Comparative human pharmacokinetics and metabolism of single-dose oral and intravenous sildenafil citrate

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Aims To characterize the absorption, metabolism and excretion of an oral and intravenous (IV) dose of radiolabelled [^{14}C]-sildenafil citrate in healthy male subjects. Specific objectives were to measure the cumulative amount of drug-related radiolabelled material excreted in the urine and faeces, to characterize urinary and faecal radioactivity as unchanged sildenafil or its metabolites, and to quantify blood and plasma total radioactivity and unchanged drug concentrations.

Methods Six healthy male subjects between the ages of 45 and 58 years were enrolled in an open-label, parallel-group study; three subjects received the oral dose and three received the IV dose. Oral drug was administered as a single dose of 50-mg [^{14}C]-sildenafil, and IV drug was administered as a single dose of 25-mg [^{14}C]-sildenafil infused over 25 min. Each dosage form contained 50 μCi of radioactivity. For radioactivity assays, whole blood, plasma, urine and faeces samples were taken predose and at specified intervals up to 5 days postdose. Plasma samples were assayed for sildenafil and the metabolites UK-103,320 and UK-150,564. Metabolite profiling was also performed in plasma, faeces and urine.

Results Absorption of sildenafil after oral administration was rapid and approximately 92% whilst the absolute bioavailability was limited to 38%, due to first-pass metabolism. Mean AUC_t values showed that sildenafil accounted for about 60% of the total circulating radioactivity in the plasma after IV administration and for 32% after oral administration. Concentrations of radioactivity in whole blood were lower than in plasma, indicating limited penetration of sildenafil into blood cells. No unchanged sildenafil was detected in either urine or faeces, demonstrating that metabolism was the major mechanism of drug clearance. The principal routes of metabolism were *N*-demethylation, oxidation and aliphatic dehydroxylation. Sildenafil was well tolerated, with treatment-related adverse events reported by three subjects. Two of these were mild, and there was one case of moderate leg pain.

Conclusions The pharmacokinetics of radiolabelled [^{14}C]-sildenafil were consistent with rapid absorption, first-pass metabolism and primarily faecal elimination of *N*-demethylated metabolites.

Keywords: sildenafil, UK-103,320, UK-150,564, pharmacokinetics, safety

Introduction

Erectile dysfunction (ED) may be caused by impaired relaxation of the smooth muscle of the corpus cavernosum, which is mediated by nitric oxide (NO) stimulation of cyclic guanosine monophosphate (cGMP) [1, 2]. Sildenafil citrate (Viagra®, Pfizer) is a selective inhibitor of cGMP-specific phosphodiesterase [3, 4], and is therefore expected to inhibit the degradation of cGMP without affecting cyclic adenosine monophosphate (cAMP). Sildenafil is an orally administered effective treatment for ED [3, 5]. It enhances the action of the endogenous NO–cGMP pathway, mediating the sequential elevation of cGMP levels, corpus cavernosum vasodilation, increased blood flow and enhanced erectile function [1, 2].

This open-label, parallel-group study was conducted to determine the absorption, metabolism and excretion

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characteristics of a single oral and a single intravenous (IV) dose of radiolabelled [^{14}C]-sildenafil in healthy male subjects. Radiolabelled drug was used due to its ease of detection and the qualitative and quantitative precision it affords in the investigation of absorption and disposition patterns of new drugs under development [6]. The study objectives included: (1) measurement of the cumulative amount of drug-related, radiolabelled material excreted in the urine and faeces; (2) characterization of urinary and faecal radioactivity as unchanged sildenafil or its metabolites; and (3) quantification of blood and plasma total radioactivity and unchanged drug concentrations, as well as those of the major circulating metabolites. Preliminary results from this study have been reported previously in comparison to animal pharmacokinetic and metabolism data for sildenafil [7]. The current study also included assessments of safety and tolerability.

Methods

Subjects and study design

Healthy men between the ages of 45 and 60 years were eligible for inclusion in the study, provided that they weighed between 60 and 90 kg, were within 15% of the permitted range for height and frame size and had regular excretory habits. They were excluded if they showed evidence of any clinically significant disease; a history of asthma or other allergic condition; previous hypersensitivity to any drug; or any clinically significant abnormality in laboratory test or physical examination findings. Subjects were also excluded if there was any evidence of drug or alcohol abuse; intention to donate blood prior to or following completion of the study; excessive tobacco use; or any condition likely to influence absorption of the drug.

Subjects who were judged by the investigator as unlikely to complete the study were excluded, as were those who had had radiation exposure of over 30 mSv from a single dose or 50 mSv from multiple doses during the previous year, and those who had been exposed to any radiation during work or for diagnostic reasons (except dental X-rays) during the previous year. The subjects gave their informed written consent, and the trial was reviewed and approved by the local ethics committee.

Before participating in the study, subjects underwent a full medical examination, including measurements of blood pressure and pulse rate, a 12-lead electrocardiogram (ECG) and a range of laboratory safety tests. Subjects remained at the study centre for a minimum of 5 full days or until radioactivity levels in the urine and faeces had fallen to less than three times the baseline level.

The oral drug was supplied as powder for reconstitution with 100 ml of sterile water and administered as a single dose of 50-mg [^{14}C]-sildenafil, followed by 140 ml of water. The IV drug was supplied as a 1-mg ml^{-1} solution and administered as a single dose of 25 mg infused over 25 min. This 25-mg IV dose was selected to provide similar plasma drug concentrations to the oral dose. Each dosage form contained 50 μCi.

Pharmacokinetic and radioactivity assessments

For radioactivity assays, whole blood, plasma, urine and faecal samples were obtained predose and at specified intervals up to 5 days postdose. For both dosage forms, 16 ml blood samples were collected into heparinized tubes. After oral administration, postdose blood samples were collected at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48 and 72 h. After IV administration, postdose blood samples were taken at 5, 10 and 25 min (the end of the infusion), and at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48 and 72 h after the start of the infusion. Subsequent samples were taken at 24-h intervals until the subject left the study centre. Additional blood volumes of 50 ml were collected at 1 and 8 h postdose for metabolite profiling.

Blood samples were prepared and aliquoted for radioactive counting, and for assays of sildenafil and the metabolites UK-103,320 and UK-150,564. The plasma assays were performed using an automated sequential trace enrichment of dialysates (ASTED) method followed by high performance liquid chromatography (HPLC) with ultraviolet detection [8]. For all three analytes, the calibration range was 1–250 ng ml^{-1}. The limits of quantification were 1 ng ml^{-1} for sildenafil, UK-103,320 and UK-150,564. The overall imprecision (CV) was $\leq$5.0% for all three analytes at concentrations of 3.0, 125 and 200 ng ml^{-1}. The inaccuracy (bias) of the assay at all concentrations ranged from −7% to 5% for all three analytes. Metabolic characterization was performed by the comparison of retention times on isocratic and gradient HPLC systems with authentic standards. To confirm metabolite identities, or to identify components for which no reference standard was available, mass spectral analysis was performed. The radioactive components were analysed by direct infusion (5 μl min^{-1} nebulizer gas pressure 40 psi) into an API III plus mass spectrometer (Perkin Elmer Sciex, Toronto, Canada) fitted with an IonSpray interface utilized in positive ion mode [7].

Urine samples were collected at 0–4, 4–8, 8–12, 12–24, 24–36, 48–72 and 72–96 h postdose and subsequently at 24-h intervals. The total volume for each collection period was measured and a 50-ml aliquot stored at −20° C until radioactive counting. All faeces were collected in the 24 h prior to dosing and at 24-h intervals after dosing. The samples were weighed and then stored at −20° C until analysis.

The following pharmacokinetic parameters were calculated for sildenafil, UK-103,320 and UK-150,564 by noncompartmental analysis from the individual concentration-time curves after oral and IV administration using WinNonlin™ Version 1.1 (Pharsight Corporation): the maximum observed plasma concentration (C_{max}); the time to achieve C_{max} (T_{max}); the apparent terminal elimination phase rate constant (k_{el}); the mean terminal half-life ($t_{1/2}$); and the area under the plasma concentration-time curve from time 0 extrapolated to infinity (AUC). Oral bioavailability (F) was calculated for sildenafil from the mean data as: $F = AUC_{oral}/dose_{oral}/AUC\ IV/dose\ IV$. C_{max}, T_{max} and area under the plasma concentration-time curve from time 0 to the time of the last measurable value (AUC_t) were calculated for blood and plasma radioactivity. Oral absorption was calculated using the formula: mean (urinary recovery/urinary + faecal recovery)$_{oral}$/mean (urinary recovery/urinary + faecal recovery)$_{IV}$.

Safety and tolerability assessments

Observed or volunteered adverse events that occurred throughout the study were assessed by the investigator for severity and recorded. Physical examination, laboratory safety tests and a 12-lead ECG were performed and supine blood pressure and pulse rate were measured predose, at 24 h postdose and at a follow-up assessment 2 weeks after dosing.

Statistical evaluation

A sample size of three subjects per treatment group was selected to conform to general requirements for radiolabel studies. No formal hypothesis testing was performed and statistics were descriptive only, as formal sample size calculation was not appropriate for this study.

Results

Subjects

Six men between the ages of 45 and 58 years old enrolled in and completed the study; three received the oral dose and three received the IV dose. All six subjects provided pharmacokinetic data and were assessed for safety. Their weights at baseline ranged from 63 to 79 kg, and none had medical histories or physical examination findings that influenced the results of the trial.

Radioactivity profiles

Concentrations of radioactivity in whole blood were lower than those in plasma, with the greatest difference following oral dosing (Table 1). Following IV administration, maximum plasma concentrations of total radioactivity occurred at the end of the 25-min infusion in two subjects, and 20 min after the end of the infusion in the other subject (Figure 1a). Radioactivity in whole blood followed the same pattern. Following oral administration, maximum concentrations of total radioactivity occurred within 1.5 h of dosing in plasma (Figure 1b) and within 1 h in whole blood in all subjects. Among the subjects receiving sildenafil IV, the geometric mean C_{max} and AUC_t ratios of total radioactivity in plasma to whole blood were 1.64 and 1.58, respectively. The respective ratios for those receiving sildenafil orally were 2.12 and 2.35.

The mean total recovery of excreted radioactivity during the sample collection period of 144 h was 88.5% after IV administration and 91.3% after oral administration (Table 2). The majority of radioactivity in the urine was excreted within the first 12 h after IV administration, and the majority of radioactivity in the faeces was excreted by 96 h after the start of the infusion. After oral administration, the majority of radioactivity in the urine was excreted within 12 h, and the majority of radioactivity in the faeces was excreted within 72 h postdose (Figure 2). Absorption of sildenafil after oral administration was calculated to be approximately 92%.

Pharmacokinetics

Plasma concentration profiles for sildenafil, UK-103,320 and UK-150,564 are shown in Figure 3(a–d). After IV administration, sildenafil C_{max} occurred at the end of the 25-min infusion in all subjects. Plasma concentrations

Table 1 Pharmacokinetic parameters for radioactivity in plasma and whole blood.

	Sildenafil 25 mg, IV		*Sildenafil 50 mg, oral*	
Parameter	*Plasma*	*Whole blood*	*Plasma*	*Whole blood*
C_{max} (ng ml^{-1})				
Subject 1	506	321	780	298
Subject 2	606	352	538	305
Subject 3	574	354	540	263
Geometric mean	560	342	610	288
AUC_t (ng h ml^{-1})				
Subject 1	1563	946	2720	1122
Subject 2	1680	889	1520	70
Subject 3	1570	1233	2783	1127
Geometric mean	1603	1012	2258	960
T_{max} (h)				
Subject 1	0.75	0.50	0.98	0.98
Subject 2	0.42	0.42	0.93	0.93
Subject 3	0.42	0.42	1.50	1.00
Arithmetic mean	0.53	0.45	1.14	0.97

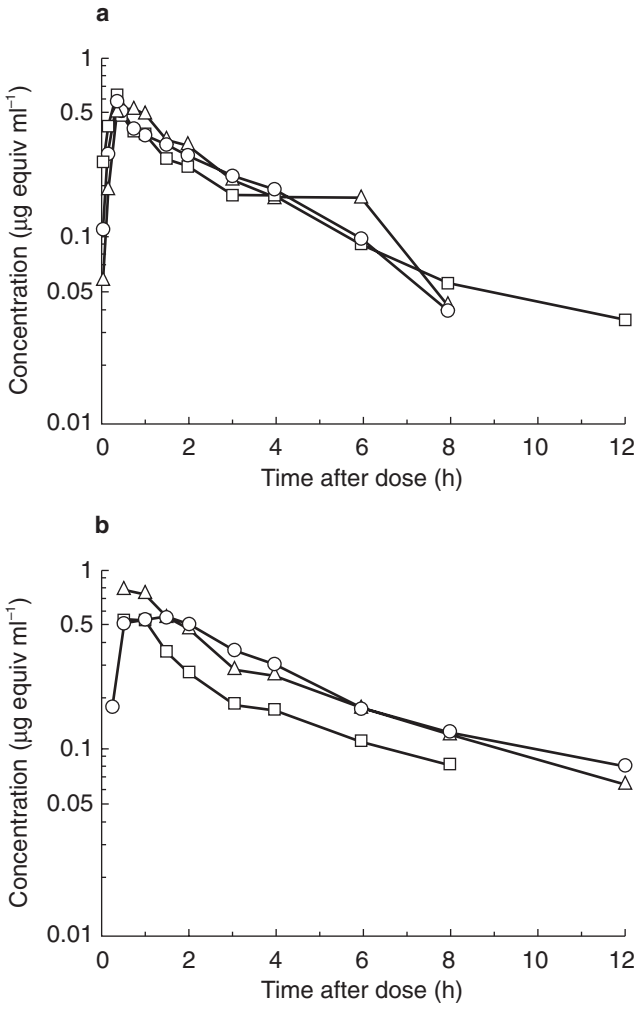

Figure 1 Individual subject profiles of total plasma radioactivity over time following a single-dose 25-mg IV (a) or 50-mg oral (b) dose of [^{14}C]-sildenafil. Subject 1 (△); Subject 2 (□); Subject 3 (○).

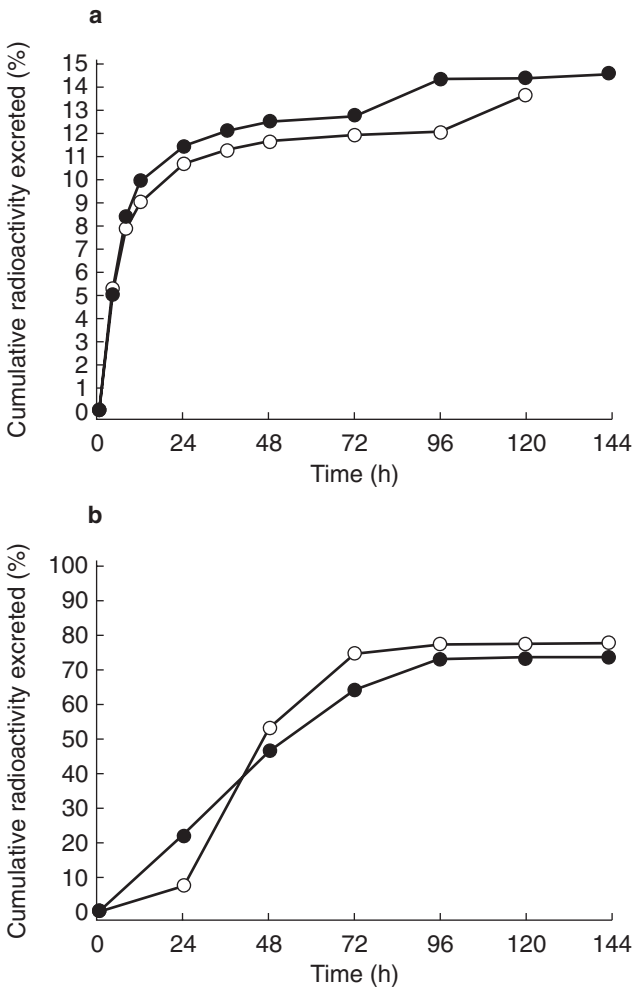

Figure 2 Mean cumulative radioactivity excreted following a single 25-mg IV (●) or 50-mg oral dose (○) of [^{14}C]-sildenafil in urine (a) and faeces (b).

Table 2 Excretion of radioactivity expressed as percentage of administered radioactivity.

	Excretion (%)	
Route	*Urinary*	*Faecal*
IV, 25 mg		
Subject 1	9.97	75.28
Subject 2	14.37	75.52
Subject 3	14.82	75.56
Arithmetic mean	13.05	75.45
Oral, 50 mg		
Subject 1	14.34	84.53
Subject 2	8.56	82.76
Subject 3	13.93	69.78
Arithmetic mean	12.28	79.03

declined in a biphasic manner with a harmonic mean terminal $t_{1/2}$ of 2.2 h (Table 3). After oral administration, plasma concentrations peaked within 1.5 h, then declined in an approximately biphasic manner with a harmonic mean terminal $t_{1/2}$ of 3.2 h (Table 4).

The geometric mean C_{max} and AUC_t ratios of plasma sildenafil to plasma total radioactivity were 0.93 and 0.60, respectively, following IV administration and 0.34 and 0.32, respectively, following oral administration. The mean AUC_t ratios indicate that sildenafil accounted for about 60% of the total circulating radioactivity in the plasma after IV administration and for 32% after oral administration. Comparison of the geometric mean AUC values between the two routes of administration gave an absolute bioavailability (F) value of 38%. The concentration-time profiles of UK-103,320 tended to parallel that of the parent drug after both IV and oral administration (Figure 3). The concentration-time profiles of UK-150,564 were similar in shape to

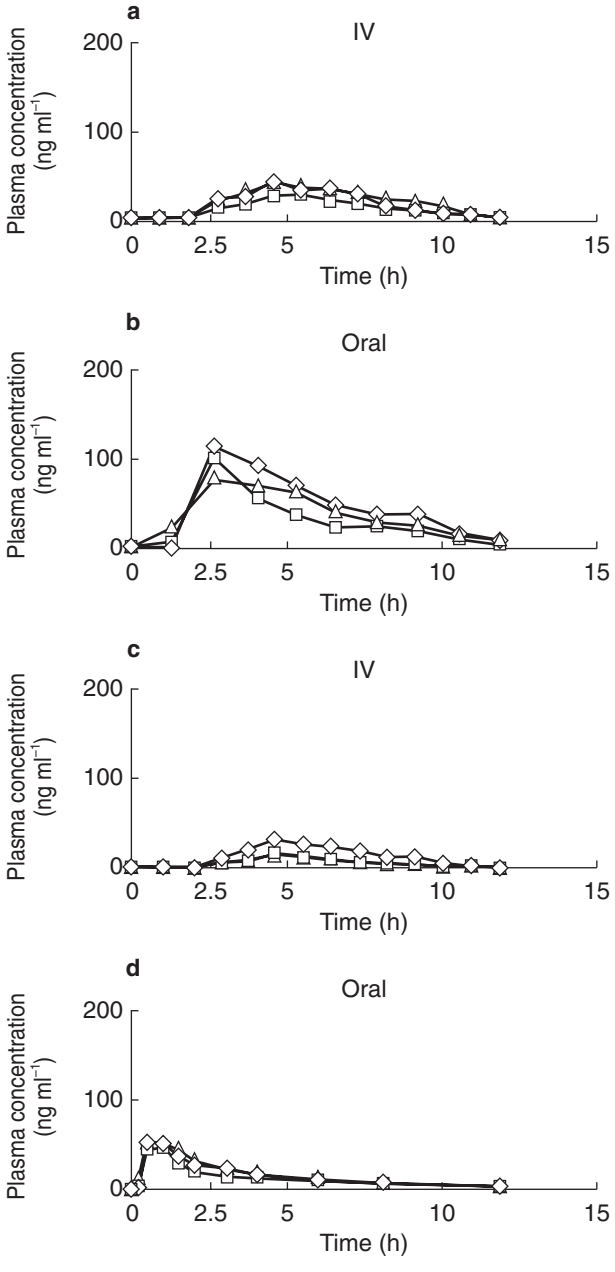

Figure 3 Individual subject profiles of UK-103,320 and UK 150,564 plasma concentrations over time following a single 25-mg IV (a,c) or 50-mg oral (b,d) dose of [^{14}C]-sildenafil. Subject 1 (◇); Subject 2 (□); Subject 3 (△).

those of the parent drug and UK-103,320, except at time points more than 8 h after administration, when a longer terminal phase became apparent (Figure 3). In both the IV and oral treatment groups, the maximum plasma concentrations of UK-150,564 occurred at approximately the same time as those of UK-103,320 (Tables 3 and 4).

The geometric mean AUC_t value for UK-103,320 was 14.4% of the equivalent parameter for sildenafil, 8.7% of that for the total circulating radioactivity following IV administration, 54% of that for sildenafil and 17% of that for total circulating radioactivity following oral administration. The geometric mean AUC_t

Table 3 Individual and mean plasma pharmacokinetics of sildenafil, UK-103,320 and UK-150,564 following IV administration of 25-mg sildenafil.

Parameter	*Sildenafil*	*UK-103,320*	*UK-150,564*
C_{max} (ng ml^{-1})			
Subject 1	455	41	31
Subject 2	565	32	15
Subject 3	540	43	15
Geometric Mean	518	38	19
AUC (ng h ml^{-1})			
Subject 1	928	119	124
Subject 2	1051	136	76
Subject 3	939	198	67
Geometric Mean	971	147	86
T_{max} (h)			
Subject 1	0.42	0.75	0.75
Subject 2	0.42	1.00	0.75
Subject 3	0.42	0.75	1.00
Arithmetic Mean	0.42	0.83	0.83
$t_{1/2}$ (h)			
Subject 1	1.69	1.97	7.24
Subject 2	3.61	2.86	7.32
Subject 3	1.96	2.18	3.59
Harmonic Mean	2.18	2.28	5.42

Table 4 Individual and mean plasma pharmacokinetics of sildenafil, UK-103,320 and UK-150,564 following oral administration of 50-mg sildenafil. (Estimates of C_{max} and T_{max} were adversely affected by mis-sampling at 0.25 and 0.5 h).

Parameter	*Sildenafil*	*UK-103,320*	*UK-150,564*
C_{max} (ng ml^{-1})			
Subject 1	278	116	50
Subject 2	164	106	50
Subject 3	195	83	47
Geometric Mean	207	101	49
AUC (ng h ml^{-1})			
Subject 1	1020	515	231
Subject 2	435	309	149
Subject 3	874	402	223
Geometric Mean	729	400	197
T_{max} (h)			
Subject 1	1.00	1.00	1.00
Subject 2	1.00	1.00	0.93
Subject 3	1.5	1.00	1.00
Arithmetic Mean	1.17	1.00	0.98
$t_{1/2}$ (h)			
Subject 1	4.52	5.11	12.80
Subject 2	2.02	7.81	4.77
Subject 3	4.45	4.55	7.06
Harmonic Mean	3.19	5.52	6.99

value for UK-150,564 was 7.9% of the equivalent parameter for sildenafil, 4.7% of that for the total circulating radioactivity following IV administration, 25% of that for sildenafil and 8% of that for total radioactivity following oral administration.

Metabolite profiles

Profiling of pooled 1-h plasma samples (oral and IV) revealed sildenafil as the major circulating radioactive component (68% of IV radioactivity and 47% of oral radioactivity). The next most slowly eluted compound from the HPLC gradient system was identified as UK-103,320 (9% of IV and 19% of oral radioactivity). UK-150,564 (7% of IV and 13% of oral radioactivity) and two other faster eluting components represented the majority of the remaining radioactivity in plasma.

The metabolite profiles of the radioactivity in the excreta were qualitatively similar for both routes of administration. The profile of radioactive components in urine revealed one major component (41% of urinary radioactivity; 5% of dosed radioactivity) an aliphatic hydroxylated metabolite. The urine contained a further eight radioactive components, each accounting for less than 1.5% of the dose. No unchanged drug was detected in urine. The profile of radioactive components in the faeces indicated one major component (28% of faecal radioactivity; 22% of dose) identified as UK-150,564. Sixteen other metabolites were identified, each accounting for $<5\%$ of the oral dose. There was no unchanged drug excreted in the faeces. Based on the profiles above, the routes of metabolism for sildenafil were proposed as shown in Figure 4.

Safety and tolerability

The study drug was well tolerated. Three subjects reported adverse events considered treatment related: one had mild nausea following IV sildenafil; one had mild penile erection for 20 min during IV infusion of sildenafil; and one had moderate leg pain 15 h after receiving oral sildenafil. There was one serious adverse event (bladder cancer), but this was not considered drug related. No clinically significant abnormalities were noted in laboratory tests or ECG parameters. None of the subjects discontinued treatment.

Discussion

In this open-label, parallel-group study, sildenafil pharmacokinetics, metabolic profile and rates and routes of excretion were characterized following the administration of single 50-mg oral doses of [^{14}C]-sildenafil and single 25-mg IV doses of [^{14}C]-sildenafil to healthy male volunteers. The use of radiolabelled oral and IV dosage forms is invaluable in the assessment of absorption (rate and extent), absolute oral bioavailability and first-pass effects [6]. Following oral administration, sildenafil was rapidly absorbed as judged by the occurrence in all subjects of maximum plasma concentrations of total radioactivity within 1.5 h. This observation concurs with a previous report of a mean T_{max} of approximately 1 h in fasted subjects, with a slight increase in T_{max} (by 0.7 h) when the sildenafil dose was taken with food [9]. Absorption of sildenafil after oral administration was approximately 92%, as would be predicted by its desirable physicochemical properties [10] and the high absorption reported in animal species [7]. The oral absolute bioavailability was found to be 38%, similar to that determined in a previous single dose pharmacokinetic trial which reported an absolute oral bioavailability value of 41% following administration of 50-mg oral doses of unlabelled sildenafil [11]. These data confirm that the oral bioavailability is determined by the first-pass metabolism of the drug, rather than incomplete absorption across the intestinal wall. The early peak of the sildenafil circulating metabolites, UK-103,320 and UK-150,564 ($T_{max} \leq 1$ h) after both oral and IV dosing confirms rapid first-pass and systemic metabolism of the parent drug. Further evidence of early biotransformation of parent drug by first-pass metabolism as well as subsequent metabolism is a lower value for the geometric mean AUC_t ratio of plasma sildenafil to plasma total radioactivity following oral administration (0.32) compared with IV administration (0.60), indicating an increase in circulating metabolites after oral administration compared with IV. However, sildenafil is the primary component circulating in plasma and this, together with the relative pharmacological potencies of the drug and metabolites [4], indicate that it is responsible for the pharmacological activity observed.

Lower concentrations of radioactivity were measured in whole blood compared to plasma, with the greatest difference observed after oral administration. After oral administration, C_{max} and AUC_t of total radioactivity in plasma was more than double that in whole blood (plasma: whole blood ratios, 2.12 and 2.35, respectively). This suggests limited penetration of sildenafil and its metabolites into blood cells. In addition, the difference between the ratios after the different routes of administration is probably due to the presence of the higher concentrations of polar metabolites after oral dosing, which would be less likely to penetrate the blood cells than the less polar parent drug.

No unchanged drug was recovered from the urine or faeces, indicating that metabolism is the major mechanism for clearance of sildenafil. This is expected for such a

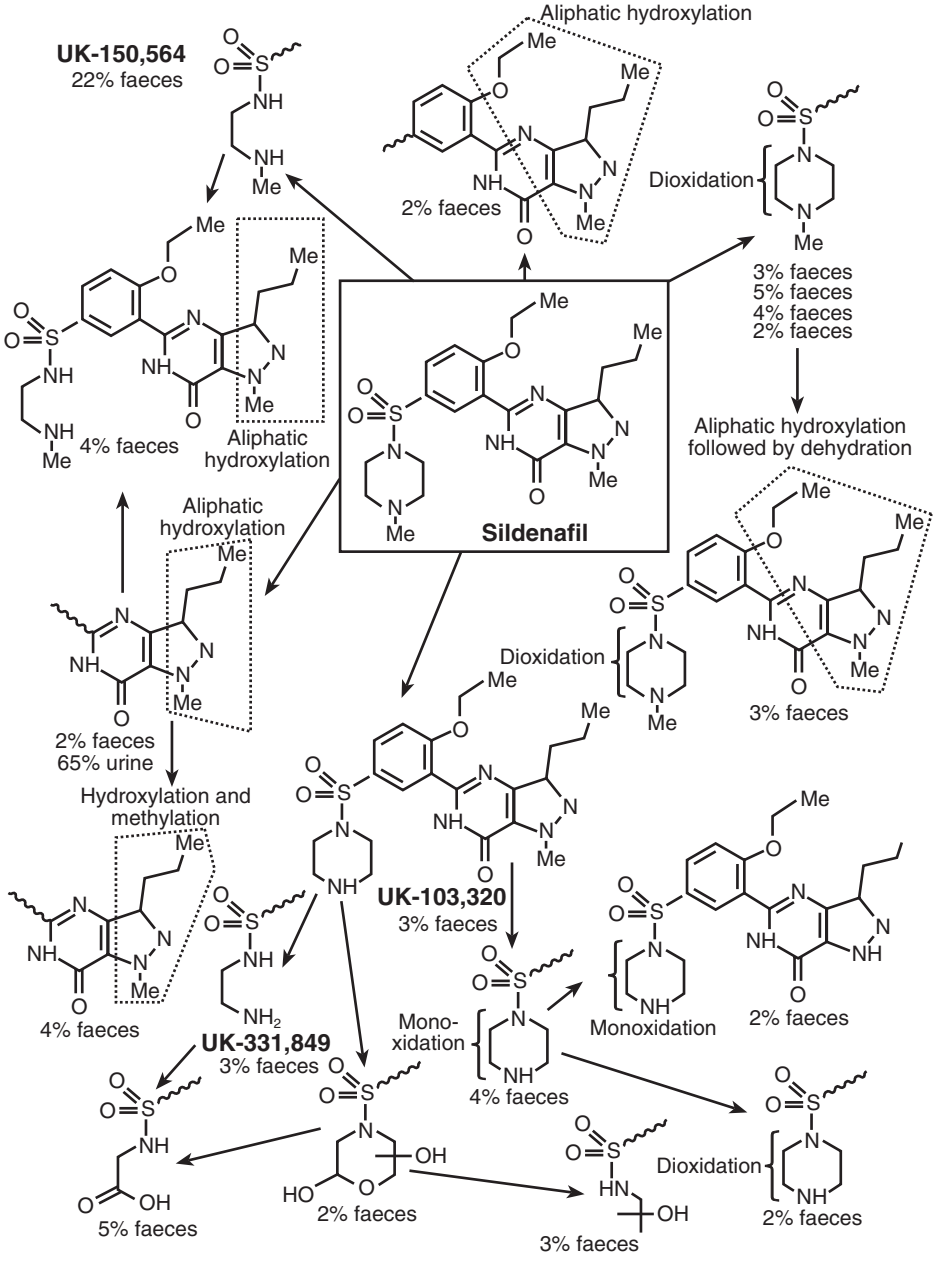

Figure 4 Proposed metabolic pathways and major metabolites for sildenafil and the respective percentages of radioactivity excreted in either the urine or faeces.

relatively lipophilic drug that has a low renal clearance and excretion due to high tubular re-absorption in the kidney. The metabolite profiles in both plasma and excreta were qualitatively similar after administration by either the oral or IV route. Several proposed pathways of metabolism were deduced. The principal routes include *N*-demethylation at the *N*-methyl piperazine and *N*-methyl pyrazole moieties, multiple oxidation and loss of a 2-carbon fragment from the piperazine ring and aliphatic dehydroxylation. These pathways have also been observed in animal species [7]. These data support the recent findings of Hyland *et al.* demonstrating that sildenafil is metabolized (*N*-demethylated) by the cytochrome P450 CYP2C9 and CYP3A4 enzymes [12].

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