JPP
Journal of
Pharmacy and Pharmacology

JPP 2009, 61: 1637–1642

Received April 21, 2009
Accepted August 27, 2009
DOI 10.1211/jpp/61.12.0008
ISSN 0022-3573

Research Paper

Effect of hepatic CYP inhibitors on the metabolism of sildenafil and formation of its metabolite, *N*-desmethylsildenafil, in rats *in vitro* and *in vivo*

Soo H. Bae[a], Soo K. Bae[b] and Myung G. Lee[a]

[a]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul and [b]Department of Clinical Pharmacology, Busan Paik Hospital, Inje University, Busan, South Korea

Abstract

Objectives It has been reported that hepatic cytochrome P450 (CYP)2C9 and CYP3A4 are responsible for the metabolism of sildenafil and formation of its metabolite, *N*-desmethylsildenafil, in humans. However, in-vivo studies in rats have not been reported.
Methods Sildenafil (20 mg/kg) was administered intravenously to rats pretreated with sulfaphenazole, cimetidine, quinine hydrochloride or troleandomycin, inhibitors of CYP2C6, CYP2C11, CYP2D subfamily and CYP3A1/2, respectively. In-vitro studies using rat liver microsomes were also performed.
Key findings The area under the plasma–concentration time curve (AUC) was increased and clearance of sildenafil decreased in rats pretreated with cimetidine or troleandomycin. The AUC ratio for *N*-desmethylsildenafil (0–4 h) : sildenafil (0–∞) was significantly decreased only in rats pretreated with cimetidine. Similar results were obtained in the in-vitro study using rat liver microsomes.
Conclusions Sildenafil is metabolised via hepatic CYP2C11 and 3A1/2, and *N*-desmethylsildenafil is mainly formed via hepatic CYP2C11 in rats. Thus, rats could be a good model for pharmacokinetic studies of sildenafil and *N*-desmethylsildenafil in humans.
Keywords CYP inhibitors; hepatic CYP2C11 and 3A1/2; *N*-desmethylsildenafil; pharmacokinetics; rats; sildenafil

Introduction

Sildenafil (UK-92,480; 1-[4-ethoxy-3-{6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo (4,3-*d*) pyrimidin-5-yl}phenylsulfonyl]-4-methylpiperazine), is an inhibitor of cGMP-specific phosphodiesterase type 5 (PDE 5) in human corpus cavernosum.

After intravenous administration of sildenafil at doses of 10, 30 and 50 mg/kg to male Sprague-Dawley rats, the total area under the plasma concentration–time curve from time zero to infinity (AUC) is dose-proportional,[1] but after its oral administration at doses of 10, 30 and 100 mg/kg the increase in AUC was disproportional, possibly due to saturation of metabolism of sildenafil in the intestinal tract.[1] After intravenous, oral, intraportal, intragastric or intraduodenal administration of sildenafil 30 mg/kg to male Sprague-Dawley rats the unabsorbed fraction from the gastrointestinal tract up to 24 h was 0.626% of the oral dose; absolute oral bioavailability was 14.6%, hepatic first-pass effect after absorption into the portal vein was 13.7% (low hepatic extraction ratio) and intestinal first-pass effect was 71% of the oral dose.[1]

Sildenafil is metabolised to *N*-desmethylsildenafil (UK-103,320; M10) via piperazine *N*-demethylation (11% in rats), UK-150,564 (M9) via piperazine *N*,*N*-deethylation (16% in rats), UK-95,340 via pyrazole *N*-demethylaion (20% in rats), M6 via aliphatic hydroxylation (2% in rats) and via other piperazine oxidation (1% in rats).[2] *N*-Desmethylsildenafil is further metabolised to UK-331,849 via ring opening of piperazine (MBA; 12% in rats) and UK-332,012 via *N*-demethylation (2% in rats).[2] Pharmacokinetic interaction studies of sildenafil and hepatic cytochrome P450 (CYP) enzyme inhibitors (such as indinavir, fluvoxamine, saquinavir, ritonavir, erythromycin, azithromycin, cimetidine, ciprofloxacin, clarithromycin and grapefruit juice) in humans have shown that sildenafil is metabolised via CYP2C9 and CYP3A4.[3–9] Studies with human B-lymphoblastoid-derived microsomes

Correspondence: Myung G. Lee, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Gwanak-Gu, Seoul 151-742, South Korea.
E-mail: leemg@snu.ac.kr

showed that *N*-desmethylsildenafil is formed via CYP3A4 (79%), CYP2C9 (20%), CYP2C19 and CYP2D6 (less than 2%).[10,11] Studies with male rat liver microsomes showed that sildenafil was metabolised via CYP2C11.[12] It has been reported that the CYP isozymes responsible for the metabolism of DA-8159 (udenafil), a new PDE 5 inhibitor, were different *in vivo* and *in vitro* in rats.[13] Thus, in-vivo rat studies were performed in the present study. It has also been reported that the CYP isozymes responsible for the metabolism of drugs differ between rats and humans,[14] and Warrington *et al*.[12] concluded that the CYP isozymes responsible for the metabolism of sildenafil were substantially different between humans and male rats. Human CYP2C9 and CYP3A4 and rat CYP2C11 and CYP3A1 have 77% and 73% protein homology, respectively.[15] The purpose of this study was to determine the CYP isozymes responsible for the metabolism of sildenafil and formation of *N*-desmethylsildenafil in rats and thus whether rats are a good model for the metabolism of sildenafil and formation of *N*-desmethylsildenafil in humans.

Materials and Methods

Chemicals

Sildenafil citrate, *N*-desmethylsildenafil and DA-8159 (internal standard for the LC/MS–MS analysis of sildenafil and *N*-desmethylsildenafil) were from Apin Chemicals (Abingdon, UK), Toronto Research Chemicals (North York, ON, Canada) and Dong-A Pharmaceutical Company (Yongin, South Korea), respectively. Sulfaphenazole (inhibitor of mainly CYP2C6 in rats[16]), cimetidine (inhibitor of mainly CYP2C11 in rats[17]), quinine hydrochloride (an inhibitor of CYP2D subfamily in rats[18]), troleandomycin (inhibitor of mainly CYP3A1/2 in rats[16]), NADPH (tetrasodium salt), tri (hydroxymethyl)aminomethane (Tris)-buffer and EDTA (disodium salt) were purchased from Sigma-Aldrich (St Louis, MO, USA). Other chemicals were of reagent or HPLC grade.

Animals

The protocols for these animal studies were approved by the Institute of Laboratory Animal Resources of Seoul National University, Seoul, South Korea. Male Sprague-Dawley rats (6–8 weeks old, 255−305 g) were purchased from Charles River Company Korea (Orient, Seoul, South Korea) and were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at 23 ± 2°C with a 12-h light–dark cycle (lights on 07:00–19:00) and relative humidity of 55 ± 5%. They were housed in metabolic cages (Tecniplast, Varese, Italy) and supplied with filtered pathogen-free air and food (Agribrands Purina Korea, Pyeongtaek, South Korea) and water *ad libitum*.

Intravenous study

The procedures used for pretreatment of rats, including cannulation (early in the morning) of the carotid artery (for blood sampling) and the jugular vein (for drug administration) were similar to a reported method.[1] Rats were divided into groups of six or seven and each group were given single intravenous injections of 80 mg (2 ml)/kg sulfaphenazole (dissolved in distilled water with a minimum amount of NaOH to achieve pH 8.0[19]) or single intraperitoneal injections of: 500 mg (5 ml)/kg troleandomycin (dissolved in 0.9% NaCl injectable solution and adjusted to pH 4.0 with HCl[16]); 20 mg (5 ml)/kg quinine hydrochloride (dissolved in 0.9% NaCl injectable solution[20]); or 150 mg (5 ml)/kg cimetidine (dissolved in 0.9% NaCl injectable solution adjusted to pH 4.0 with HCl[21]). Control rats received an intraperitoneal or intravenous injection of 5 ml/kg of 0.9% NaCl injectable solution. Rats had free access to food and water during the pretreatment period.

Experiments were performed just after the injection of sulfaphenazole,[19] 90 min after injection of cimetidine[21] and 2 h after injection of quinine or troleandomycin.[16]

Sildenafil (sildenafil citrate dissolved just before use in distilled water with a minimum amount of HCl and adjusted to a final pH of approximately 4 with NaOH[1]) at a dose of 20 mg/kg sildenafil base was administered by intravenous infusion over 1 min via the jugular vein of the control and pretreatment groups. Blood samples (approximately 0.12 ml) were collected via the carotid artery at 0 (control), 1 (end of the infusion), 5, 15, 30, 60, 90, 120, 180 and 240 min after the start of sildenafil infusion. Heparinised 0.9% NaCl injectable solution (20 units/ml; 0.3 ml) was used to flush the cannula immediately after blood sampling to prevent clotting. Blood samples were immediately centrifuged and 50 μl of plasma stored at −70°C until LC/MS–MS analysis of sildenafil and *N*-desmethylsildenafil. After 24 h, rats were exsanguinated and sacrificed by cervical dislocation.

Measurement of enzyme kinetics *in vitro*

The procedures used were similar to a reported method.[22] To identify the hepatic CYP isozymes responsible for the metabolism of sildenafil and formation of *N*-desmethylsildenafil in rats, specific chemical inhibitors of CYP isozymes were used. Livers (approximately 8 g) of control rats ($n = 4$) were homogenised (Ultra-Turrax T25; Janke & Kunkel, IKA-Labortechnik, Staufeni, Germany) in approximately 20 ml ice-cold buffer (0.154 mol/l KCl/50 mmol/l Tris-HCl in 1 mmol/l EDTA (pH 7.4) at 4°C in an ice bath. The homogenates were centrifuged at 10 000*g* for 30 min and the supernatant centrifuged at 100 000*g* for 90 min. The protein content of hepatic microsomes was measured using a reported method.[23]

The maximum velocity (V_{max}) and apparent Michaelis–Menten constant (K_m; the concentration at which the rate is half the V_{max}) for the disappearance of sildenafil and the formation of *N*-desmethylsildenafil were determined after incubating microsomal fractions (equivalent to 0.2 mg), 10 μl methanol containing final concentrations of 1, 2, 5, 10, 20, 50 and 100 μmol/l sildenafil citrate with and without 5 μl methanol containing a final concentration of 50 μmol/l cimetidine, 10 μmol/l sulfaphenazole, 50 μmol/l troleandomycin or 3 μmol/l quinine hydrochloride,[18] and 50 μl 0.1 mol/l phosphate buffer (pH 7.4) containing 1 mol/l NADPH. The volume was adjusted to 0.5 ml with 0.1 mol/l phosphate buffer (pH 7.4). The components were incubated in an oscillating water bath (37°C, 500 oscillations/min). For the studies on the mechanism-based inhibitor (troleandomycin) and controls, the microsomes, CYP inhibitor and NADPH

were preincubated for 15 min and 20 units catalase were added to prevent auto-inactivation of CYP isozymes during preincubation of microsomes with NADPH.[24] All microsomal incubations were within the linear range of the reaction rate. Reactions were terminated after 5 min' incubation by the addition of 0.2 ml acetonitrile to 0.1 ml of sample.

The kinetic constants (K_m and V_{max}) for the disappearance of sildenafil and the formation of *N*-desmethylsildenafil were calculated from the Michaelis–Menten equation using a non-linear regression method[25] and intrinsic clearance (CL_{int}) calculated by dividing V_{max} by K_m.

LC/MS–MS analysis of sildenafil and *N*-desmethylsildenafil

Concentrations of sildenafil and *N*-desmethylsildenafil were determined using an LC/MS–MS method developed in our laboratories. Briefly, 200 μl acetonitrile containing 4 μg/ml DA-8159 (internal standard) was added to 50 μl of sample. After vortex mixing and centrifugation at 9000*g* for 10 min at 4°C, the supernatant was transferred to a clean tube and 6 μl was injected directly onto a reversed-phase HPLC column (Luna C_{18}; 50 mm × 2.0 mm ID; particle size 3 μm; Phenomenex, Torrance, CA, USA). The mobile phase was 10 mmol/l ammonium acetate (pH 5.2) and acetonitrile (25 : 75, v/v) at a flow rate of 0.2 ml/min, delivered using an Agilent 1200 series HPLC system (Wilmington, DE, USA). The column and autosampler were maintained at 40°C and 4°C, respectively.

An LC/MS–MS analysis was performed using a PE SCIEX API4000 LC/MS–MS system (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionisation interface used to generate positive ions, $[M+H]^+$. The optimised ion spray voltage and temperature were set at 5500 V and 500°C, respectively. The typical ion source parameters – declustering potential, collision energy, entrance potential and collision cell exit potential – were 60, 35, 10 and 14 V, respectively. Nitrogen was used as the nebuliser gas, curtain gas and collision-activated dissociation gas. Quantification was performed by multiple reactions monitoring of the protonated precursor ion and the related product ion for sildenafil and *N*-desmethylsildenafil, using the internal standard method with peak area ratios and a weighting factor of $1/x^2$. The mass transitions used for sildenafil, *N*-desmethylsildenafil and internal standard were *m/z* 475.3→100.1, 460.9→283.2 and 517.2→283.1, respectively, with a dwell time of 150 ms per transition. Quadrupoles Q1 and Q3 were set on unit resolution. The analytical data were processed by Analyst software (version 1.4.1; Applied Biosystems). The retention times of sildenafil, *N*-desmethylsildenafil and DA-8159 (internal standard) were approximately 1.2, 0.97 and 0.93 min, respectively. This assay was linear over concentration ranges of 5–1000 ng/ml, with a lower limit of quantification of 5 ng/ml for both sildenafil and *N*-desmethylsildenafil. The coefficient of variation for the assay precision was < 14.7% and the accuracy was > 94.2%.

Pharmacokinetic analysis

The total AUC for sildenafil (from time zero to infinity) or to the last measured time (4 h) in plasma ($AUC_{0-4\ h}$) for *N*-desmethylsildenafil was calculated using the trapezoidal rule with extrapolation.[26] The area from the last datum point to time infinity (for AUC) was calculated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods[27] were used to calculate the time-averaged total body clearance and the terminal half-life using non-compartment analysis (WinNonLin; professional edition version 2.1; Pharsight, Mountain View, CA, USA).[28] For comparison, the metabolite ratio, $AUC_{N\text{-desmethylsidenafil, 0–4 h}}/AUC_{sidenafil}$, was estimated. The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined directly from the experimental data.

Statistical analysis

A P value below 0.05 was deemed to be statistically significant using an unpaired *t*-test. All the results are expressed as mean ± SD except for T_{max}, which is given as median (ranges).

Results

Pharmacokinetics studies

The mean arterial plasma concentration–time profiles of sildenafil and *N*-desmethylsildenafil after intravenous administration of sildenafil to rats pretreated with CYP inhibitors are shown in Figure 1. The relevant pharmacokinetic parameters are listed in Table 1. Compared with control rats, the terminal half-lives of sildenafil and *N*-desmethylsildenafil were significantly increased (by 36.3% and 111%) and C_{max} of *N*-desmethylsildenafil decreased (by 55.7%) in sulfaphenzole-treated rats.

The AUC and terminal half-life of sildenafil were significantly increased (by 84.3% and 28.8%, respectively) and clearance decreased (by 49.5%) in cimetidine-treated rats compared with control rats, and the terminal half-life and T_{max} of *N*-desmethylsildenafil were significantly increased (by 69.9% and 88.6%). The $AUC_{N\text{-desmethylsildenafil, 0–4 h}}/AUC_{silenafil}$ ratio was significantly decreased by 31.8% in the treated rats.

The AUC and terminal half-life of sildenafil were significantly increased (by 30.8% and 19.8% respectively) and clearance decreased (by 27.7%) in troleandomycin-treated rats compared with control rats, and the terminal half-life of *N*-desmethylsildenafil was significantly increased (by 301.8%).

The pharmacokinetic parameters of both sildenafil and *N*-desmethylsildenafil were similar in quinine-treated rats and quinine control rats.

These data suggest that hepatic CYP2C11 and 3A1/2 are involved in the metabolism of sildenafil and that CYP2C11 is involved in the formation of *N*-desmethylsildenafil in rats.

Enzyme kinetics

The V_{max}, K_m and CL_{int} for the disappearance of sildenafil in hepatic microsomes are listed in Table 2. V_{max} was slower in cimetidine-treated (52.2% decrease; $P = 0.168$) and troleandomycin-treated rats (38.8% decrease; $P < 0.05$) than their respective controls, suggesting that the maximum velocity for the disappearance of sildenafil (primarily metabolism)

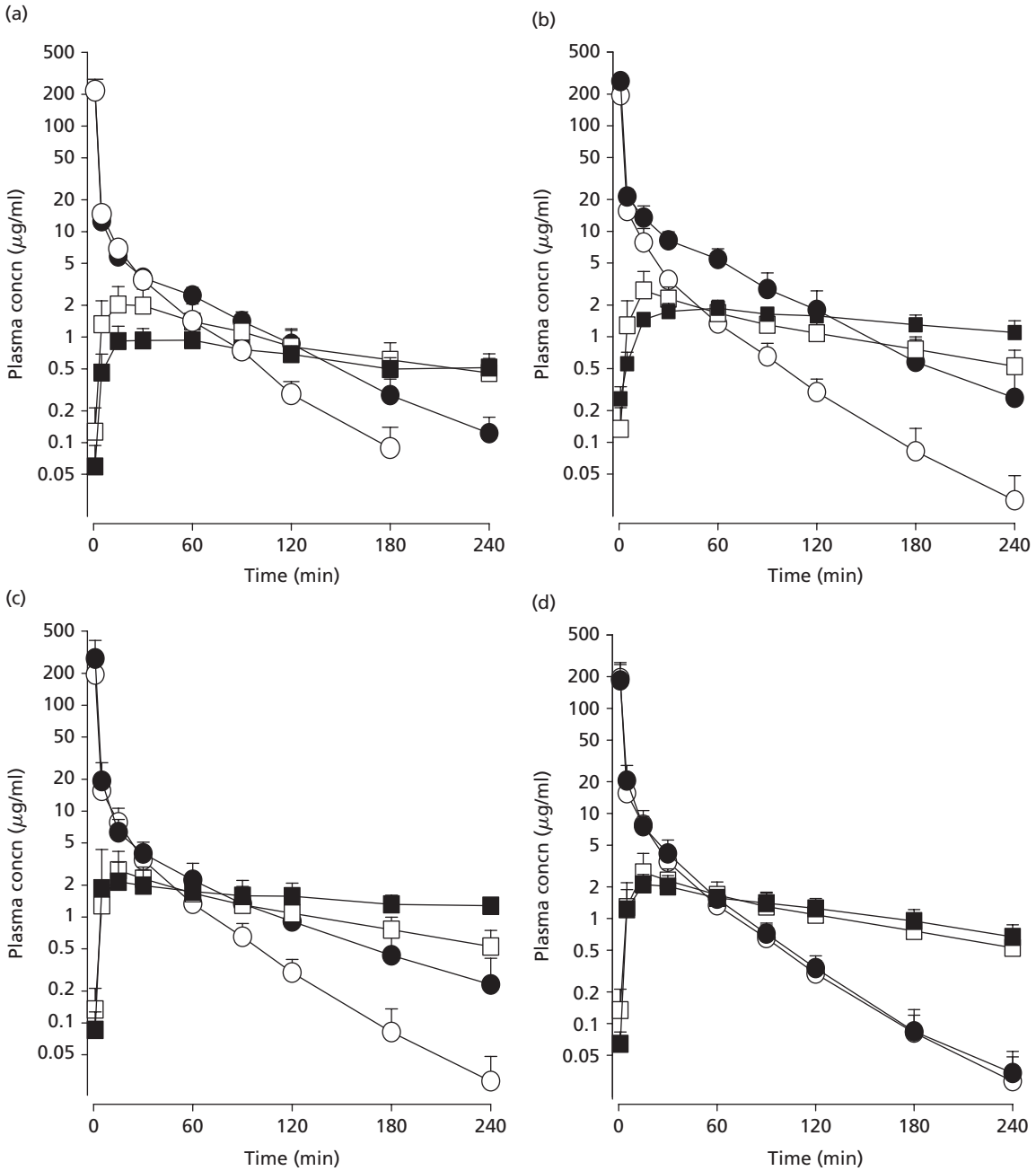

Figure 1 Mean plasma concentration–time profiles of sildenafil and *N*-desmethylsildenafil after intravenous infusion of sildenafil. Sildenafil (20 mg/kg) was administered to control rats (closed symbols) and rats pretreated with enzyme inhibitors (open symbols): (a) sulfaphenazole; (b) cimetidine; (c) troleandomycin; (d) quinine hydrochloride. Circles, sildenafil; squares, *N*-desmethylsildenafil. Values are means ± SD.

was slowed by cimetidine and troleandomycin. However, K_m values were unaltered, suggesting that the affinity of enzymes for sildenafil was not altered by CYP inhibitors. As a result, values for CL_{int} were significantly lower in cimetidine- and troleandomycin-treated rats than controls (decreases of 40.0% and 24.3%, respectively), suggesting that the formation of sildenafil metabolites was decreased by cimetidine and troleandomycin. These data suggest that inhibition of sildenafil metabolism by cimetidine or troleandomycin was non-competitive.

The V_{max}, K_m and CL_{int} values for the formation of *N*-desmethylsildenafil in hepatic microsomes are also listed in Table 2. CL_{int} was decreased significantly only in cimetidine-treated rats compared with controls (26.0% decrease), suggesting that the formation of *N*-desmethylsildenafil was slowed by cimetidine. Chang *et al*.[29] reported that rat hepatic microsomes incubated with a low concentration (50 μmol/l; the same concentration used in this study) of cimetidine resulted in the inhibition of CYP2C11 but had no effect on CYP2A1, 2B1/2 or 3A1/2.

Table 1 Pharmacokinetic parameters of sildenafil and *N*-desmethylsildenafil after intravenous administration of sildenafil

	SPZ control (n = 6)	SPZ (n = 6)	CMT/QN/TRL control (n = 7)	CMT (n = 7)	QN (n = 6)	TRL (n = 7)
Sildenafil						
AUC (μg min/ml)	722 ± 77.2	780 ± 58.1	727 ± 159	1340 ± 227‡	739 ± 192	951 ± 212*
Terminal half-life (min)	31.7 ± 5.69	43.2 ± 4.64†	30.9 ± 6.23	39.8 ± 8.50*	71.4 ± 97.7	47.7 ± 11.6†
CL (ml/min per kg)	28.0 ± 3.14	25.8 ± 1.88	30.3 ± 7.45	15.3 ± 2.72‡	28.4 ± 6.07	21.9 ± 4.65*
N-desmethylsildenafil						
$AUC_{0-4\ h}$ (μg/min per ml)	275 ± 117	213 ± 41.4	334 ± 105	433 ± 76.4	349 ± 98.0	396 ± 123
C_{max} (μg/ml)	2.24 ± 0.881	0.993 ± 0.269†	2.90 ± 1.29	1.89 ± 0.291	2.28 ± 0.565	2.99 ± 1.97
T_{max} (min)	15 (5–30)	22.5 (15–120)	15 (15–30)	30 (15–60)*	15 (15–30)	15 (5–60)
AUC ratio (%)	19.0 ± 7.56	13.5 ± 2.67	23.9 ± 6.09	16.3 ± 2.37†	24.7 ± 8.44	21.7 ± 7.89

Sildenafil (20 mg/kg) was intravenously administered to rats pretreated with cimetidine (CMT; 150 mg/kg), sulfaphenazole (SPZ; 80 mg/kg), quinine hydrochloride (QN; 20 mg/kg) or troleandomycin (TRL; 500 mg/kg). Values are mean ± SD except T_{max}, which is given as median (range). AUC, area under the plasma concentration–time curve (from time 0 to infinity for sildenafil); CL, clearance; C_{max}, maximum plasma concentration; T_{max}, time to C_{max}; AUC ratio, $AUC_{N\text{-desmethylsildenafil, 0–4 h}}/AUC_{sildenafil}$. $^{*}P < 0.05$, $^{\dagger}P < 0.01$; $^{\ddagger}P < 0.001$ vs respective control.

Table 2 V_{max}, K_m and CL_{int} for the disappearance of sildenafil and the formation of *N*-desmethylsildenafil in rats pretreated with cimetidine, sulfaphenazole, quinine hydrochloride or troleandomycin and respective control rats

	CMT, SPZ, QN control (n = 4)	CMT (n = 4)	SPZ (n = 4)	QN (n = 4)	TRL control (n = 4)	TRL (n = 4)
Disappearance of sildenafil						
V_{max} (nmol/min per mg)	2.49 ± 1.33	1.19 ± 0.825	3.16 ± 1.67	2.07 ± 1.54	4.54 ± 0.0583	2.78 ± 0.696†
K_m (μmol/l)	8.14 ± 6.24	5.66 ± 3.77	12.9 ± 6.62	7.04 ± 4.55	19.1 ± 1.36	13.3 ± 7.11
CL_{int} (ml/min per mg)	0.345 ± 0.0763	0.207 ± 0.00812*	0.243 ± 0.0366	0.260 ± 0.0820	0.239 ± 0.0172	0.181 ± 0.0311*
Formation of *N*-desmethylsildenafil						
V_{max} (nmol/min per mg)	14.0 ± 2.38	11.5 ± 5.39	9.63 ± 1.58	10.6 ± 1.94	9.96 ± 2.14	8.28 ± 3.79
K_m (μmol/l)	42.0 ± 7.79	34.5 ± 10.5	39.7 ± 5.88	35.3 ± 6.78	32.5 ± 9.29	26.3 ± 13.6
CL_{int} (ml/min per mg)	0.308 ± 0.0246	0.228 ± 0.00844*	0.244 ± 0.0372	0.287 ± 0.0262	0.312 ± 0.0293	0.333 ± 0.0479

Values are means ± SD. CMT, cimentidine; SPZ, sulfaphenazole; QN, quinine hydrochloride; TRL, troleandomycin; V_{max}, maximum velocity; K_m apparent Michaelis–Menten constant (concentration at which rate is half the V_{max}); CL_{int}, intrinsic clearance; values are given per mg microsomal protein. $^{*}P < 0.05$ and $^{\dagger}P < 0.01$ vs respective control.

The above data suggest that sildenafil was metabolised mainly by hepatic CYP2C11 and 3A1/2, and that *N*-desmethylsildenafil was formed mainly by hepatic CYP2C11 in rats.

Discussion

Shin *et al.*[1] reported that the AUC of sildenafil was proportional to dose after intravenous administration of 10, 30 and 50 mg/kg to rats. Thus, an intravenous dose of sildenafil, 20 mg/kg, was chosen in the present study. Shin *et al.*[1] also reported that the percentage of the intravenous dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h following doses of 10, 20, and 30 mg/kg was almost negligible: less than 0.897%. Thus, the clearance of sildenafil listed in Table 1 could represent its metabolic clearance.

Compared with controls, the AUC of sildenafil was significantly greater and clearance significantly slower in cimetidine- and troleandomycin-treated rats, and the $AUC_{N\text{-desmethylsildenafil, 0–4 h}}/AUC_{sildenafil}$ ratio was significantly lower in the cimetidine-treated rats (Table 1). Similar results have also been obtained from the in-vitro study (Table 2). These data suggest that hepatic CYP2C11 and 3A1/2 are responsible for the metabolism of sildenafil, and that *N*-desmethylsildenafil is mainly formed via CYP2C11 in rats.

In a preliminary study, sildenafil was administered to rats pretreated with dexamethasone phosphate (inducer of mainly CYP3A1/2 in rats[15]); the AUC of sildenafil and the $AUC_{N\text{-desmethylsildenafil, 0–4 h}}/AUC_{silenafil}$ ratio were significantly smaller than in controls (decreases of 19.0% and 86.4%, respectively). The significantly smaller ratio could have been due to the further metabolism of *N*-desmethylsildenafil in rats.[2] The metabolites of *N*-desmethylsildenafil were not measured in the present study so it was not easy to find the CYP isozyme(s) responsible for the formation of *N*-desmethylsildenafil using CYP inducers. Hence, CYP inhibitors were used.

The various inhibitors studied have activities towards various CYP isoforms,[2] although only the main CYP isoform inhibited is stated. Thus, these results are confined to the main CYP isoforms.

The present results will play an important role in explaining possible differences in the pharmacokinetic parameters of sildenafil and *N*-desmethylsildenafil in various rat disease models in which CYP2C11 and/or 3A1/2 are changed, such as in rats with protein–calorie malnutrition,[30]

acute renal failure induced by uranyl nitrate[31] or diabetes mellitus induced by alloxan or streptozotocin,[32] and in studies of the pharmacokinetic effects of lipopolysaccharide endotoxin induced by *Escherichia coli*[33] or *Klebsiella pneumoniae*.[34] These results could also explain possible drug interactions between sildenafil and other drugs that are primarily metabolised via CYP2C11 and/or 3A1/2.

The hepatic CYP isozymes responsible for the metabolism of sildenafil in humans (CYP2C9 and 3A4) and in rats (CYP2C11 and 3A1/2) and formation of *N*-desmethylsildenafil in humans (CYP2C9 and 3A4) and in rats (CYP2C11) are very similar. This suggests that rats could be a good model for pharmacokinetic studies of sildenafil and *N*-desmethylsildenafil in humans.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This study was supported in part by a grant from the 2008 BK21 Project for Applied Pharmaceutical Life Sciences.

References

1. Shin HS *et al*. Pharmacokinetics of sildenafil after intravenous and oral administration in rats: hepatic and intestinal first-pass effects. *Int J Pharm* 2006; 320: 64–70.
2. Walker DK *et al*. Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. *Xenobiotica* 1999; 29: 297–310.
3. Merry C *et al*. Interaction of sildenafil and indinavir when co-administered to HIV-positive patients. *AIDS* 1999; 13: F101–F107.
4. Muirhead GJ *et al*. Pharmacokinetic interactions between sildenafil and saquinavir/ritonavir. *Br J Clin Pharmacol* 2000; 50: 99–107.
5. Jetter A *et al*. Effects of grapefruit juice on the pharmacokinetics of sildenafil. *Clin Pharmacol Ther* 2002; 71: 21–29.
6. Muirhead GJ *et al*. The effects of steady-state erythromycin and azithromycin on the pharmacokinetics of sildenafil in healthy volunteers. *Br J Clin Pharmacol* 2002; 53(Suppl. 1): 37S–43S.
7. Wilner K *et al*. The effects of cimetidine and antacid on the pharmacokinetic profile of sildenafil citrate in healthy male volunteers. *Br J Clin Pharmacol* 2002; 53(Suppl. 1): 31S–36S.
8. Hesse C *et al*. Fluvoxamine affects sildenafil kinetics and dynamics. *J Clin Psychopharmacol* 2005; 25: 589–592.
9. Hedaya MA *et al*. The effect of ciprofloxacin and clarithromycin on sildenafil oral bioavailability in human volunteers. *Biopharm Drug Dispos* 2006; 27: 103–110.
10. Warrington JS *et al*. *In vitro* biotransformation of sildenafil (Viagra): identification of human cytochromes and potential drug interactions. *Drug Metab Dispos* 1999; 28: 392–397.
11. Hyland R *et al*. Identification of the cytochrome P450 enzymes involved in the *N*-demethylation of sildenafil. *Br J Clin Pharmacol* 2001; 51: 239–248.
12. Warrington JS *et al*. *In vitro* biotransformation of sildenafil (Viagra) in the male rat: the role of CYP2C11. *Drug Metab Dispos* 2002; 30: 655–657.
13. Shim HJ *et al*. Species differences in the formation of DA-8164 after intravenous and/or oral administration of DA-8159, a new erectogenic, to mice, rats, rabbits, dogs and humans. *Biopharm Drug Dispos* 2005; 26: 161–166.
14. Elsherbiny ME *et al*. The metabolism of amiodarone by various CYP isoenzymes of human and rat, and the inhibitory influence of ketoconazole. *J Pharm Pharm Sci* 2008; 11: 147–159.
15. Lewis DFV. P450 substrate, specificity and metabolism. In: *Cytochrome P450. Structure, Function and Metabolism.* Bristol, PA: Taylor & Francis, 1996: 123.
16. Sinclair J *et al*. Acetaminophen hepatotoxicity precipitated by short-term treatment of rats with ethanol and isopentanol: protection by triacetyloleandomycin. *Biochem Pharmacol* 2000; 59: 227–232.
17. Kobayashi K *et al*. Selectivities of human cytochrome P450 inhibitors toward rat P450 isoforms: study with cDNA-expressed systems of the rat. *Drug Metab Dispos* 2003; 31: 833–836.
18. Kobayashi S *et al*. The specificity of inhibition of debrisoquine 4-hydroxylase activity by quinidine and quinine in the rat is the inverse of that in man. *Biochem Pharmacol* 1989; 38: 2795–2799.
19. Ogiso T *et al*. Pharmacokinetic drug interactions between ampiroxicam and sulfaphenazole in rats. *Biol Pharm Bull* 1999; 22: 196–199.
20. Tomkins DM *et al*. Effects of cytochrome P450 2D1 inhibition on hydrocodone metabolism and its behavioral consequences in rats. *J Pharmacol Exp Ther* 1997; 280: 1374–1382.
21. Levine M *et al*. *In vivo* cimetidine inhibits hepatic CYP2C6 and CYP2C11 but not CYP1A1 in adult male rats. *J Pharmacol Exp Ther* 1998; 284: 493–499.
22. Oh EY *et al*. Pharmacokinetic and pharmacodynamic consequences of inhibition of terazocin metabolism via CYP3A1 and/or 3A2 by DA-8159, an erectogenic, in rats. *Br J Pharmacol* 2007; 151: 24–34.
23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–254.
24. Huskey SW *et al*. Identification of human cytochrome P450 isozymes responsible for the *in vitro* oxidative metabolism of finasteride. *Drug Metab Dispos* 1995; 31: 833–836.
25. Duggleby RG. Analysis of enzyme progress curves by nonlinear regression. *Methods Enzymol* 1995; 249: 61–90.
26. Chiou WL. Critical evaluation of the potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J Pharmacokinet Biopharm* 1978; 6: 539–546.
27. Gibaldi M, Perrier D. *Pharmacokinetics,* 2nd edn. New York: Marcel-Dekker, 1982.
28. Kim SH *et al*. Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J Pharmacokinet Biopharm* 1993; 21: 1–17.
29. Chang T *et al*. Selective inhibition of rat hepatic microsomal cytochrome P-450. II. Effect of the *in vitro* administration of cimetidine. *J Pharmacol Exp Ther* 1992; 260: 1450–1455.
30. Cho MK *et al*. Suppression of rat hepatic cytochrome P450s by protein–calorie malnutrition: complete or partial restoration by cysteine or methionine supplementation. *Arch Biochem Biophys* 1999; 372: 150–158.
31. Moon YJ *et al*. Effects of acute renal failure on the pharmacokinetics of chlorzoxazone in rats. *Drug Metab Dispos* 2003; 31: 776–784.
32. Kim YC *et al*. Pharmacokinetics of theophylline in diabetes mellitus rat: induction of CYP1A2 and CYP2E1 on 1,3-dimethyluric acid formation. *Eur J Pharm Sci* 2005; 26: 114–123.
33. Yang KH, Lee MG. Effects of endotoxin derived from *Escherichia coli* lipopolysaccharide on the pharmacokinetics of drugs. *Arch Pharm Res* 2008; 31: 1073–1086.
34. Yang KH, Lee MG. Effects of lipopolysaccharide on pharmacokinetics of drugs. *Toxicol Res* 2007; 23: 289–299.