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Pharmacokinetics of sildenafil after intravenous and oral administration in rats: Hepatic and intestinal first-pass effects

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Abstract

Pharmacokinetics of sildenafil after intravenous and oral administration at various doses and first-pass effect at 30 mg/kg were evaluated in rats. After intravenous administration (10, 30, and 50 mg/kg), the dose-normalized AUC values were proportional to intravenous doses studied. However, after oral administration (10, 30, and 100 mg/kg), the dose-normalized AUC values increased significantly with increasing doses, possibly due to saturation of metabolism of sildenafil in rat intestinal tract. After oral administration (30 mg/kg), approximately 0.626% was not absorbed and F was 14.6%. The AUC after intragastric administration was significantly smaller (71.4% decrease) than that after intraportal administration, however, the values were not significantly different between intragastric and intraduodenal administration. The above data suggested that intestinal first-pass effect of sildenafil was approximately 71% of oral dose in rats. The AUC values after intraportal administration were significantly smaller (49% decrease) than that after intravenous administration. This suggested that hepatic first-pass effect of sildenafil after absorption into the portal vein was approximately 49% of oral dose in rats (approximately 49% was equivalent to approximately 13.7% of oral dose). The low F of sildenafil at a dose of 30 mg/kg in rats could be mainly due to considerable intestinal first-pass effect.

Keywords: Sildenafil; Pharmacokinetics; Hepatic and intestinal first-pass effects; Rats

1. Introduction

Sildenafil (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-*d*] pyrimidin-5-yl) phenylsulphonyl]-4-methyl piperazine; a weak basic drug with a pK_a of 6.5) is an inhibitor of the cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE 5) found in human corpus cavernosum. Sildenafil citrate is marketed as Viagra® for the treatment of male erectile dysfunction. The following pharmacokinetic parameters of sildenafil were reported in humans and animals (Walker et al., 1999). After single intravenous administration of sildenafil in mice (1.0 mg/kg), male and female rats (4.0 mg/kg), dogs (1.0 mg/kg), and humans (0.35 mg/kg), the terminal half-lives were not determined due to insufficient data, 0.3 ± 0.1, 1.9 ± 0.1, 5.2 ± 2.1, and 2.4 ± 1.0 h, respectively, and the corresponding time-averaged total body clearance (CL) values were 91, 48 ± 11, 13 ± 1, 12 ± 4, and 6.0 ± 1.1 ml/min/kg. After single oral administration of sildenafil in mice (10.0 mg/kg), male and female rats (1.0 mg/kg), dogs (1.0 mg/kg), and humans (0.68 mg/kg), the extent of absolute oral bioavailability (F) values were 17, 23, 44, 54 ± 13, and 38%, respectively. The low F values could be due to pre-systemic hepatic metabolism in all species. However, the reasons for the low F values of sildenafil seemed not to be reported in humans and animals. Hence, this study was performed to find the reasons for the low F values of sildenafil using rats as an animal model.

The purpose of this study is to report the pharmacokinetics of sildenafil after intravenous administration at doses of 10, 30, and 50 mg/kg and oral administration at doses of 10, 30, and 100 mg/kg, and the first-pass (hepatic and gastrointestinal)

Abbreviations: $Ae_{0-24\,h}$, total amount excreted in 24-h urine; AUC, total area under the plasma concentration–time curve from time zero to time infinity; CL, time-averaged total body clearance; CL_{NR}, time-averaged nonrenal clearance; CL_R, time-averaged renal clearance; C_{max}, peak plasma concentration; F, extent of absolute oral bioavailability; $GI_{24\,h}$, total amount recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h; MRT, mean residence time; T_{max}, time to reach a C_{max}; V_{ss}, apparent volume of distribution at steady state

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effect of sildenafil after intravenous, intraportal, intragastric, and intraduodenal administration at a dose of 30 mg/kg in male Sprague–Dawley rats.

2. Materials and methods

2.1. Chemicals

Sildenafil citrate and testosterone (an internal standard of high-performance liquid chromatographic, HPLC, analysis of sildenafil) were purchased from APIN Chemicals Limited (Oxfordshire, UK) and Sigma–Aldrich Corporation (St. Louis, MO), respectively. Other chemicals were of reagent grade or HPLC grade.

2.2. Animals

Male Sprague–Dawley rats (weighing 240–330 g) of 7 or 8 weeks of age were purchased from the Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea). Animals were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea) at a temperature between 20 and 23 °C with 12-h light (07:00–19:00) and dark (19:00–07:00) cycles and a relative humidity of 50%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air with food (Agribrands Purina Korea, Pyeongtaek, South Korea) and water ad libitum. The protocol of this animal study was approved by the Institutional Animal Care and Use Committee, Seoul National University.

2.3. Intravenous and oral administration of sildenafil in rats

The procedures for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and/or the jugular vein (only intravenous study for drug administration) of each rat were similar to previously reported methods (Kim et al., 1993). Experiments were started after 4–5 h recovery period from light ether anesthesia. Sildenafil citrate (dissolved in distilled water with a minimum amount of HCl and adjusted to a final pH of approximately 4 with NaOH) at doses of 10 mg/kg ($n = 8$), 30 mg/kg ($n = 10$), and 50 mg/kg ($n = 9$) as free sildenafil was infused (total infusion volume of approximately 0.9 ml) via the jugular vein over 1-min in rats. An approximately 0.22-ml aliquot of blood sample was collected via the carotid artery at 0 min (to serve as a control), 1 min (at the end of the infusion), 5, 15, 30, 60, 90, 120, 180, and 240 min after intravenous administration of sildenafil. After centrifugation of blood sample, a 0.1-ml aliquot of plasma sample was stored in a −70 °C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until the HPLC analysis of sildenafil. An approximately 0.3-ml aliquot of heparinized 0.9% NaCl-injectable solution (20 units/ml) was used to flush the cannula immediately after each blood sampling to prevent blood clotting. At the end of 24 h, each metabolic cage was rinsed with 25 ml of distilled water and the rinses were combined with the 24-h urine. After measuring the exact volume of the combined urine sample, two 0.1-ml aliquots of the combined urine sample were stored in a −70 °C freezer until the HPLC analysis of sildenafil. At the same time (24 h), each rat was sacrificed by cervical dislocation and then the entire gastrointestinal tract (including its contents and feces) was removed, transferred into a beaker containing 100 ml of methanol (to facilitate the extraction of sildenafil) and cut into small pieces using scissors. After manual shaking and stirring with a glass rod for 1 min, two 0.1-ml aliquots of the supernatant were collected from each beaker and stored in a −70 °C freezer until the HPLC analysis of sildenafil.

Sildenafil citrate (the same solution that was used in the intravenous studies) at doses of 10 mg/kg ($n = 9$), 30 mg/kg ($n = 8$), and 100 mg/kg ($n = 8$) as free sildenafil was administered orally (total oral volume of approximately 1.5 ml) using a feeding tube in rats after overnight fasting with free access to water. An approximately 0.22-ml aliquot of blood sample was collected via the carotid artery at 0, 5, 15, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 min after oral administration of sildenafil. The plasma, 24-h urine, and gastrointestinal tract samples were similarly handled as those of the intravenous studies. The oral doses of sildenafil in this study were much higher than the clinical dose to obtain complete pharmacokinetics of sildenafil in rats.

2.4. Measurement of hepatic first-pass effect of sildenafil in rats

The carotid artery and the jugular vein of each rat were cannulated under light ether anesthesia (Kim et al., 1993). At the same time, the pyloric vein instead of the portal vein was cannulated to minimize impaired blood flowing in the portal vein (Kim et al., 1997) by the modified Suzuki method (Xu et al., 1992). Sildenafil citrate (the same solution that was used in the intravenous studies) at a dose of 30 mg/kg as free sildenafil was infused (total infusion volume of approximately 1 ml) over 30 min into the jugular vein and the pyloric vein for intravenous ($n = 9$) and intraportal ($n = 10$) administration, respectively, after 4–5 h recovery from light ether anesthesia with the assistance of an infusion pump (model 2400-006; Harvard Instrument, Southnatick, MA). At the same time, an equal volume (approximately 1 ml) of the vehicle was also infused over 30 min via the pyloric vein for intravenous study and via the jugular vein for intraportal study. An approximately 0.22-ml aliquot of blood sample was collected via the carotid artery at 0, 15, 30 min (at the end of the infusion), 31, 35, 45, 60, 90, 120, 150, 210, 270, and 330 min after infusion of sildenafil. A 0.1-ml aliquot of plasma sample was stored in a −70 °C freezer until the HPLC analysis of sildenafil.

2.5. Measurement of gastric and intestinal first-pass effects of sildenafil in rats

Rats were fasted overnight with free access to water. The carotid artery and the pyloric vein of each rat were cannulated after abdominal incision under light ether anesthesia (Kim et al., 1997). For intraportal infusion ($n = 7$), an approximately 0.75 ml aliquot of the vehicle was instilled into the stomach and

duodenum, respectively, using a 29-guage needle, and sildenafil citrate (the same solution that was used in the intravenous studies) at a dose of 30 mg/kg as free sildenafil was infused (total infusion volume of approximately 1 ml) over 30 min into the pyloric vein of rats with the assistance of an infusion pump. For intraduodenal instillation ($n = 7$), approximately 0.75 and 1-ml aliquots of the vehicle were instilled into the stomach and infused over 30 min via the pyloric vein, respectively, and sildenafil citrate at a dose of 30 mg/kg as free sildenafil was instilled (total instilled volume of approximately 0.75 ml) into the duodenum. For intragastric administration ($n = 6$), approximately 1 and 0.75-ml aliquots of the vehicle were infused over 30 min via the pyloric vein and instilled into the duodenum, respectively, and sildenafil citrate at a dose of 30 mg/kg as free sildenafil was instilled (total instilled volume of approximately 0.75 ml) into the stomach. Other procedures were similar to those described to measure the hepatic first-pass effect of sildenafil in rats.

2.6. HPLC analysis of sildenafil

Concentrations of sildenafil in the above samples were determined by the modification of the reported HPLC method (Shim et al., 2002). A 50-μl aliquot of 0.1N borax, a 50-μl aliquot of acetonitrile containing 200 μg/ml testosterone (an internal standard), and a 1-ml aliquot of ether were added to a 100-μl aliquot of sample. After vortex-mixing and centrifugation, the ether layer was collected and dried under a gentle stream of nitrogen gas. A 125-μl aliquot of the mobile phase was added to reconstitute the residue, and a 60-μl aliquot was injected directly onto a reversed-phase (C_{18}) HPLC column. The mobile phase, 20 mM KH_2PO_4:acetonitrile (68:32, v/v) was run at a flow-rate of 1.3 ml/min, and the column effluent was monitored by an ultraviolet detector set at 292 nm at room temperature. The retention times of sildenafil and testosterone were approximately 5.8 and 17.0 min, respectively. The detection limits of sildenafil in rat plasma and urine were 20 and 30 ng/ml, respectively. The coefficients of variation of the assay were below 11.5% for plasma and 10.5% for urine sample.

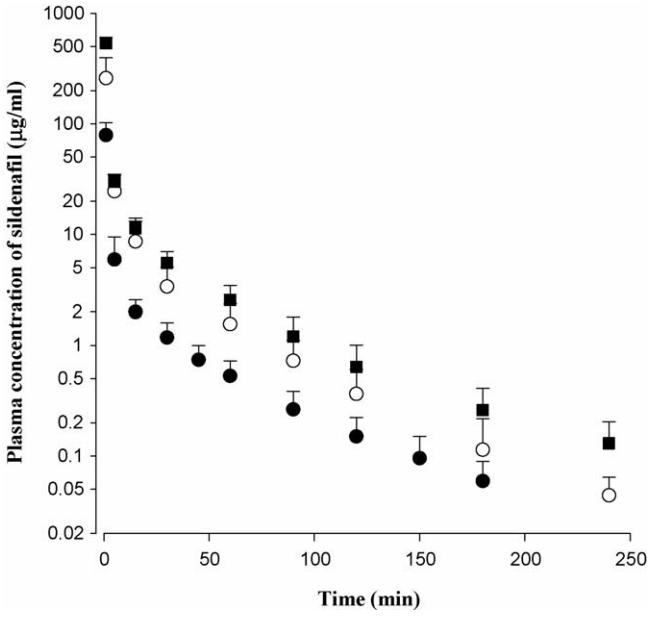

Fig. 1. Mean arterial plasma concentration–time profiles of sildenafil after 1-min intravenous infusion at doses of 10 mg/kg (●; $n = 8$), 30 mg/kg (○; $n = 10$), and 50 mg/kg (■; $n = 9$) in rats. Vertical bars represent S.D.

2.7. Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule–extrapolation method; this method uses the logarithmic trapezoidal rule recommended by Chiou (1978) for the calculation of the area during the phase of a declining level in plasma and the linear trapezoidal rule for the phase of a rising level in plasma. The area from the last datum point to time infinity was estimated by dividing the last measured concentration in plasma by the terminal rate constant.

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, terminal half life, first moment of AUC (AUMC), mean residence time (MRT), apparent vol-

Table 1
Pharmacokinetic parameters of sildenafil after 1-min intravenous administration at doses of 10, 30, and 50 mg/kg in rats

Parameter	10 mg/kg ($n = 8$)	30 mg/kg ($n = 10$)	50 mg/kg ($n = 9$)
Body weight (g)	282 ± 56.6	286 ± 23.4	262 ± 11.7
AUC[a] (μg min/ml)	260 ± 52.7	882 ± 214	1520 ± 181
Terminal half-life (min)	40.8 ± 4.38	38.0 ± 11.0	45.4 ± 13.6
MRT (min)	20.0 ± 6.67	16.0 ± 5.72	16.9 ± 4.14
V_{ss} (ml/kg)	681 ± 327	487 ± 272	535 ± 105[b]
CL (ml/min/kg)	38.5 ± 8.30	34.0 ± 8.92	32.8 ± 3.90
CL_R (ml/min/kg	0.120 ± 0.494	0.0864 ± 0.0514[c]	0.207 ± 0.108
CL_{NR} (ml/min/kg)	38.2 ± 8.12	33.9 ± 8.89	32.6 ± 3.88
$Ae_{0-24\,h}$ (% of dose)	0.897 ± 0.856	0.321 ± 0.110[c]	0.767 ± 0.284
$GI_{24\,h}$ (% of dose)	BD[d]	BD	0.106 ± 0.104

Each value represents the mean ± S.D.

[a] AUC was normalized to the dose of 10 mg/kg when statistical analysis was performed.

[b] 50 mg/kg was significantly different ($P < 0.05$) from 10 mg/kg.

[c] 30 mg/kg was significantly different ($P < 0.05$) from 10 mg/kg.

[d] Below the detection limit.

ume of distribution at steady state (V_{ss}), and F (Kim et al., 1993). The peak plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) after oral administration were directly read from the experimental data.

The harmonic mean method was employed to calculate the mean values of V_{ss} (Chiou, 1979), terminal half-life (Eatman et al., 1977), and each clearance (Chiou, 1980).

2.8. Statistical analysis

A P-value of less than 0.05 was considered to be statistically significant using a t-test between the two means for the unpaired data, or a Duncan's multiple range test of social package of statistical sciences (SPSS) posteriori analysis of variance (ANOVA) among the three means for the unpaired data. All data are expressed as mean ± S.D.

3. Results

3.1. Pharmacokinetics of sildenafil after intravenous administration in rats

The mean arterial plasma concentration–time profiles of sildenafil after intravenous administration at doses of 10, 30, and 50 mg/kg in rats are shown in Fig. 1, and some relevant pharmacokinetic parameters are listed in Table 1. Note that the AUC values of sildenafil were proportional to intravenous doses studied. For example, the dose-normalized (based of 10 mg/kg) AUC values were 260 ± 52.7, 294 ± 71.3, and 304 ± 36.2 μg min/ml for 10, 30, and 50 mg/kg, respectively. The other pharmacokinetic parameters of sildenafil listed in Table 1 were also independent of intravenous doses studied, except V_{ss}, CL_R, and percentage of intravenous dose of sildenafil excreted in 24-h urine as unchanged drug ($Ae_{0-24\,h}$).

3.2. Pharmacokinetics of sildenafil after oral administration in rats

The mean arterial plasma concentration–time profiles of sildenafil after oral administration at doses of 10, 30, and 100 mg/kg in rats are shown in Fig. 2, and some relevant pharmacokinetic parameters are listed in Table 2. After oral administration, sildenafil was absorbed rapidly from rat gastrointestinal tract; the drug was detected in plasma from the first blood sampling time (5 min) and rapidly reached T_{max} values; 11.1, 17.5, and 38.1 min for 10, 30, and 100 mg/kg, respectively. Note that the dose-normalized AUC values increased with increasing doses. For example, the dose-normalized (based on 10 mg/kg) AUC values were 16.5 ± 6.95, 43.0 ± 19.8, and 71.3 ± 33.5 μg min/ml for 10, 30, and 100 mg/kg, respectively; each value was significantly different. This could be due to saturation of metabolism of sildenafil in rat intestinal tract as will be discussed later. For comparison, the F values were estimated based on AUC of intravenous dose at 30 mg/kg. As expected from AUC values, the F values also increased with increasing doses; the values were 5.61, 14.6, and 24.3% for 10,

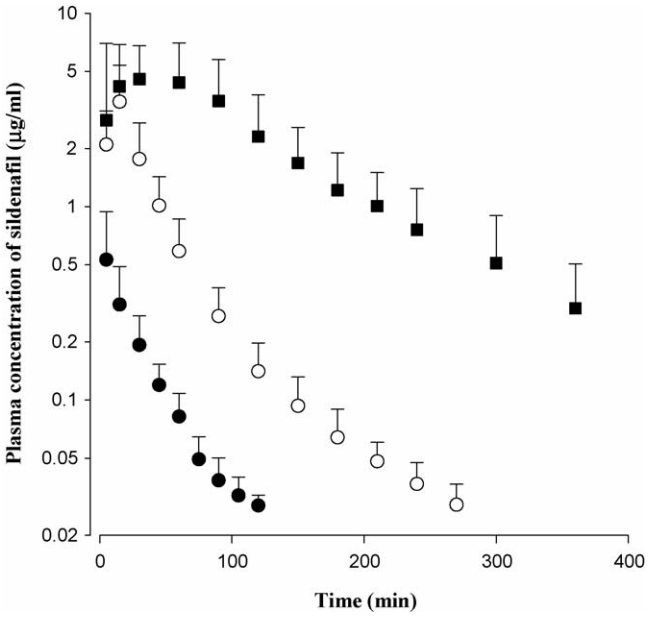

Fig. 2. Mean arterial plasma concentration–time profiles of sildenafil after oral administration at doses of 10 mg/kg (●; $n = 9$), 30 mg/kg (◯; $n = 8$), and 100 mg/kg (■; $n = 8$) in rats. Vertical bars represent S.D.

Table 2
Pharmacokinetic parameters of sildenafil after oral administration at doses of 10, 30, and 100 mg/kg in rats

Parameter	10 mg/kg ($n = 9$)	30 mg/kg ($n = 8$)	100 mg/kg ($n = 8$)
Body weight (g)	249 ± 3.78	299 ± 13.0[a]	250 ± 7.56
AUC[b] (μg min/ml)	16.5 ± 6.95[c]	129 ± 59.5	713 ± 335
Terminal half life (min)	31.3 ± 18.4[c]	59.8 ± 27.8	95.6 ± 24.2
C_{max}[b] (μg/ml)	0.555 ± 0.425	3.58 ± 1.79[a]	5.89 ± 3.42
T_{max} (min)	11.1 ± 8.84	17.5 ± 11.6	38.1 ± 29.1[d]
CL_R (ml/min/kg)	1.02 ± 2.41	1.27 ± 2.28	0.288 ± 0.188
$Ae_{0-24\,h}$ (% of dose)	0.256 ± 0.205	0.597 ± 0.214[a]	0.201 ± 0.0487
$GI_{24\,h}$ (% of dose)	2.55 ± 3.05	0.626 ± 0.978	0.675 ± 1.27
F (%)	5.61	14.6	24.3

Each value represents the mean ± S.D.
[a] 30 mg/kg was significantly different ($P < 0.05$) from 10 and 100 mg/kg.
[b] AUC and C_{max} were normalized to the dose of 10 mg/kg when statistical analysis was performed.
[c] Each dose was significantly different ($P < 0.05$).
[d] 100 mg/kg was significantly different ($P < 0.05$) from 10 and 30 mg/kg.

30, and 100 mg/kg, respectively. The other pharmacokinetic parameters of sildenafil listed in Table 2 were dose-dependent except CL_R and total amount recovered from the gastrointestinal tract (including its contents and feces) at 24 h ($GI_{24\,h}$).

3.3. Measurement of hepatic first-pass effect of sildenafil in rats

The mean arterial plasma concentration–time profiles of sildenafil after intravenous and intraportal administration at a dose of 30 mg/kg in rats are shown in Fig. 3A. The AUC values, 665 ± 119 and 339 ± 157 μg min/ml for intravenous and intraportal administration, respectively, were significantly different between two routes of administration. This suggested that the hepatic first-pass effect of sildenafil after absorption into the portal vein could be approximately 49% in rats.

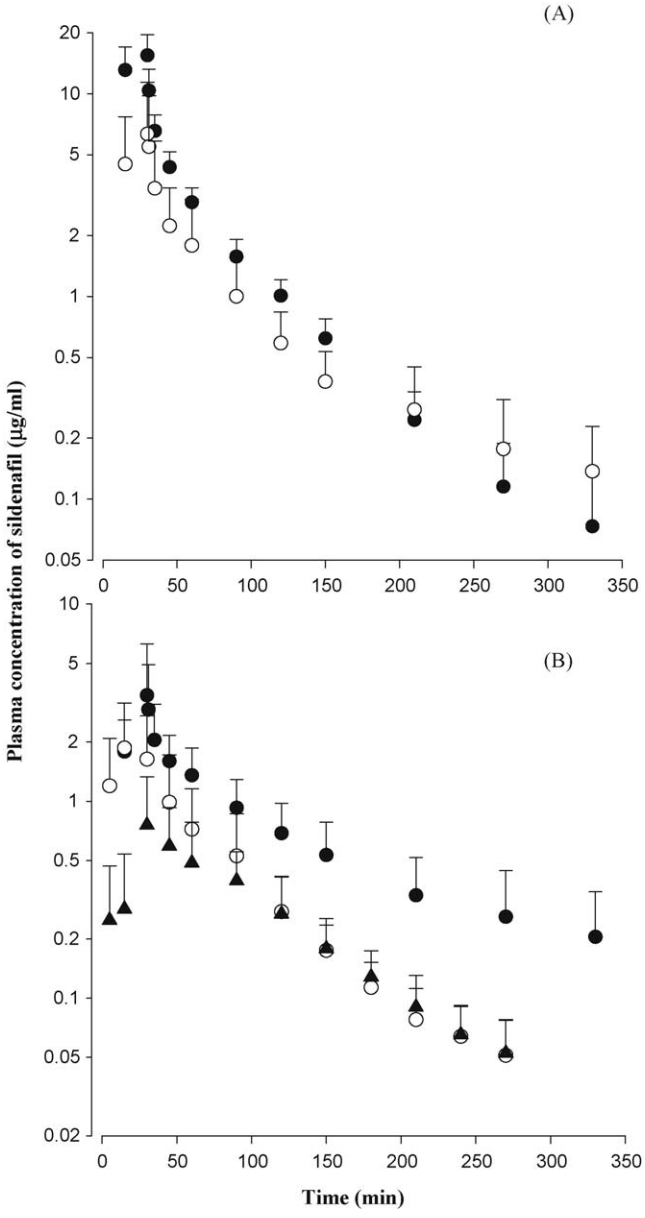

Fig. 3. Mean arterial plasma concentration–time profiles of sildenafil after intravenous (●; $n = 9$) and intraportal (○; $n = 10$) administration (A), and intragastric (▲; $n = 6$), intraduodenal (○; $n = 7$), and intraportal (●; $n = 7$) administration (B) at a dose of 30 mg/kg in rats. Vertical bars represent S.D.

3.4. Measurement of gastric and intestinal first-pass effects of sildenafil in rats

The mean arterial plasma concentration–time curves of sildenafil after intraportal, intragastric, and intraduodenal administration at a dose of 30 mg/kg in rats are shown in Fig. 3B. The AUC values of sildenafil after intragastric and intraduodenal administration (82.5 ± 24.3 and 131 ± 58.7 μg min/ml, respectively) were significantly smaller than that after intraportal administration (288 ± 124 μg min/ml). However, the AUC values were not significantly different between intragastric and intraduodenal administration. The above data suggested that the intestinal first-pass effect of sildenafil is approximately 71.4% and gastric first-pass effect of sildenafil is almost negligible, if any, in rats.

4. Discussion

After intravenous administration of sildenafil in rats, the CL_R values were estimated as free (unbound to plasma proteins) fractions of sildenafil in plasma based on the plasma protein binding of sildenafil in rat plasma (the protein binding of sildenafil to rat plasma was independent of sildenafil concentrations ranging from 0.01–10 μg/ml; the mean value was 95%; Walker et al., 1999) and CL_R values of sildenafil (Table 1); the values were estimated to be 2.40, 1.73, and 4.14 ml/min/kg for 10, 30, and 50 mg/kg, respectively. The 1.73–4.14 ml/min/kg were slower than the reported glomerular filtration rate in rats, 5.24 ml/min/kg (Davies and Morris, 1993). The above data indicated that sildenafil is mainly reabsorbed in rat renal tubules especially at low dose of sildenafil. Considering the CL_R values of sildenafil (Table 1) and reported renal blood flow rate of 36.8 ml/min/kg (Davies and Morris, 1993) and hematocrit of approximately 45% (Mitruka and Rawnsley, 1981) in rats, the estimated renal extraction ratios of sildenafil (CL_R of sildenafil/renal plasma flow rate; only for urinary excretion of unchanged sildenafil) were 0.593, 0.427, and 1.02% for 10, 30, and 50 mg/kg, respectively. The above data indicated that sildenafil is a low renal extraction ratio drug in rats.

The F of sildenafil after oral dose at 30 mg/kg in rats was only 14.6% (Table 2). After oral administration of sildenafil at a dose of 30 mg/kg in rats, the $GI_{24\,h}$ was only 0.626% (Table 2). For comparison, the mean 'true' fraction of oral dose unabsorbed (F_{unabs}) at oral dose of 30 mg/kg was estimated by the reported equation (Lee and Chiou, 1983); the estimated F_{unabs} was 0.626%, suggesting that gastrointestinal absorption of sildenafil was almost complete in rats. Since 0.626% of orally administered sildenafil at a dose of 30 mg/kg was not absorbed from rat gastrointestinal tract up to 24 h and F-value was only 14.6%, approximately 85% (100% − 0.626% − 14.6%) of orally administered sildenafil at a dose of 30 mg/kg could be eliminated by the first-pass effect in rats.

After intravenous administration of sildenafil at doses of 10–50 mg/kg in rats, the CL of 32.8–38.5 ml/min/kg (based on plasma data; Table 1) were considerably slower than the reported cardiac output in rats, 296 ml/min/kg, based on blood data (Davies and Morris, 1993). This suggested that the first-pass effects of sildenafil in the lung and heart could be almost negligible, if any, in rats.

After intragastric instillation of sildenafil at a dose of 30 mg/kg in rats, the AUC of sildenafil was 28.6% of that after intraportal administration, and the AUC values were comparable between intragastric and intraduodenal administration. This suggested that intestinal first-pass effect of sildenafil could be approximately 71.4% and gastric first-pass effect of sildenafil is almost negligible, if any, in rats. Therefore, it could be concluded that approximately 28% (100% − 71.4% − 0.626%) of orally administered sildenafil at a dose of 30 mg/kg could be absorbed into the portal vein.

After intraportal administration of sildenafil at a dose of 30 mg/kg in rats, the AUC of sildenafil was 51.0% of that after intravenous administration at a dose of 30 mg/kg. Hence, the hepatic first-pass effect of sildenafil after absorption into the portal vein could be approximately 49% in rats (the approximately 49% is equivalent to approximately 13.7% of oral dose, since approximately 28% of oral dose was absorbed into the portal vein).

The considerable intestinal first-pass effect of furosemide, azosemide, YH439 (a hepatoprotective agent), YJA-20379-8 (a new reversible proton pump inhibitor), ipriflavone, bumetanide, KR-31543 (a new neuroprotective agent for ischemia-reperfusion damage), SR-4668 (a candidate for diabetic neuropathy), KR-60436 (a new reversible proton pump inhibitor), and oltipraz in rats, and midazolam and saquinavir in humans were reported (Yu et al., 2003, and references therein).

After intravenous administration of sildenafil in rats, the AUC values were dose-independent (Table 1), suggesting that hepatic first-pass effect of sildenafil were not saturated. However, after oral administration of sildenafil in rats, the AUC values were dose-dependent (Table 2). This could be due to saturation of metabolism of sildenafil in rat intestine. The intestinal first-pass effect of sildenafil at a dose of 30 mg/kg was 71.4% as mentioned earlier. Hyland et al. (2001) reported that the hepatic microsomal cytochrome P450 (CYP) 3A4 and to a lesser extent CYP2C9 are involved in the metabolism of sildenafil to form *N*-demethylsildenafil (major circulating metabolite of sildenafil) based on human liver microsomes. Warrington et al. (2002) reported that CYP2C11 contributes to a major way for the formation of *N*-desmethylsildenafil based on liver microsomes of male Fisher 344 rats. CYP3A1(23)/2 also had some activity to form *N*-desmethylsildenafil (Warrington et al., 2002). Human CYP2C9 in the liver and intestine and rat CYP2C11 have 77% homology, and human CYP3A4 in the liver and gastrointestinal tract and rat CYP3A23 have 73% homology (Lewis, 1996). The CYP3A23 (Kaminsky and Fasco, 1991) and 2C11 (Lindell et al., 2003) are present in rat small intestine. Recently, it was obtained from our laboratories that sildenafil was mainly metabolized via CYP3A23 and not via CYP2C11 in male Sprague–Dawley rats. For example, the AUC of sildenafil was significantly greater (60.9% increase) in rats pretreated with troleandomycin (a main inhibitor of CYP3A1/2 in rats; Correia, 1995), but not changed in rats pretreated with sulfaphenazole (a main inhibitor of CYP2C11 in rats; Ogiso et al., 1999).

In conclusion, the low F of sildenafil (14.6%) after oral administration at a dose of 30 mg/kg in rats was mainly due to considerable intestinal first-pass effect (approximately 71% of oral dose). Since the dose-normalized AUC values of sildenafil increased with increasing oral doses (Table 2), the first-pass effects of sildenafil are expected to be changed with different oral doses in rats. However, the changes seemed not to be considerable, because the F values were low; 5.61, 14.6, and 24.3% for oral doses of 10, 30, and 100 mg/kg, respectively (Table 2).

Acknowledgement

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