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Research Paper

Faster clearance of mirodenafil in rats with acute renal failure induced by uranyl nitrate: contribution of increased protein expression of hepatic CYP3A1 and intestinal CYP1A1 and 3A1/2

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Abstract

Objectives It has been reported that mirodenafil is primarily metabolized via hepatic cytochrome P450 (CYP) 1A1/2, 2B1/2, 2D1 and 3A1/2 in rats. It has also been reported that the protein expression of hepatic CYP3A1 and intestinal CYP1A1 and 3A1/2 increases and that of hepatic CYP2D1 decreases in rats with acute renal failure induced by uranyl nitrate (U-ARF rats). Thus, the pharmacokinetics of mirodenafil were studied in control and U-ARF rats.

Methods The pharmacokinetic parameters of mirodenafil and SK3541 (a metabolite of mirodenafil) were compared after the intravenous and oral administration of mirodenafil at a dose of 20 mg/kg to U-ARF and control rats.

Key findings After interavenous administration of mirodenafil to U-ARF rats, the total area under the concentration–time curve (AUC) of mirodenafil was significantly smaller (36.5% decrease) than controls, possibly due to the significantly faster non-renal clearance (66.1% increase; because of increase in the protein expression of hepatic CYP3A1) than controls. After the oral administration of mirodenafil to U-ARF rats, the AUC of mirodenafil was also significantly smaller (47.8% decrease) due to the increase in the protein expression of hepatic CYP3A1 and intestinal CYP1A1 and 3A1/2 compared with controls.

Conclusions After both intravenous and oral administration of mirodenafil to U-ARF rats, the $AUC_{SK3541}/AUC_{mirodenafil}$ ratios were comparable with that in controls and this could be due to further metabolism of SK3541 in rats.

Introduction

The phosphodiesterase type 5 (PDE-5) inhibitors are used to improve male erectile dysfunction by binding cyclic guanosine monophosphate and maintaining sufficient cellular levels in the smooth muscles.[1,2] Thus, mirodenafil, 5-ethyl-2-{5-[4-(2-hydroxyethyl)piperazine-1-sulfonyl]-2-phenyl}-7-propoxypropyl-3,5-dihydro-pyrrolo-[3,2-d]pyrimidin-4-one, was developed (SK Chemicals, Seoul, South Korea) to alleviate drawbacks of PDE-5 inhibitors, such as headache, flushing, nasal congestion and dyspepsia.[3] The IC50 of mirodenafil (0.338 nM) for the inhibition of PDE-5 was lower than that of other PDE-5 inhibitors, such as sildenafil and tadalafil (an internal report). Mirodenafil has recently been marketed in South Korea as Mvix (100 mg tablets; SK Chemicals).

Studies with rat liver microsomes have shown that mirodenafil (SK3530) is converted to twelve metabolites, including SK3541, 5-ethyl-2-(5-(piperazin-1-sulfonyl)-2-n-propoxy-phenyl)-7-n-propyl-3,5-dihydro-4*H*-pyrrolo-[3,2-d]-pyri-midin-4-one and SK3544, 2-(4-(3-(5-ethyl-7-n-propyl-3,5-dihydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-onyl)-4-propoxyphenyl)-sulfonylpiperazin-1-yl)-ethyl-hydrogen sulfate, as a sulfate conjugate of mirodenafil.[4] SK3541 is further metabolized to nine metabolites.[4]

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Choi *et al.*[5] reported that mirodenafil was mainly metabolized via hepatic cytochrome P450 (CYP) 1A1/2, 2B1, 2D1 and 3A1/2 (not via CYP2C6/11) in male Sprague–Dawley rats after pretreatment with various CYP isozyme inducers and inhibitors. It has also been reported that following the intravenous, oral, intraportal, intragastric and intraduodenal administration of mirodenafil at a dose of 20 mg/kg to male Sprague–Dawley rats, the extent of absolute oral bioavailability (F) was 29.4%, the unabsorbed fraction from the gastrointestinal tract for up to 24 h was 2.59% of the oral dose and gastrointestinal and hepatic first-pass effects were 54.3% and 21.3% of the oral dose, respectively.[5]

Moon *et al.*[6] reported that in male Sprague–Dawley rats with acute renal failure induced by uranyl nitrate (U-ARF rats), the protein expression and mRNA levels of hepatic CYP1A2 and 2B1/2 were not altered, but those of CYP3A1 and 2D increased (4 fold increase) and decreased (73.7% decrease), respectively, compared with controls. The mRNA levels of hepatic CYP3A1 and 3A2, however, were not altered compared with controls. This may result from protein stabilization of CYP3A1 (i.e. a decrease in protein turnover). Also Lee *et al.*[7] reported that compared with controls, the protein expression of intestinal CYP1A and 3A subfamilies increased (300% and 156% increase, respectively) in U-ARF rats. Thus, it could have been expected that the pharmacokinetic parameters of intravenous and oral mirodenafil would be altered in U-ARF rats.

Although the pharmacokinetic changes of drugs in U-ARF rats have been reported,[6,7] no studies on mirodenafil with respect to hepatic and intestinal CYP isozyme changes in U-ARF rats have yet been reported. Dachille *et al.*[8] reported that erectile dysfunction is very common in patients with renal failure. Thus, mirodenafil was chosen in this study using U-ARF rats as an animal model. The purpose of this study was to investigate the pharmacokinetic changes of mirodenafil, SK3541 and SK3544 after the intravenous and oral administration of mirodenafil to U-ARF rats compared with controls.

Materials and Methods

Chemicals

Mirodenafil, SK3541 and SK3544 were supplied by SK Chemicals. Uranyl nitrate and polyethylene glycol 400 (PEG 400) were products from BDH Chemicals (Poole, UK) and Showa Chemical Company (Tokyo, Japan), respectively. Sildenafil (internal standard for the high-performance liquid chromatographic (HPLC) analysis of mirodenafil, SK3541 and SK3544), the reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt) and tri(hydroxymethyl)aminomethane (Tris)-buffer were purchased from Sigma–Aldrich Corporation (St Louis, USA). Other chemicals were of reagent or HPLC grade.

Animals

The protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats (5–7 weeks old, 200–285 g) were purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea). The procedures used for keeping and handling of the rats were similar to reported methods.[6,7]

Induction of acute renal failure in rats by uranyl nitrate injection

Uranyl nitrate (freshly dissolved in 0.9% NaCl injectable solution) at a dose of 5 mg (1 ml)/kg was injected once via the tail vein of rats to induce acute renal failure.[6] The same volume of 0.9% NaCl injectable solution was injected into the control rats.

Intravenous and oral studies

The procedures used for the pretreatments of rats, including the cannulation of the jugular vein (for drug administration in the intravenous study) and the carotid artery (for blood sampling), were similar to a reported method.[9] No rat was restrained in this study.

Mirodenafil (dissolved in PEG 400–distilled water, 1 : 1 v/v) at a dose of 20 mg (2 ml)/kg was infused via the jugular vein over 1 min to control (n = 9) and U-ARF (n = 12) rats. A blood sample (approximately 0.12 ml) was collected via the carotid artery at 0 (control), 1 (end of the infusion), 5, 15, 30, 60, 90, 120, 180, 240 and 360 min after the start of the infusion of mirodenafil. A blood sample was immediately centrifuged and 50 μl of a plasma sample was stored at –70°C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, USA) until used for the HPLC analysis of mirodenafil, SK3541 and SK3544.[10] The procedures used for the preparation and handling of the 24-h urine sample ($Ae_{0-24\ h}$) and the entire gastrointestinal tract (including its contents and faeces) sample at 24 h ($GI_{24\ h}$) were similar to a reported method.[11]

Mirodenafil (the same solution used in the intravenous study) at a dose of 20 mg (6 ml)/kg was administered orally using a gastric gavage tube to control (n = 8) and U-ARF (n = 8) rats. A blood sample was collected at 0, 5, 15, 30, 60, 90, 120, 180, 240 and 360 min after the oral administration of mirodenafil. Other procedures were similar to those for the intravenous study.

Measurement of V_{max}, K_m and CL_{int} for the disappearance of mirodenafil and in the formation of SK3541 in hepatic and intestinal microsomes

The procedures used for the preparation of hepatic[6] and intestinal[7] microsomes were similar to reported methods. Protein contents in hepatic and intestinal microsomes were measured using a reported method.[12]

The V_{max} (the maximum velocity) and the K_m (apparent Michaelis–Menten constant; the concentration at which the rate is one-half of the V_{max}) for the disappearance of mirodenafil and in the formation of SK3541 were determined after incubating the above microsomal fractions (equivalent to 0.5 and 1.0 mg protein for the hepatic and intestinal microsomes, respectively), 5 μl of methanol containing the final mirodenafil concentrations of 1, 5, 10, 20, 50 and 100 μM (for the hepatic microsomes) or 5, 10, 20, 50 and 100 μM (for the intestinal microsomes), and 50 μl of 0.1 M phosphate buffer

(pH 7.4) containing 1 mM NADPH. The final volume was adjusted to 0.5 ml by adding 0.1 M phosphate buffer (pH 7.4) in a water-bath shaker (37°C, 50 oscillations per min (opm)). Incubation times were 5, 10, 15, 45 and 60 min, respectively. All of the above microsomal incubation conditions were in the linear range of the reaction rate. The reaction was terminated by addition of 1 ml of acetonitrile containing 5 μg/ml of sildenafil (internal standard) after 15 and 60 min incubation for the hepatic and intestinal microsomes, respectively. Mirodenafil and SK3541 were measured by the HPLC method.[10]

The kinetic constants (K_m and V_{max}) for the disappearance of mirodenafil and in the formation of SK3541 were calculated using a non-linear regression method.[13] The intrinsic clearance (CL_{int}) values for the disappearance of mirodenafil and in the formation of SK3541 were calculated by dividing the V_{max} by the K_m.

Measurement of rat plasma protein binding of mirodenafil using equilibrium dialysis

Protein binding values of mirodenafil to fresh plasma from control and U-ARF rats ($n = 5$ for each group) were measured using equilibrium dialysis.[11] Plasma (1 ml) was dialysed against 1 ml of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran ('the buffer') in a 1-ml dialysis cell (Spectrum Medical Industries, Los Angeles, USA) using a Spectra/Por 4 membrane (MW cut-off 12–14 kDa; Spectrum Medical Industries). After 24-h incubation, two 50-μl volumes were collected from each compartment and stored at –70°C until used for the HPLC analysis of mirodenafil.[10]

HPLC analysis of mirodenafil, SK3541 and SK3544

Concentrations of mirodenafil, SK3541 and SK3544 in the samples were determined using an HPLC method developed in our laboratories.[10] Briefly, 75 μl of acetonitrile containing 1 μg/ml sildenafil (internal standard) was added to 50 μl of sample. After vortex-mixing for 1 min and centrifugation (15 000 *g*, 10 min), 50 μl of the supernatant was directly injected onto a reversed-phase (C_{18}; Symmetry; 100 mm × 4.6 mm i.d.; particle size, 3.5 μm; Waters, Milford, USA) HPLC column. The mobile phase, 20 mM ammonium acetate–acetonitrile (52 : 48, v/v), was run at a flow rate of 1.4 ml/min, and the column eluent was monitored using an ultraviolet detector at 254 nm at room temperature. The retention times of SK3544, sildenafil (internal standard), SK3541 and mirodenafil were approximately 4.0, 5.6, 7.0 and 8.3 min, respectively. The detection limits of mirodenafil in rat plasma and urine samples were both 0.03 μg/ml. The corresponding values of SK3541 and SK3544 in rat plasma and urine samples were all 0.1 μg/ml. The coefficients of variation (intra- and inter-day) were below 9.83%.

Pharmacokinetic analysis

Standard methods[14] were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, USA): the total area under the plasma concentration–time curve from time zero to time infinity (AUC; Chiou 1978),[15] the time-averaged total body, renal, and non-renal clearances (CL, CL_R and CL_{NR}, respectively), the terminal half-life, the first moment of AUC (AUMC), the mean residence time (MRT), the apparent volume of distribution at steady state (V_{ss}) and the F.[9] The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the extrapolated data.

Statistical analysis

$P < 0.05$ was deemed to be statistically significant using Student's t-test between the two means for the unpaired data. All results are expressed as mean ± SD, except median (ranges) for T_{max}.

Results

Pharmacokinetics of mirodenafil, SK3541 and SK3544 after the intravenous administration of mirodenafil to rats

For the intravenous administration of mirodenafil at a dose of 20 mg/kg to control and U-ARF rats, the mean arterial plasma concentration–time curves of mirodenafil and SK3541 are shown in Figures 1a and 1b, respectively. The relevant pharmacokinetic parameters including SK3544 are listed in Table 1. Compared with controls, changes in the pharmacokinetic parameters of mirodenafil in U-ARF rats were as follows: the AUC was significantly smaller (36.5% decrease), V_{ss} was significantly larger (102% increase), CL and CL_{NR} were significantly faster (65.8% and 66.1% increase, respectively), CL_R was significantly slower (39.4% decrease) and $Ae_{0-24\ h}$ was significantly smaller (62.7% decrease). The CL_R/CL ratio was 0.449% and 0.164% for control and U-ARF rats, respectively. Thus, changes in the CL_R could not affect other pharmacokinetic parameters of mirodenafil.

After the intravenous administration of mirodenafil, formation of SK3541 was rapid; SK3541 was detected in the plasma at the second blood sampling time point (15 min) and rapidly reached T_{max} (15 min) for both groups of rats. Compared with controls, changes in the pharmacokinetic parameters of SK3541 in U-ARF rats were as follows: the AUC was significantly smaller (28.4% decrease), C_{max} was significantly lower (44.3% decrease) and terminal half-life was significantly longer (60.6% increase). The $Ae_{0-24\ h}$ and $GI_{24\ h}$ of SK3544 (expressed in terms of the intravenous dose of mirodenafil) listed in Table 1 were not significantly different between the two groups of rats. SK3544 was below the detection limit in the plasma for all blood samples collected from both groups of rats.

Pharmacokinetics of mirodenafil, SK3541 and SK3544 after oral administration of mirodenafil to rats

For the oral administration of mirodenafil at a dose of 20 mg/kg to both groups of rats, the mean arterial plasma concentration–time curves of mirodenafil and SK3541 are shown in Figures 2a and 2b, respectively. The relevant pharmacokinetic parameters, including those of SK3544, are listed in Table 2. Absorption of mirodenafil from the rat gastrointestinal tract was rapid; mirodenafil was detected

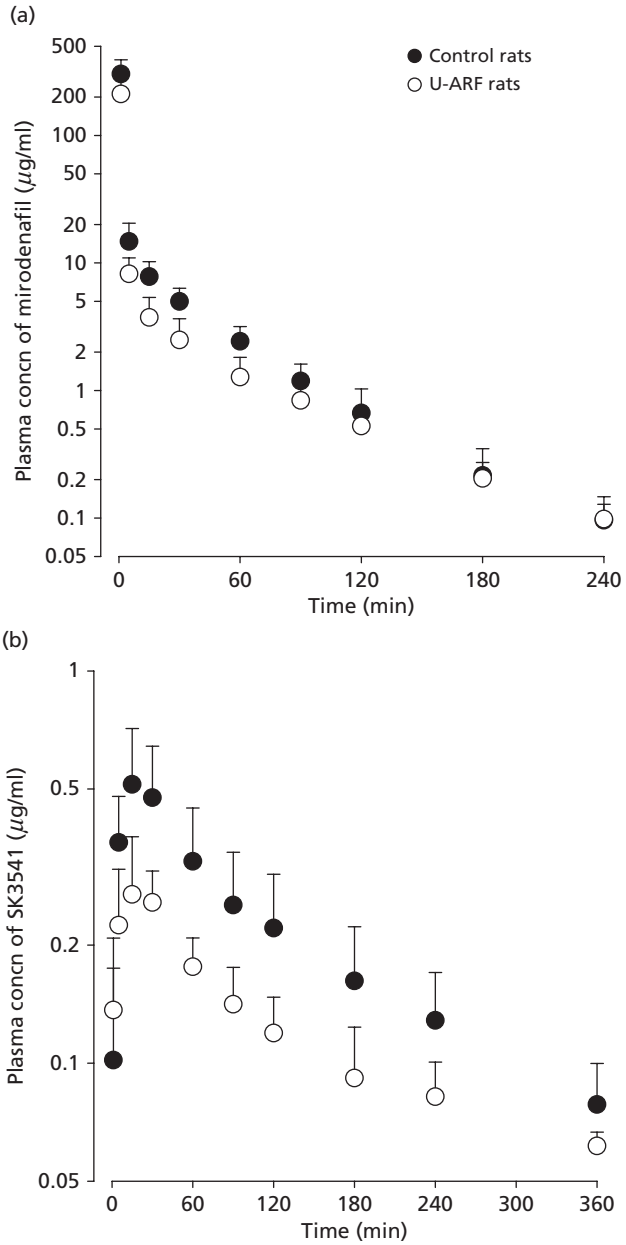

Figure 1 Mean arterial plasma concentration–time profiles of (a) mirodenafil and (b) SK3541 after intravenous administration of mirodenafil. Mirodenafil was administered as a 1-min intravenous infusion at a dose of 20 mg/kg to control (n = 9) and rats with uranyl nitrate-induced renal failure (U-ARF) (n = 12) rats. Vertical bars represent SD.

in the plasma at the second sampling time point (15 min) and rapidly reached T_{max} (30 min) for both groups of rats. Compared with controls, changes in the pharmacokinetic parameters of mirodenafil in U-ARF rats were as follows: the AUC was significantly smaller (47.8% decrease), CL_R became significantly slower (48.9% decrease) and $Ae_{0-24\ h}$ was significantly smaller (78.0% decrease).

After the oral administration of mirodenafil, the formation of SK3541 was also rapid; SK3541 was detected in the plasma at the second blood sampling time point (15 min) and rapidly reached T_{max} (60 min) for both groups of rats. Compared with controls, changes in the pharmacokinetic parameters of SK3541 in U-ARF rats were as follow: the AUC was significantly smaller (35.9% decrease), CL_R was significantly slower (65.5% decrease) and $Ae_{0-24\ h}$ (expressed in terms of the oral dose of mirodenafil) was significantly smaller (65.6% decrease). The $Ae_{0-24\ h}$ and $GI_{24\ h}$ of SK3544 (expressed in terms of the oral dose of mirodenafil), listed in Table 2, were not significantly different between two groups of rats. SK3544 was also below the detection limit in the plasma for all blood samples collected from both groups of rats.

V_{max}, K_m and CL_{int} for the disappearance of mirodenafil and in the formation of SK3541 in hepatic and intestinal microsomes

The V_{max}, K_m, and CL_{int} for the disappearance of mirodenafil and in the formation of SK3541 in hepatic microsomes from both groups of rats are listed in Table 3. Compared with controls, both the V_{max} and the K_m values for the disappearance of mirodenafil and the formation of SK3541 were comparable with those in U-ARF rats, suggesting that the maximum velocity for the disappearance (primarily metabolism) of mirodenafil and the formation of SK3541, and the affinity of the enzyme(s) for the mirodenafil were not altered in U-ARF rats. However, compared with controls, the CL_{int} values for both the disappearance of mirodenafil and the formation of SK3541 were significantly faster and considerably slower (21.6% increase and 12.5% decrease, respectively) in U-ARF rats, suggesting that the metabolism of mirodenafil and the formation of SK3541 increased and decreased, respectively, in U-ARF rats.

The V_{max}, K_m, and CL_{int} for the disappearance of mirodenafil and the formation of SK3541 in intestinal microsomes from both groups of rats are also listed in Table 3. Compared with controls, the V_{max} and K_m for the disappearance of mirodenafil were significantly smaller (87.5% and 79.7% decrease, respectively) in U-ARF rats. Compared with controls, the CL_{int} for the disappearance of mirodenafil became considerably faster (60.0% increase) in U-ARF rats, suggesting that the metabolism of mirodenafil increased in U-ARF rats. Compared with controls, the V_{max}, K_m and CL_{int} in the formation of SK3541 were comparable in U-ARF rats.

Rat plasma protein binding of mirodenafil using equilibrium dialysis

The protein binding value of mirodenafil to fresh plasma from U-ARF rats, 85.8 ± 14.2%, was significantly smaller (5.30% decrease) than that of 90.6 ± 9.35% in control rats. The adsorption of mirodenafil to the equilibrium dialysis apparatus, which included the semi-permeable membrane, was almost negligible, and 93.9–115% of the spiked amounts of mirodenafil were recovered from both plasma and 'the buffer' compartments. The binding of mirodenafil to 4% human serum albumin was independent of mirodenafil concentrations ranging from 1 to 100 μg/ml; the mean value was 92.1%.[5] Thus, a 1 μg/ml concentration of mirodenafil was chosen for this plasma protein binding study.

Table 1 Pharmacokinetic parameters of mirodenafil, SK3541 and SK3544 after the intravenous administration of mirodenafil to control and U-ARF rats

Parameter	Control (n = 9)	U-ARF (n = 12)
Initial body weight (g)	244 ± 5.27	244 ± 4.98
Final body weight (g)	277 ± 9.39	250 ± 6.89*
Mirodenafil		
AUC (μg min/ml)	949 ± 211	603 ± 187*
Terminal half-life (min)	40.2 ± 5.62	46.6 ± 8.15
MRT (min)	22.8 ± 8.46	27.2 ± 5.97
V_{ss} (ml/kg)	506 ± 241	1020 ± 555**
CL (ml/min per kg)	21.9 ± 4.22	36.3 ± 11.5***
CL_R (ml/min per kg)	0.0983 ± 0.0331	0.0596 ± 0.0281***
CL_{NR} (ml/min per kg)	21.8 ± 4.20	36.2 ± 11.5***
$Ae_{0-24\ h}$ (% of mirodenafil dose)	0.445 ± 0.0998	0.166 ± 0.0644*
$GI_{24\ h}$ (% of mirodenafil dose)	0.245 ± 0.262	0.442 ± 0.480
SK3541		
AUC (μg min/ml)	88.5 ± 27.8	63.4 ± 11.7**
C_{max} (μg/ml)	0.539 ± 0.184	0.300 ± 0.0723**
T_{max} (min)	15 (15–30)	15 (15–30)
Terminal half-life (min)	160 ± 40.7	257 ± 121**
CL_R (ml/min/kg)	0.0514 ± 0.0312	0.0690 ± 0.0810
$Ae_{0-24\ h}$ (% of mirodenafil dose)	0.0474 ± 0.0323	0.0468 ± 0.0563
$GI_{24\ h}$ (% of mirodenafil dose)	0.0725 ± 0.0274	0.0740 ± 0.0415
$AUC_{SK3541}/AUC_{mirodenafil}$ (%)	9.52 ± 3.21	11.5 ± 4.40
SK3544		
$Ae_{0-24\ h}$ (% of mirodenafil dose)	0.656 ± 0.739	0.715 ± 0.438
$GI_{24\ h}$ (% of mirodenafil dose)	9.37 ± 5.79	8.78 ± 8.00

Mirodenafil was administered to control and U-ARF rats at a dose of 20 mg/kg. U-ARF, uranyl nitrate-induced acute renal failure; AUC, total area under the plasma concentration–time curve from time zero to time infinity; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged non-renal clearance; V_{ss}, apparent volume of distribution at steady state; MRT, mean residence time; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}; $Ae_{0-24\ h}$, percentage of the dose excreted in the 24-h urine; $GI_{24\ h}$, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h. Values are mean ± SD, except median (ranges) for T_{max}. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with controls.

Discussion

It is well known that acute renal failure is evident in U-ARF rats, as demonstrated by a higher plasma level of urea nitrogen, slower creatinine clearance, smaller urine output and smaller body weight gain compared with control rats.[6,7,16] Kidney histology also supports the induction of ARF; there is extensive acute tubular necrosis involving the distal convoluted tubules.[16]

The AUCs of mirodenafil were dose-proportional after its intravenous (5, 10 and 20 mg/kg) and oral (10 and 20 mg/kg) administration to male Sprague–Dawley rats.[5] Thus, a dose of 20 mg/kg of mirodenafil was chosen for this study.

The contribution of the gastrointestinal (including the biliary) excretion of unchanged mirodenafil to the CL_{NR} of the drug was almost negligible; the $GI_{24\ h}$ values were 0.245% and 0.442% of the intravenous dose for control and U-ARF rats, respectively (Table 1). However, the smaller values of $GI_{24\ h}$, 0.245% and 0.442%, were not likely due to the chemical and enzymatic degradation of mirodenafil in rats' gastric fluids; mirodenafil was stable for up to 24 h incubation in various buffer solutions having pHs ranging from 1 to 13 and in three rats' gastric juices having pHs of 3, 4 and 3.5, respectively.[5] Moreover, it has been reported that after the intravenous administration of mirodenafil at a dose of 20 mg/kg to the control rats with bile duct cannulation, the 24-h biliary excretion of unchanged mirodenafil was almost negligible, 0.181 ± 0.0341%.[5] Thus, the CL_{NR} of mirodenafil listed in Table 1 could have represented its metabolic clearance. Additionally, changes in the CL_{NR} of mirodenafil could have represented changes in its metabolism in rats.

After the intravenous administration of mirodenafil to U-ARF rats, the AUC of mirodenafil was significantly smaller, possibly as a result of the significantly faster CL than controls (Table 1). The faster CL of mirodenafil could have mainly been due to its faster CL_{NR} than controls because the contribution of the CL_R to the CL of the drug was almost negligible (Table 1). The hepatic first-pass effect of mirodenafil after absorption into the portal vein was 21.4% in male Sprague–Dawley rats.[5] Because mirodenafil is a drug with a low hepatic extraction ratio, its hepatic clearance depends more on the CL_{int} than on the hepatic blood flow rate in rats.[17] Thus, the faster CL_{NR} of mirodenafil in U-ARF rats (Table 1) could have been due to the faster hepatic CL_{int} for the disappearance of mirodenafil (Table 3) because of the increase in the protein expression of hepatic CYP3A1.[6] This suggests that the contribution of decrease in the protein expression of CYP2D1 in U-ARF rats to the hepatic metabolism of mirodenafil was not considerable

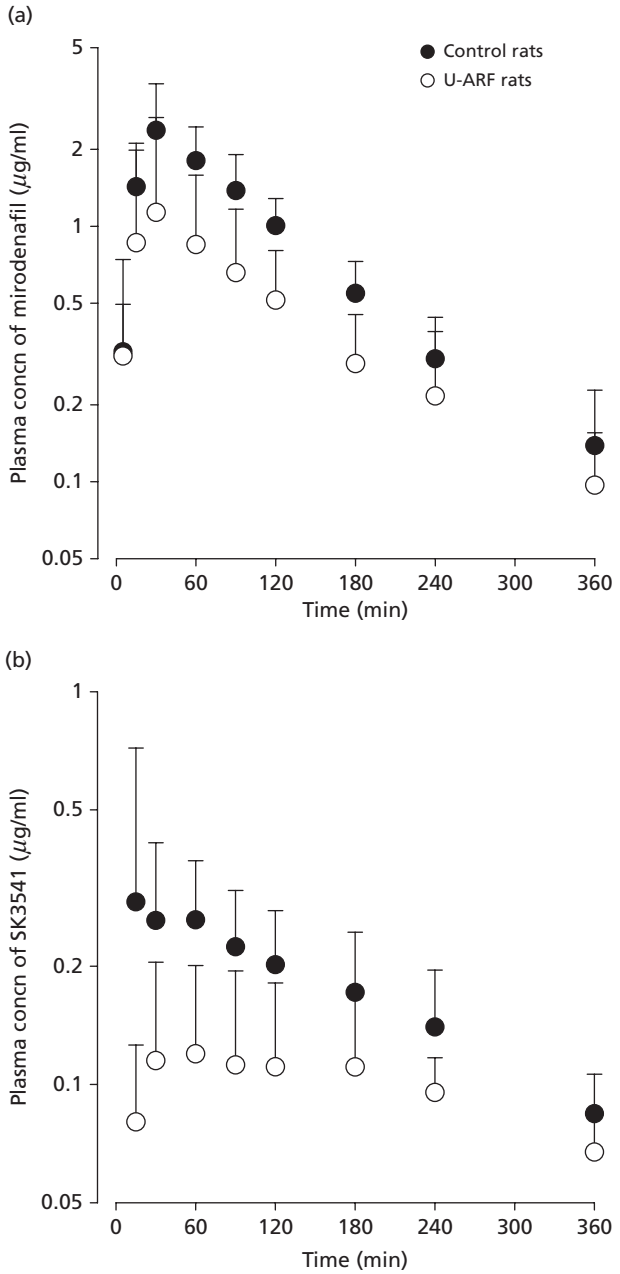

Figure 2 Mean arterial plasma concentration–time profiles of (a) mirodenafil and (b) SK3541 after the oral administration of mirodenafil. Mirodenafil was administered at a dose of 20 mg/kg to control ($n = 8$) and rats with uranyl nitrate-induced renal failure (U-ARF) ($n = 8$) rats. Vertical bars represent SD.

compared with the increase in the protein expression of CYP3A1.[6]

After the intravenous administration of mirodenafil to U-ARF rats, the V_{ss} of mirodenafil was significantly larger than controls (Table 1). This could have been due to the significantly greater (51.5% increase) free fractions of mirodenafil in U-ARF rats.

After the oral administration of mirodenafil to U-ARF rats, the AUC of the drug was also significantly smaller than

Table 2 Pharmacokinetic parameters of mirodenafil, SK3541 and SK3544 after the oral administration of mirodenafil to control and U-ARF rats

Parameter	Control ($n = 8$)	U-ARF ($n = 8$)
Initial body weight (g)	250 ± 2.67	249 ± 4.96
Final body weight (g)	274 ± 7.91	255 ± 16.5*
Mirodenafil		
AUC (μg min/ml)	289 ± 51.8	151 ± 95.3**
C_{max} (μg/ml)	2.46 ± 1.15	1.37 ± 1.45
T_{max} (min)	30 (15–90)	30 (15–90)
Terminal half-life (min)	81.6 ± 23.2	86.6 ± 34.9
CL_R (ml/min per kg)	0.0789 ± 0.0345	0.0403 ± 0.0255*
$Ae_{0-24\ h}$ (% of mirodenafil dose)	0.116 ± 0.0592	0.0255 ± 0.0123*
$GI_{24\ h}$ (% of mirodenafil dose)	1.44 ± 1.29	3.61 ± 4.06
F (%)	30.5	25.0
SK3541		
AUC (μg min/ml)	74.4 ± 22.0	47.7 ± 24.4*
C_{max} (μg/ml)	0.369 ± 0.336	0.145 ± 0.0752
T_{max} (min)	60 (30–180)	60 (30–90)
Terminal half-life (min)	139 ± 37.5	183 ± 75.6
CL_R (ml/min per kg)	0.237 ± 0.141	0.0818 ± 0.0756*
$Ae_{0-24\ h}$ (% of mirodenafil dose)	0.182 ± 0.108	0.0626 ± 0.0579*
$GI_{24\ h}$ (% of mirodenafil dose)	0.275 ± 0.279	0.0649 ± 0.0331
$AUC_{SK3541}/AUC_{mirodenafil}$ (%)	25.5 ± 5.98	32.8 ± 7.62
SK3544		
$Ae_{0-24\ h}$ (% of mirodenafil dose)	1.82 ± 1.13	1.81 ± 3.00
$GI_{24\ h}$ (% of mirodenafil dose)	10.3 ± 6.42	14.3 ± 13.9

Mirodenafil was administered to control and U-ARF rats at a dose of 20 mg/kg. U-ARF, uranyl nitrate-induced acute renal failure; AUC, total area under the plasma concentration–time curve from time zero to time infinity; CL_R, time-averaged renal clearance; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}; $Ae_{0-24\ h}$, percentage of the dose excreted in the 24-h urine; $GI_{24\ h}$, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h; F, extent of absolute oral bioavailability. Values are mean ± SD, except median (ranges) for T_{max}. *$P < 0.05$ and **$P < 0.01$ compared with controls.

controls (Table 2). However, this was not likely due to the decrease in the absorption of mirodenafil from the gastrointestinal tract in U-ARF rats. After the oral administration of mirodenafil, the $GI_{24\ h}$ values were 0.444% and 3.61% of the dose for control and U-ARF rats, respectively (Table 2). It is possible that this unchanged mirodenafil, 0.444% and 3.61%, might be partly attributed to the gastrointestinal (including the biliary) excretion of the absorbed drug. Based on the linear pharmacokinetics,[5] the 'true' mean fractions of the oral dose unabsorbed ('F_{unabs}') in this study could be estimated by the following equations:[18]

$$0.0144 = \text{'}F_{unabs}\text{'} + (0.345 \times 0.0245) \text{ for control rats} \quad (1)$$

$$0.0361 = \text{'}F_{unabs}\text{'} + (0.250 \times 0.0442) \text{ for U-ARF rats} \quad (2)$$

in which 0.345 (0.250) and 0.0245 (0.0442) are the F (Table 2) and $GI_{24\ h}$ values after the intravenous administration of the drug (Table 1) in control rats (U-ARF rats), respectively. The 'F_{unabs}' values thus estimated were 0.595% and 2.51% for control and U-ARF rats, respectively, indicating

Table 3 V_{max}, K_m and CL_{int} for the disappearance of mirodenafil and in the formation of SK3541 in microsomes prepared from the livers and intestines of control and U-ARF rats

Preparation	Parameter	Control (n = 5)	U-ARF (n = 5)
Hepatic microsomes	Disappearance of mirodenafil		
	V_{max} (nmol/min per mg protein)	50.9 ± 21.7	41.5 ± 28.8
	K_m (μM)	3.13 ± 1.38	2.91 ± 1.66
	CL_{int} (ml/min per mg protein)	0.0611 ± 0.00151	0.0743 ± 0.00974*
	Formation of SK3541		
	V_{max} (nmol/min per mg protein)	113 ± 28.3	169 ± 65.0
	K_m (μM)	9.66 ± 2.15	12.8 ± 4.87
	CL_{int} (ml/min per mg protein)	0.0866 ± 0.0107	0.0758 ± 0.00451**
Intestinal microsomes	Disappearance of mirodenafil		
	V_{max} (nmol/min per mg protein)	220 ± 135	27.5 ± 11.4*
	K_m (μM)	0.336 ± 0.198	0.0683 ± 0.0419*
	CL_{int} (ml/min per mg protein)	0.00175 ± 0.000719	0.00280 ± 0.00103***
	Formation of SK3541		
	V_{max} (nmol/min per mg protein)	75.7 ± 71.1	50.7 ± 24.8
	K_m (μM)	0.319 ± 0.430	0.167 ± 0.0461
	CL_{int} (ml/min per mg protein)	0.00312 ± 0.00283	0.00359 ± 0.00109

U-ARF, uranyl nitrate-induced acute renal failure; V_{max}, maximum velocity; K_m, apparent Michaelis–Menten constant; CL_{int}, intrinsic clearance. Values are mean ± SD. *$P < 0.05$, **$P = 0.0690$ and ***$P = 0.0950$ compared with controls.

that the contribution of the gastrointestinal (including the biliary) excretion of the absorbed drug to the total drug recovered from the gastrointestinal tract following the oral administration of the drug was almost negligible, 0.0144% and 0.0361% for control and U-ARF rats, respectively. Thus, approximately 97% of the oral dose was absorbed from the gastrointestinal tract for both groups of rats.

After the oral administration of mirodenafil to U-ARF rats, the AUC of the drug was significantly smaller (47.8% decrease) than in controls (Table 2). This could have been due to increase in intestinal metabolism of mirodenafil in addition to increase in hepatic metabolism of mirodenafil in U-ARF rats. Lee *et al.*[7] reported that the protein expression of the intestinal CYP1A and 3A1/2 significantly increased in U-ARF rats compared with controls. It has been reported that the CYP1A and 3A subfamily are most abundant, but the CYP2B1 and 2D subfamily are expressed at a very low level in rats' intestine.[19,20] Note that the terminal-half lives of mirodenafil after the oral administration of the drug were considerably longer than those after the intravenous administration (Table 1). This could have been due to the flip-flop pattern of oral administration of mirodenafil in rats.[5]

Unexpectedly, after both intravenous and oral administration of mirodenafil to U-ARF rats, the formation of SK3541 was comparable between the two groups of rats; although the AUCs of SK3541 were significantly greater than controls, the $AUC_{SK3541}/AUC_{mirodenafil}$ ratios were comparable between two groups of rats (Tables 1 and 2). This could have been due to the fact that SK3541 is further metabolized.[4] For example, after the intravenous administration of mirodenafil to rats pretreated with 3-methylcholanthrene (a main inducer of CYP1A1/2 in rats), orphenadrine citrate (a main inducer of CYP2B1/2 in rats) and dexamethasone phosphate (a main inducer of CYP3A1/2 in rats), the $AUC_{SK3541}/AUC_{mirodenafil}$ ratios were also comparable with controls.[5] Moreover, unknown metabolites were found in HPLC chromatograms after 20 min incubation of SK3541 in hepatic microsomes.[5]

The CYP isozyme changes in humans with renal insufficiency have not been thoroughly studied. The dissimilarities between rat and human CYP isozymes have been reported; the species-specific isoforms of CYP1A, 2C, 2D and 3A show appreciable differences.[21] However, homologies (%) of proteins between human and rat CYP isozymes have been reported.[22] Thus, the present rat data should be carefully extrapolated to humans.

Conclusions

In conclusion, after the intravenous administration of mirodenafil to U-ARF rats, the CL_{NR} (AUC) of mirodenafil was significantly faster (smaller) than in controls. After the oral administration of mirodenafil to U-ARF rats, the AUC of mirodenafil was also significantly smaller than in controls. These results could have been due to the increase in the protein expression of hepatic CYP3A1 or intestinal CYP1A1 and 3A1/2 compared with controls.

Declarations

Conflict of interest

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

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