma. The study duration was as follows: sumatriptan – 10 h, phenobarbital – 336 h and for the other three drugs 24 h. The time for each dose administration was recorded. Intravenous doses were given as an infusion over 30 min and the start of infusion was taken as $t = 0$. Samples were stored between −20 °C and −80 °C and transported from the clinic to the AMS facility on solid carbon dioxide.

2.5. Drug measurement by HPLC and AMS

Plasma samples were either aliquoted directly for total ^{14}C analysis by AMS (60 μL) or extracted for HPLC analysis. Plasma extractions were performed in essentially the same way for all drugs using 100 μL sample with 200 μL acetonitrile in the presence of 30 μg/mL non-labelled drug as an internal standard using a 96-well protein precipitation plate under vacuum (Sirocco, obtained from Waters, UK). The supernatant was evaporated to complete dryness using N_2 at 40 °C and reconstituted in 200 μL HPLC mobile phase starting conditions (see below) prior to injection (typically 50 μL) on HPLC.

For clarithromycin, a Hypersil BDS C18, 5 μm, 4.6 × 250 mm (Phenomenex) column was eluted with 10% mobile phase A (acetonitrile: 0.02 M KH_2PO_4: triethylamine, 20:80:0.5 (v/v/v) pH 7.0) and 90% mobile phase B (water) for 5 min, followed by a linear gradient to 100% B over 23 min. The flow rate was 1 mL/min and the UV response was monitored at 205 nm. For sumatriptan, the column was a Hypersil BDS C18, 5 μm, 4.6 × 250 mm (Phenomenex) eluted isocratically with acetonitrile: 0.05 M KH_2PO_4, 16: 84 (v/v) pH 3.8 at 1 mL/min and 30 °C, with UV detection at 282 nm. For propafenone, the HPLC column was a Hypersil BDS C18, 5 μm, 4.6 × 250 mm (Phenomenex) eluted isocratically with 0.05 M KH_2PO_4 and methanol (3:7 v/v) at 1 mL/min and 30 °C, with UV detection at 210 nm. For paracetamol and phenobarbital, a Hypersil BDS C18, 5 μm, 4.6 × 250 mm was eluted with 0.05 M KH_2PO_4: CH_3COOH: methanol – 100: 0.1: 5 (v/v/v), pH 6.5 isocratically at 1 mL/min and 30 °C. Chromatographic standards were monitored at 210 nm for paracetamol and 230 nm for phenobarbital.

The HPLC eluate was collected as a series of fractions in 96-well plates and those fractions corresponding to the retention time of the peak of interest (located by UV detection from the non-labelled drug spiked into plasma) were pooled. In order to determine the drug concentration in mass per volume of sample from the ^{14}C:^{12}C ratio data obtained from AMS analysis, the HPLC fractions were accurately isotopically diluted with the addition of 1.627 mg of liquid paraffin. The isotopically diluted samples were graphitised and analysed by AMS as described previously (Zhou et al., 2009). Calculation of the drug concentration from the isotope ratio measurements by AMS and internal standardization against the UV response for the non-labelled drug was conducted as previously described (Lappin et al., 2008).

2.6. HPLC–UV and electrochemical analysis

For clarithromycin, sumatriptan and propafenone, non-labelled therapeutic oral doses were administered in dose period 3 (see Table 1). The resulting plasma drug concentrations were measured using HPLC with UV or electrochemical detection as described below.

For clarithromycin, a Inertsil ODS-2 C18 (150 × 4.6 mm, 5 μm) column was isocratically eluted at 43 °C with phosphate buffer (0.02 M, pH 6.8): acetonitrile: methanol (50:40:10, v/v/v) at 1.0 mL/min, with electrochemical detection at +0.90 V. For sumatriptan a Pursuit C18 (150 × 4.6 mm, 3 μm) column was isocratically eluted at 40 °C with phosphate buffer (25 mM, pH 3.9): acetonitrile (17: 3 v/v) at 0.5 mL/min, with UV detection at 228 nm. For propafenone, a Microsphere C18 (100 × 4.6 mm, 3 μm) column was isocratically eluted at 50 °C with acetonitrile: methanol: water (5:2.5:68 v/v/v) containing 0.01% sulphuric acid at 1.5 mL/min, with UV detection at 209 nm. The retention times for clarithromycin, sumatriptan, and propafenone were typically 14.3, 4.8, 12.5 min, respectively.

2.7. Liquid scintillation counting (LSC)

Radiolabelled dose preparations were analysed by LSC using a Packard TriCarb counter. Samples were admixed with Gold Star scintillant (Meridian, Epsom, Surrey, UK) and counted for up to 1 hour or 2%2σ, whichever came first.

2.8. Pharmacokinetics

PK parameters were calculated from data expressed as mass of unchanged drug/mL plasma using WinNonLin (Pharsight California, USA) version 3.3. AUCs were calculated using trapezoid approximation, with linear interpolation in the constant and ascending parts of the concentration-time profiles and logarithmic interpolation in the descending region. The (absolute) oral bioavailability of the microdose was calculated as the AUC ratio of parent compound following the oral and iv microdoses, and that for the therapeutic dose as the ratio of dose-normalised parent AUC of the therapeutic dose to that for the iv tracer dose.

3. Results

All subjects completed the study and treatments were well tolerated. Where crossover studies were performed, based on radioactive measurements of plasma samples taken prior to each dose administration, there was no evidence of drug related compounds present from a previous dose. Figs. 1a–3a display semilog plots of the dose-normalized mean plasma concentration versus time profiles following the oral and iv microdoses, oral therapeutic dose, and associated iv tracer dose for clarithromycin, sumatriptan, and propafenone, respectively. Figs. 1b–3b show the corresponding mean plasma drug and total ^{14}C concentration-time profiles following the oral and iv microdoses, Fig. 4 those for paracetamol, and Fig. 5 displays data for phenobarbital following an oral microdose. Calculated PK parameters for all 5 drugs are listed in Table 2.

3.1. Clarithromycin

The LOQ for the LC AMS assay was 10 pg/mL, with 11.6 pg/mL the lowest plasma concentration measured, whereas the LOQ for the HPLC–UV assay was 50 ng/mL, with 72 ng/mL the lowest concentration measured. For the oral microdose and therapeutic dose the final 2 time points (16 and 24 h) were <LOQ. One sample at 24 h was <LOQ for the iv 100 μg dose.

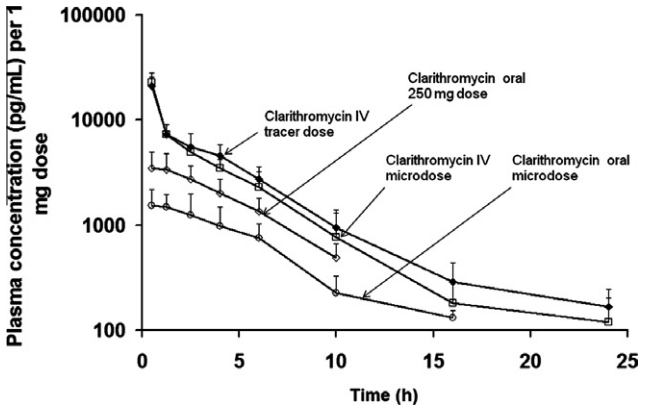

Fig. 1a. Semilog plots of mean plasma clarithromycin concentration-time profiles following a single oral dose of 100 μg (○), a 30 min iv infusion of 100 μg (□), a single oral dose of 50 mg (◇) and a 30 min iv infusion of 100 μg with a simultaneous oral dose of 250 mg (♦). Data are dose normalised to a 1 mg dose and error bars are +1 standard deviation.

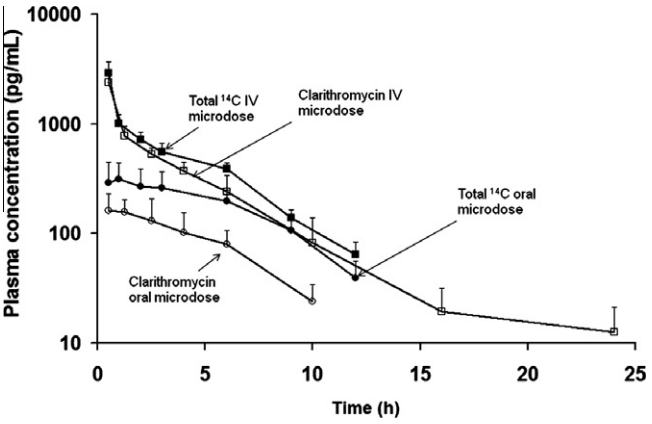

Fig. 1b. Semilog plot of mean plasma clarithromycin concentrations following a single oral dose of 100 μg (○), a 30 min iv infusion of 100 μg (□). Data for total ^{14}C are also shown following the oral dose (●) and iv dose (■). Error bars are +1 standard deviation.

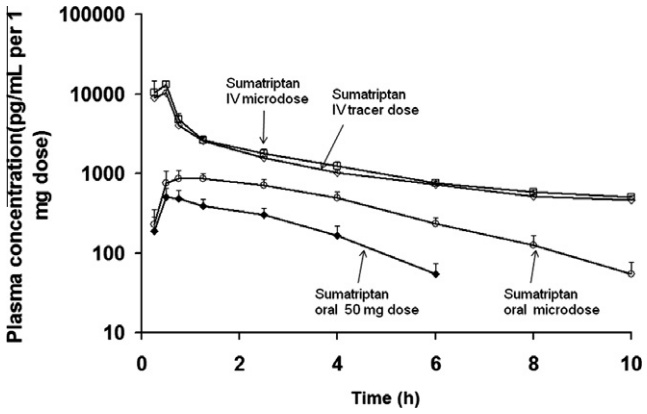

Fig. 2a. Semilog plots of mean plasma sumatriptan concentration-time profiles following a single oral dose of 100 μg (○), a 30 min iv infusion of 100 μg (□), a single oral dose of 50 mg (♦) and a 30 min iv infusion of 100 μg with a simultaneous oral dose of 50 mg (◇). Data are dose normalised to a 1 mg dose and error bars are +1 standard deviation.

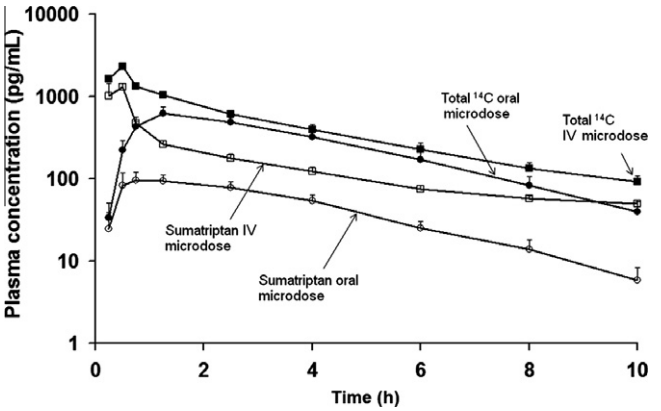

Fig. 2b. Semilog plots of mean plasma sumatriptan concentration-time profiles following a single 100 μg microdose given orally (○) and as a 30 min iv infusion (□). Data for total ^{14}C are also shown following the oral (●) and iv (■) doses. Error bars are +1 standard deviation.

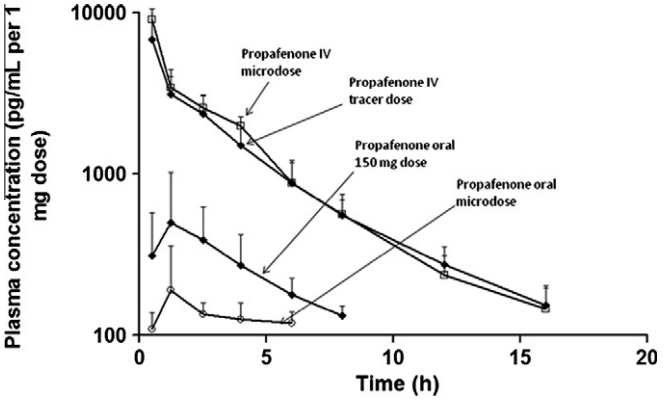

Fig. 3a. Semilog plots of mean plasma propafenone concentration-time profiles following a single oral dose of 100 μg (○), a 30 min iv infusion of 100 μg (□), a single oral dose of 150 mg (♦) and a 30 min iv infusion of 100 μg with a simultaneous oral dose of 150 mg (◇). Data are dose normalised to a 1 mg dose and error bars are +1 standard deviation.

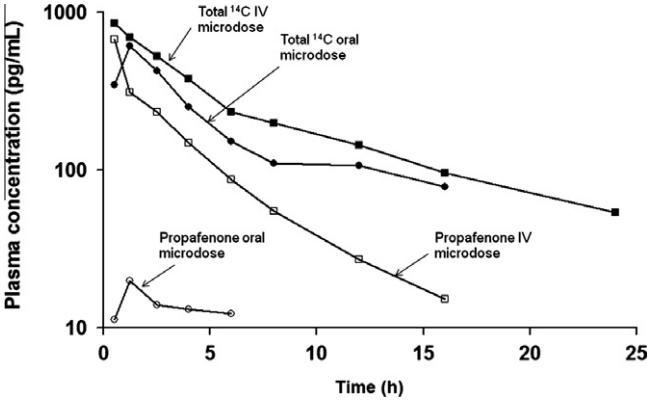

Fig. 3b. Semilog plots of mean plasma propafenone concentration-time profiles following a 100 μg administered as a single oral microdose (○) and a iv infusion over 30 min (□). Data for total ^{14}C are also shown following the oral dose (●) and iv dose (■). Error bars are +1 standard deviation.

The shape of the plot for the iv microdose was similar to that of the iv ^{14}C-tracer dose given along with the oral therapeutic dose. Also, values for V, V_{ss} CL, and terminal $t_{½}$ were virtually identical, with means of 136 L, 90 L, 22 L/h, and 4.3 h, respectively. Despite the 2500-fold difference in dose, the dose-normalized curves for oral micro- and 250-mg therapeutic doses have the same shape and have AUCs within a factor of 2, with oral bioavailability of

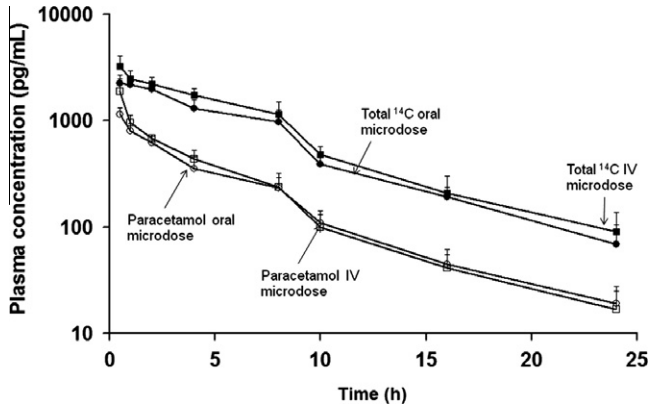

Fig. 4. Semilog plot of mean plasma paracetamol concentrations following a single oral dose of 100 μg (○) and a 30 min iv infusion of 100 μg (□). Data for total ^{14}C are also shown following the oral dose (●) and iv dose (■). Error bars are +1 standard deviation.

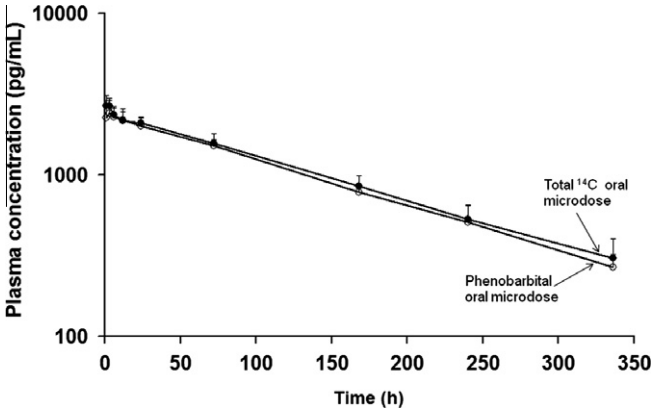

Fig. 5. Semilog plot of mean plasma phenobarbital concentrations following a single oral dose of 100 μg (○). Data for total ^{14}C are also shown following (●). Error bars are +1 standard deviation.

22% and 39%, respectively (Table 2). The parent drug to total radioactivity AUC ratio after oral and iv microdosing, was 0.30 and 0.66, respectively. The corresponding metabolites (difference between total and drug) to drug AUC ratios (given by AUC(drug)/AUC(total) − 1) for the oral and iv microdoses are 2.3 and 0.52.

3.2. Sumatriptan

The LOQ for the LC AMS assay was 3 pg/mL; the lowest plasma concentration was 4 pg/mL. The LOQ for the HPLC–UV assay was 2 ng/mL with the lowest concentration measured being at the LOQ. For the oral microdose 2 data points were excluded at the 10 h collection as being <LOQ (the mean for 10 h was calculated using $n = 4$). One datum point for the iv microdose at 10 h was <LOQ (mean calculated using $n = 5$). For the oral 50 mg dose, all data for the final two sampling times (8 and 10 h) were <LOQ.

The plots of the plasma time profiles for the iv microdose and the iv ^{14}C-tracer dose given along with the oral 50-mg therapeutic dose are virtually superimposable, with V, V_{ss}, CL, and terminal $t_{½}$ of approximately 411 L, 270 L, 50 L/h, and 6 h, respectively. Despite the 500-fold difference in dose, the dose-normalized curves for oral micro- and 50-mg therapeutic doses have a similar shape with a t_{max} close to 1 h and estimated respective terminal $t_{½}$s of 1.9 h and 1.4 h. Also, a clear difference in the AUC ratio, parent drug to total radioactivity, was seen, this being 0.17 and 0.43 for the oral and iv microdoses, respectively, corresponding to a metabolites-to-drug AUC ratios of 4.33 and 1.33. Furthermore, the estimated oral bioavailability of sumatriptan following the microdose (20%) was somewhat higher than found for the 50 mg therapeutic dose (7.6%).

3.3. Propafenone

The LOQ for the LC AMS assay was 10 pg/mL, with 11.5 pg/mL the lowest plasma concentration measured. The LOQ for the HPLC–UV assay was 10 ng/mL, with 18 ng/mL the lowest concentration measured. The last time point that concentration could be measured was 12 h for the 100 μg iv dose and 6 h for the 100 μg oral dose. For the 150 mg dose, data after 8 h were <LOQ.

The parent drug plots for the iv microdose and iv tracer dose given with the oral non-labelled dose were virtually superimposable, yielding a common estimate of CL (45 L/h) and very similar values of V, of 273 L and 214 L, respectively and V_{ss} of 272 and 209 L respectively. The estimated terminal $t_{½}$ was 3.8 h for the oral microdose, 2.6 h for the 150 mg oral dose, and approximately 5 h for the iv dose, whether given as a microdose or a tracer dose superimposed on the oral therapeutic dose. The shapes of the oral microdose and therapeutic dose curves for parent drug were also very similar. The mean oral bioavailability of propafenone following the microdose (5.8%) was somewhat lower than found with the 150 mg therapeutic dose (13%). A marked increase in the ratio of total radioactivity to parent drug was seen after oral than iv

Table 2
Calculated pharmacokinetic parameters for test drugs (%CV in parentheses).

Drug	Treatment	$t_{½}$ (h)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng h/mL)	V (L)	V_{ss} (L)	CL (L/h)	F (%)
Clarithromycin	Oral 100 μg	4 (32)	0.19 (35)	1.2 (61)	0.99 (38)				22 (47)
	IV 100 μg	4.1 (18)			4.78 (21)	136 (26)	88 (20)	23 (18)	
	IV 100 μg + oral 250 mg	4.5 (32)			5.44 (19)	136 (41)	92 (77)	21 (20)	
	Oral 250 mg	3.4 (46)	958 (34)	0.96 (85)	4905 (36)				39 (37)
Sumatriptan	Oral 100 μg	1.9 (34)	0.1 (21)	1.2 (62)	0.44 (16)				20 (11)
	IV 100 μg	6.5 (17)			2.2 (9.3)	426 (16)	272 (48)	46 (11)	
	IV 100 μg + oral 50 mg	5.6 (28)			2.1 (7.8)	397 (23)	268 (54)	50 (8.6)	
	Oral 50 mg	1.4 (24)	26 (25)	0.75 (37)	76 (21)				7.6 (21)
Propafenone	Oral 100 μg	3.8 (32)	0.015 (13)	2.2 (62)	0.12 (42)				5.8 (38)
	IV 100 μg	5.4 (47)			1.9 (17)	273 (29)	272 (47)	49 (15)	
	IV 100 μg + oral 150 mg	4.7 (28)			2.2 (22)	214 (12)	209 (54)	44 (23)	
	Oral 150 mg	2.6 (14)	98 (78)	1.1 (27)	399 (58)				13 (68)
Paracetamol	Oral 100 μg	5.8 (33)	1.1 (16)	0.5 (0)	4.8 (19)				88 (21)
	IV 100 μg	4.6 (21)			5.4 (17)	123 (23)	90 (13)	19 (19)	
Phenobarbital	Oral 100 μg	108 (13)	2.6 (17)	5 (76)	366 (11)				

Table 3
Pharmacokinetic parameters from the literature (values in parentheses are standard deviation). po – oral dose.

Drug	Dose	*t*½ (h)	cmax	V (L)	CL (L/h)	F (%)	Reference
Clarithromycin	250 mg (i.v.) 100–1200 mg (po)	2.8 (0.61) iv 2.52 (2.2) po	788 ± 269	125 (27)	31.1 (8.1)	51 (16)	[a]
Sumatriptan	3 mg (i.v.) 50 mg (po)	1.6 (1.5) iv 1.92 (0.94) po	25.1 (13.8)	170 (115)	69.5 (28)	14.1 (6.6)	[b]
Propafenone	35–70 mg (i.v.) 150 mg (po)	3.0 (0.8) iv 3.9 (1.8) po	121 (78)	248 (44)	61.5 (16.6)	12.7 (9.8)	[c]
Paracetamol	500–1400 mg (i.v.) 1 g (po)	2.5 (0.4) iv 2.6 (0.4) po	14.9 (5.9)	66.5 (8.8)	19.7 (3.9)	89 (9.8)	[d]
Phenobarbital	130–218 mg (i.v.) 30-240 mg (po)	99.2 (16) iv 99.3 (18.2) po	2.48 (0.48)	46.6 (2.3)	0.28 (0.06)	97.5 (12)	[e]

[a] Davey (1991), Chu et al. (1992a–d and Fraschini et al. (1993).
[b] Fowler et al. (1991), Dechant and Clissold (1992), Scott (1994), Lacey et al. (1995), Duquesnoy et al. (1998), Perry and Markham (1998), Moore et al. (2002) and Ferrari et al. (2008).
[c] Hollmann et al. (1983), Siddoway et al. (1984), Arboix et al. (1985), Giani et al. (1988), Haefeli et al. (1990), Vozeh et al. (1990), Hii et al. (1991), and Komura and Iwaki (2005).
[d] Albert et al. (1974), Rawlins et al. (1977), Clements et al. (1978), Douglas et al. (1978), Perucca and Richens (1979), Prescott (1980), Abernethy et al. (1982), Divoll et al. (1982a,b), Forrest et al. (1982).
[e] Viswanathan et al. (1978,1979), Nelson et al. (1982), Wilensky et al. (1982) and Yska et al. (2000).

microdosing, especially pronounced during the earliest times when (Fig. 3a). Parent drug to total radioactivity AUC ratio after oral than iv dosing was 0.026 and 0.42, respectively, following microdose administration, resulting in a corresponding metabolites-to-drug AUC ratios of 37.5 and 1.38.

3.4. Paracetamol

The LOQ for the LC AMS assay was 10 pg/mL and the lowest concentration measured was 9.3 pg/mL. The estimated V, V_{ss}, CL, and terminal $t_{½}$ following the iv microdose were 123 L, 90 L, 19 L/h, and 4.6 h, respectively. The C_{max} and t_{max} following the oral microdose were 1.1 ng/mL and 0.5 h, respectively, with a similar terminal $t_{½}$ (5.8 h) to the iv microdose, and oral bioavailability of 88%.

3.5. Phenobarbital

The LOQ for the LC AMS assay was 5 pg/mL; the lowest plasma concentration measured was 201 pg/mL. Following the oral microdose, the mean t_{max} was 5 h, and terminal $t_{½}$ 108 h.

4. Discussion

The current study extends the results of the CREAM trial (Lappin et al., 2006a,b) by examining the prediction of PK linearity in humans with a further 5 representative drugs for which existing methods of prediction based on animal data or *in vitro* methods still raise considerable uncertainty. To assess how well the PK following a microdose predicts that at a therapeutic dose, a definition of predictability is necessary. A widely accepted criterion for both allometric scaling and *in vitro*-to-*in vivo* extrapolation is one in which the human prediction is within ±2-fold of the actual value (Beaumont and Smith, 2009). Normally, the metric evaluated is a PK parameter such as CL, V, or oral bioavailability, although arguably shape of profile is often equally important to predict. Certainly, C_{max} and trough concentration at the end of a dosing interval at steady state on multiple dosing are important for some drugs, and in such cases a twofold deviation in CL and V does not necessarily translate into a corresponding twofold deviation in these metrics. Furthermore, caution should be exercised in the application of a defined tolerance (such as 2-fold) given that most pharmacokinetic parameters are bounded. For example, percent bioavailability lies between zero and 100 and organ clearance between zero and organ blood flow. If, for arguments sake, the reference value for bioavailability is 15%, application of a ±2-fold tolerance seems reasonable but if the reference value is 70% then this application would clearly be inappropriate.

Accordingly, a balanced perspective is needed when viewing the success of prediction, which varies with the drug as well as its therapeutic application.

A note on terminology used in this paper is also appropriate. The term microdose refers to the situation in which a subpharmacologic dose (⩽100 μg) of compound is administered to a subject (in whom the compound is not present in the body), irrespective whether or not it is isotopically labeled. In contrast, as used here, a tracer dose is a small amount of an isotopically-labeled compound administered to a subject in whom are substantial amounts of the compound, which may be endogenous, or exogenously administered. The intent of the tracer is to literally trace, and not perturb, the fate of the existing pool of compound (Sarapa et al., 2005; Lappin et al., 2006a,b). The doses of the microdose and tracer dose may be the same, as in the current study, but the intent of the two administrations are clearly different.

In the current study paracetamol and phenobarbital were administered only as microdoses as there is a large body of published therapeutic dose data for comparison, with relatively small intersubject variability. For clarithromycin, sumatriptan, and propafenone there are either less published data, or evidence of considerable intersubject variability, and for these compounds oral microdose PK was compared to therapeutic oral dose data in the same subjects. In addition, for these three compounds comparison of PK was made between an iv microdose and an iv tracer dose given simultaneously with the oral therapeutic dose, to assess linearity of disposition kinetics and determine the absolute bioavailability of the therapeutic dose, respectively. For reference, Table 3 lists literature values for the PK of these compounds following therapeutic doses.

4.1. Clarithromycin

Clarithromycin was chosen to exemplify a relatively large molecular weight (748 g/mol) moderately lipophilic (logP 2.3) compound that is both a P-gp substrate and undergoes extensive metabolism via CYP3A4, and which exhibits limited oral bioavailability (ca. 50%). Previously, structurally related erythromycin had been chosen for study in the CREAM trial, which intrinsically should have been a suitable probe compound, but it was too unstable in the acidic gastric environment to allow evaluation of the abovementioned features. Clarithromycin is relatively acid stable

at pH >2 (Erah et al., 1997) and hence was expected to overcome the deficiency experienced with erythromycin.

The virtual superposition of the iv PK curves given as microdose and tracer indicates that the presence of the amount absorbed systemically (approximately 100 mg) following the oral 250 mg therapeutic dose does not affect the disposition kinetics of clarithromycin, including hepatic clearance. Also, the resultant PK parameters of 136 L and 22 L/h compare reasonably closely with reported mean literature values of 180 L and 31 L/h, for V and CL, respectively (Table 3). The $t_{½}$ in the current study, 3.4–4.5 h irrespective of the dosing modality, is somewhat longer than reported, 2.8 ± 0.6 h (Davey, 1991).

While within the two-fold limitation for acceptance of prediction, the higher oral bioavailability for the 250 mg versus the microdose (39% vs. 22%) signifies some saturation during absorption at the therapeutic dose, which is supported by a still higher value (55%) for a 500 mg oral dose (Fraschini et al., 1993). Apart from the lack of change in hepatic clearance with dose, evidence in favour of gut wall rather than the liver being the major site of loss and saturation of oral bioavailability is as follows. With a renal clearance of 7.7 L/h (corresponding to a fraction excreted unchanged, f_e, of 0.35) and a blood-to-plasma ration of 0.63 (Ferrero et al., 1990), the non-renal blood clearance of clarithromycin is 22.7 L/h ((22–7.7 L/h)/0.63). Assuming this is due to hepatic elimination, and given that hepatic blood flow is 90 L/h, yields an estimated hepatic extraction ratio (E_H) of 0.25 and a corresponding expected oral bioavailability of 75%, if all loss had been due to hepatic extraction. This contrasts with the observed oral bioavailability of 22% for the microdose, signifying that some 70% of the administered dose fails to reach the portal blood intact. Lack of solubility can be discounted for the microdose, and while some of the loss may be due to poor passive intestinal permeability it is more likely to be due a combination of moderate passive permeability and high PgP efflux (Varma and Panchagnula, 2005). Although calculations based on radioactivity data should be viewed with some caution, they do suggest that some loss in bioavailability is also due to gut wall metabolism. Had loss of bioavailability been solely due to intestinal efflux the expected ratio of AUC of radioactivity in plasma associated with metabolites (difference between total and unchanged compound) to that of parent drug following oral administration $[AUC(m)/AUC(d)]_{oral}$ would be $[1 + E_H/((1 - f_e)(1 - E_H))] \times [AUC(m)/AUC(d)]_{iv}$ (Pang and Durk, 2010) or 1.51 × $(AUC(m)/AUC(d)_{iv}$. Whereas, experimentally, with $(AUC(m)/AUC(d)_{oral} = 2.3$, and $AUC(m)/AUC(d)_{iv} = 0.52$, the observed ratio is 4.4, implying additional formation of metabolites in the gut. Exact quantitative conclusions as to the relative contributions of gut wall metabolism and poor intestinal permeability/efflux to loss of bioavailability cannot be made, not least because some formed gut wall metabolites may be preferentially effluxed into the intestine and eliminated in faeces rather than entering portal blood.

The source of the increase in oral bioavailability between the microdose and therapeutic dose is unclear, although it is likely to be due to saturation in either CYP3A metabolism or P–gP efflux in the gut wall. A decrease in hepatic extraction associated with CYP3A metabolism is unlikely to explain the rise from 22% to 39%. Even if hepatic metabolism was totally saturated, and extraction ratio was reduced from 0.25 to zero (and there is no such evidence given that the PK profiles for iv microdose and tracer dose in the presence of the therapeutic dose are essentially superimposable), bioavailability would only increase from 22% to 29%.

Previous microdose studies have been performed with midazolam (Lappin et al., 2006a,b), a probe substrate for cytochrome 3A4, and fexofenadine (Yamane et al., 2007; Lappin et al., 2010), a probe substrate for P–gP, and there was a high concordance between the oral microdose and therapeutic dose PK in both cases. This confirms that nonlinearity is a function of dose relative to the saturation kinetics of a compound and that each compound should be considered on a case-by-case basis.

4.2. Sumatriptan

Sumatriptan is primarily metabolized by monoamine oxidase-A, a mitochondrial enzyme, with little evidence of cytochrome P450 involvement (Dixon et al., 1994). In contrast to CYP450 little is currently known about the conditions of the *in vitro* systems of monoamine oxidase (stability of enzyme, intersystem scaling factors) needed to ensure robust and accurate scaling to predict human CL or degree of first-pass loss of orally administered compound.

The similarity in the PK of the iv microdose and iv tracer indicates that the disposition kinetics is not affected by the presence of systemic drug associated with the oral therapeutic dose. Moreover, the mean values of V and CL (270 L and 50 L/h) compare favourably with mean literature values of 170 L and 70 L/h, respectively (Lacey et al., 1995). Whether the difference in V is real is open to question. The range of previously reported values is relatively wide at between 90 and 320 L (Scott, 1994). The estimate is heavily dependent on the reported terminal $t_{½}$, which varies between 1 and 8 h (Lacey et al., 1995), with the longer quoted values tending to be in studies where observations were continued for longer times. The estimated $t_{½}$ for the iv administrations in the current study was consistently towards the upper value, at approximately 6 h. Following the oral microdose (100 μg) and therapeutic dose (50 mg) the terminal $t_{½}$ is similar, 1.9 and 1.4 h, respectively, which was close to the 1 h value generally reported for oral administration (Table 3). As mentioned above, the difference in estimates between oral and iv administration lies in the duration of quantifiable measurements.

The cause of the low oral bioavailability of sumatriptan is unclear, although as shown below that unlike clarithromycin, a substantial component is hepatic extraction. Sumatriptan is not known to be a substrate for P-gP, and in part the low bioavailability may be due to poor inherent intestinal permeability associated with its effective low lipophilicity (log $D_{7.4}$ – 1.5), with bioavailability (and appearance in plasma of metabolite) decreasing along the intestine from jejenum to cecum (Warner et al., 1995). Adopting the same approach as with clarithromycin, the AUC ratio of metabolite-to-drug suggests that while some of the loss of bioavailability is due to hepatic extraction, evidence of some gut wall metabolism also exists. Thus, assuming a blood-to-plasma ratio of 1 (in the absence of any literature reports) and given that $f_e = 0.22$ (Lacey et al., 1995), the hepatic blood clearance of sumatriptan, and hence extraction ratio, is 39 L/h and 0.43, respectively, yielding an expected oral bioavailability due to first pass hepatic loss of 57% compared with an observed value of 20% for the oral microdose. Based on these data, $[AUC(m)/AUC(d)]_{oral}$ should be 1.96 $(1 + 0.43/((1 - 0.22)(1 - 0.43))$ times $[AUC(m)/AUC(d)]_{iv}$, whereas the observed ratio is much larger, 3.7, given that the $[AUC(m)/AUC(d)]_{oral} = 4.88$ and $[AUC(m)/AUC(d)]_{iv} = 1.33$. Further support for some gut wall metabolism is the declining ratio of plasma AUC of metabolite to parent drug on placement of sumatriptan on increasingly lower parts of the intestinal tract (Warner et al., 1995). If formation of metabolite were only post intestinal absorption this AUC ratio would be expected to be constant, independent as where absorption occurs.

However, the higher bioavailability following the oral microdose (20%) than 50 mg therapeutic dose (7.6%), slightly outside the two-fold limit, was unexpected, and is the first case reported with microdosing, although neither value was entirely inconsistent with that reported in the literature of 14% (Scott, 1994). A possible reason is saturation of an uptake transporter, although there is no

evidence to support such an uptake mechanism let alone it being saturated.

4.3. Propafenone

This drug was chosen to represent compounds known to exhibit dose dependent pharmacokinetics over the therapeutic dose range, in this case due to first pass saturation of CYP2D6 metabolism (Hollmann et al., 1983). The ultimate aim is to combine microdose data and *in vitro* data characterizing the saturable process to predict the nonlinear pharmacokinetics observed at therapeutic doses. Reported here are the microdose data.

As with the other compounds studied the virtual superimposition of the iv curves following a microdose and a tracer dose superimposed on an oral therapeutic dose indicates that overall the disposition kinetics of propafenone is linear over the range of systemic exposure. In addition, the estimated CL, V, V_{ss} and $t_{½}$ of 45L/h, 214–273 L, 209–272 L and 5 h, respectively, compare closely to reported values of 61 L/h, 248 L and 3.0 h (Hollmann et al., 1983).

Oral bioavailability was observed to rise modestly from 5.8% to 13% on increasing the dose from 100 μg to 150 mg, a 1500-fold increase in dose. This finding of a dose dependency is in agreement with the literature, in that oral bioavailability of a 150 mg oral dose was previously reported as approximately 10%, rising further to around 50% at doses of 450 mg (Hollmann et al., 1983). Further supporting first pass metabolic loss as the cause of the low oral bioavailability is the much lower parent drug to total radioactivity plasma concentration and AUC ratios after oral than iv dosing following microdose administration, with for example little difference between the two concentrations within the first sample (0.5 h) after the iv dose compared to a 40-fold difference after oral administration (Fig. 3b).

Adopting the same approach as with clarithromycin, the AUC ratio of metabolite-to-drug raises the possibility that while hepatic extraction contributes significantly to loss of bioavailability, the suggestion of some gut wall metabolism also exists. Thus, given that the blood-to-plasma ratio of propafenone is 0.70 (Komura and Iwaki, 2005) and that f_e is approximately zero, with no effect of renal failure on the clearance of propafenone (Burgess et al., 1989) k its hepatic blood clearance, and hence extraction ratio, is 64 L/h and 0.71, respectively, yielding an expected oral bioavailability due to first pass hepatic loss of 29% compared with an observed value of 5.8% for the oral microdose. Based on these data $[AUC(m)/AUC(d)]_{oral}$ should be 3.4 $(1 + 0.71/(1 - 0.71)$ times that of $[AUC(m)/AUC(d)]_{iv}$, whereas the observed ratio, 27.2, (given that $[AUC(m)/AUC(d)]_{oral} = 37.5$ and $[AUC(m)/AUC(d)]_{iv} = 1.38$) is very much larger. And, although a larger predicted AUCs ratio than 3.4 would result by say using a lower hepatic blood than 90 L/h, it is difficult to see the ratio approaching the value of 27, implying some gut wall metabolism occurs, presumably by CYP2D6 there (Paine et al., 2006). Again, as with clarithromycin and sumatriptan, it is difficult to quantify how much of the observed dose dependency in oral bioavailability is due to saturation of gut wall or liver metabolism. For both propafenone and sumatriptan, hepatic blood clearance is higher and approach blood flow and if saturation due absorption does occur during absorption, owing to high portal concentration, while it will affect bioavailability is anticipated to have minimal effect on clearance.

4.4. Paracetamol

Paracetamol represents a drug that undergoes extensive sulphate and glucuronide conjugation and is given in large doses. Although there are some reports of reasonably good prediction of clearance of such compounds based on *in vitro* metabolic data (Miners et al., 2006; Cubitt et al., 2011; Halifax et al., 2010) the number are currently too limit to be confident yet with this methodology.

Comparison of the oral microdose data (Table 2), suitably scaled, and literature therapeutic dose data indicates that the microdose predicts well the therapeutic dose PK despite the 14,000-fold range of doses. This result was consistent with a recent microdose study conducted with paracetamol in Japanese subjects (Tozuka et al., 2010). Thus, the mean value for C_{max} for the 100 μg oral microdose was 1.1 ng/mL which if scaled proportionately to a 1400 mg dose, would be 15.4 μg/mL, which is close to that reported of 20 μg/mL for this albeit high therapeutic dose (Forrest et al., 1982). The half-life observed in the 100 μg dose study of 4.6 h (iv) and 5.8 h (oral) compares reasonably well with reported values of 2–4 h following therapeutic doses (Table 3). Microdose CL (19 L/h), signifying a moderate extraction ratio, is also very close to the reported therapeutic dose value (21 L/h), but the somewhat longer $t_{½}$ observed in the microdose study reflects the larger estimated volume of distribution of 123 L, compared to 66.5 L previously reported (Forrest et al., 1982), although still within a factor of two. The shapes of the plasma-concentration time plots for the oral and iv doses (Figs. 1a and 1b) were also in general agreement with those seen at therapeutic doses (Crighton et al., 1998). In addition, the oral bioavailability gained from the microdose data of 88% is virtually identical to that generally accepted (Forrest et al., 1982; Brunton et al., 2006). The predicted value is somewhat lower. Thus, although not specifically reported, with minimal plasma binding and an equal distribution between blood cells and plasma water (Pang and Terrell, 1981) the anticipated blood-to-plasma ratio is close to 1. Accordingly, the hepatic blood clearance of paracetamol is 19 L/h for this extensively metabolised compound, that is minimally renally excreted unchanged (Morris and Levy, 1984) which yields a predicted oral bioavailability due to hepatic extraction of 79%, approximately 10% lower than observed.

4.5. Phenobarbital

Phenobarbital was chosen as representative of compound with very high metabolic stability such that *in vitro* metabolic intrinsic clearance, needed in the prediction of *in vivo* CL, cannot be estimated with any confidence. Overall, the microdose scales well to the therapeutic dose implying linear PK over the dose range. Thus, the mean phenobarbital C_{max} for the microdose study was 2.6 ng/mL, which if scaled to a 240-mg therapeutic dose (a factor of 2400) would result in a plasma concentration of 2.4 μg/mL, which compares well with the reported 5.5 μg/mL (Nelson et al., 1982). Moreover, assuming $F = 1$ for the oral microdose, as found for the therapeutic dose (Nelson et al., 1982), then CL (dose/AUC) = 4.6 mL/min, which compares very closely to the reported value, 4.3 mL/min (Nelson et al., 1982). Finally, the $t_{½}$ of 108 h (Figs. 2a and 2b and Table 2), is consistent with the literature (Table 3), as is the shape of the curve.

4.6. Overall conclusions

Because iv data allow characterization of the disposition kinetics of a compound and defines its behaviour in the body once absorbed systemically, it can be invaluable in decision making during future development of the compound for separating disposition from absorption issues. For those drugs administered intravenously (clarithromycin, sumatriptan, propafenone and paracetamol) the PK of the microdose agreed very well with that observed with the tracer dose superimposed on systemic drug concentrations associated with an oral therapeutic dose, and with the literature, inferring that the disposition PK of these compounds is linear and hence independent of the plasma concentration over the range of the oral therapeutic doses studied. This supports sim-

ilar findings with the additional 18 compounds available in the public domain to date (Lappin and Garner, 2008). An exception is that of warfarin for which discrepancies in the shape and terminal half-life between oral microdose and therapeutic doses was observed (Lappin et al., 2006a,b). This behaviour of warfarin is typical of compounds displaying target mediated disposition kinetics, which share in common saturation of a high-affinity, low capacity tissue binding site at therapeutic doses that is manifest in the plasma profile owing to the small volume of distribution of such compounds (Mager, 2006).

Therapeutic non-labelled doses were administered in solution as per the oral microdoses to avoid differences in dissolution from solid forms which might limit the rate of absorption. The oral microdose predicts reasonably well the bioavailability after an oral therapeutic dose and even for the exceptions, such as sumatriptan and propafenone, the values were only marginally outside the 2-fold criterion set for accuracy of prediction. Whether such deviations would have resulted in an inappropriate decision to take a compound forward into full Phase I assessment will vary with the compound. Interestingly, of the 26 drugs so far examined at a microdose (Lappin, 2010), with the exception of warfarin (Lappin et al., 2006a,b), any nonlinearity in PK has been due to saturation of one or more processes during systemic absorption. Here, having both parent drug and metabolite data (made possible in the current study using ^{14}C-labelled compound) allows further insight into the events occurring during absorption, which for clarithromycin, sumatriptan and propafenone implies a significant contribution of gut wall to loss in oral bioavailability and which for clarithromycin may be the predominant site of saturation rather than the liver. And, given the general lack of difference between the iv kinetics of the microdose and tracer dose given with the oral therapeutic dose, this may also be so for other drugs showing an increasing dose-dependent oral bioavailability due to first pass loss, although it is not possible to completely separate quantitatively gut wall from hepatic events from such data alone.

The drugs exemplified in the current report have been marketed for some years and therefore the doses are relatively high compared to many, although by no means all, modern drugs coming through the development pipeline, which tend to exhibit greater potency than in the past and therefore are often administered at lower doses where enzyme and transporter saturation may be less of an issue, in which case a microdose would be expected to predict therapeutic dose PK well.

5. Conflict of interest statement

Dr. Lappin is an employee of Xceleron and together with Professor Garner holds stock in the Company. Dr. Oosterhuis is an employee of PRA International. Dr. Jochemsen is an employee of Laboratoires Servier. Professor Emeritus Rowland is the chair of the Scientific Advisory Board of Xceleron, but holds no stock in the company.

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