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Short communication

Determination of mirodenafil and sildenafil in the plasma and corpus cavernous of SD male rats

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ABSTRACT

The purpose of the present study was to determine sildenafil and a novel PDE-5 inhibitor, mirodenafil in the plasma and corpus cavernosum tissue of rats to compare their pharmacokinetic properties. The concentrations of mirodenafil and sildenafil in the rat plasma and corpus cavernosum tissue samples were analyzed using LC–MS/MS after a single oral administration at a dose of 40 mg/kg to rats. Although the T_{max}, $T\lambda_{1/2}$ and MRT were not different between mirodenafil and sildenafil, the C_{max} and AUC of mirodenafil were significantly higher than those of sildenafil in the plasma and corpus cavernosum tissue. Consequently mirodenafil remained longer than sildenafil in the plasma and tissue. This may provide pharmacokinetic evidence for assessment of the in vivo efficacy of mirodenafil and sildenafil.

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, Viagra®) is a well-known phosphodiesterase type-5 (PDE-5) inhibitor with a high efficacy for the treatment of male erectile dysfunction (MED). However, clinically important adverse effects of sildenafil such as headache, facial flushing, dyspepsia, and visual disturbance have been reported [1–3]. To alleviate these adverse effects of sildenafil, novel sildenafil derivatives including vardenafil, tadalafil, avanafil, and udenafil have been developed as Viagra® alternatives [1]. Mirodenafil (Mvix®) is also one of the sildenafil derivatives synthesized as a Viagra® alternative. Mirodenafil showed higher efficacy than sildenafil in the *in vitro* pharmacological test (internal data) and recently, has come into market.

However, obvious pharmacokinetic differences between sildenafil and later drugs including mirodenafil such as a faster time-to-onset, longer half-life time and better safety profile are required to be the post-Viagra® and to be considered a truly better option for patients [1]. In this context, we compared pharmacokinetics of these two drugs in the plasma and corpus cavernosum tissue of rats to assess their in vivo efficacy.

In the present study, sildenafil and mirodenafil in rat plasma and corpus cavernosum samples were determined using LC–MS/MS. Based on the quantitative results, plasma pharmacokinetics and distribution in the target tissue of these two drugs were evaluated.

2. Materials and methods

2.1. Chemicals

Mirodenafil HCl and sildenafil citrate were provided by SK Chemical Ltd (Suwon, Korea) with a chemical purity of 99% (Fig. 1). KJH-1045 as an internal standard used in this study was provided from Chemoinformatics Center, Korea Institute of Science and Technology (Seoul, Korea). Ammonium formate was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile (ACN) was HPLC-grade from Mallinckrodt Baker Inc. (NJ, USA). All other chemicals were of analytical grade and used as received.

2.2. Animal treatments

Specific pathogen-free male Sprague–Dawley (SD) rats (250–280 g) were obtained from the Samtako Inc. (Osan, Korea). The animals received at 5–6 weeks of age were acclimated for at least 1 week. Upon arrival, animals were randomized and housed three per cage. The animal quarters were strictly maintained at 23 ± 3 °C and 50 ± 10% relative humidity. A 12 h light and dark cycle was used with an intensity of 150–300 lx. All animal procedures

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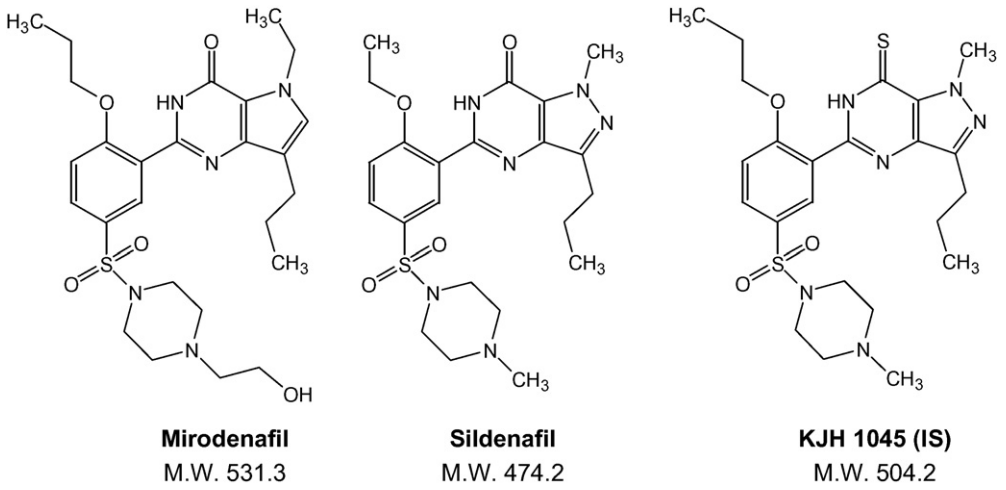

Fig. 1. Chemical structures of mirodenafil, sildenafil and IS (KJH-1045).

were followed based on a guideline recommended by the Society of Toxicology (USA) in 1989.

Mirodenafil HCl was dissolved in 40% PEG solution in water at a dose of 40 mg/kg as a free form [3]. Sildenafil citrate dissolved in distilled water at a dose of 40 mg/kg as a free form and adjusted to a final pH of approximately 4–4.5 with HCl. Then, these solutions were orally administered to rats ($n = 5$) [2]. The rats were fasted overnight before drug administration and until 6 h after dosing.

2.3. Pharmacokinetic experiment and distribution

After an oral administration of male SD rats with mirodenafil HCl and sildenafil citrate at 40 mg/kg, approximately 6 ml of blood was obtained by heart puncture and rat corpus cavernosum immediately removed at the time points of 1, 2, 4, 6, 10 and 12 h after dosing. Plasma samples were harvested after centrifugation of the blood samples at 3000 × *g* for 10 min at 4 °C and stored frozen at −70 °C until analyzed. The corpus cavernosum was rapidly dissected, washed with 0.1 M PBS buffer, weighed and stored frozen at −70 °C until analyzed.

An Internal standard solution (5 μl of 1 μg/ml of KJH-1045) was added to the 100 μl of plasma samples, 100 μl of 50 mM NaOH added, and 700 μl of ethylacetate added for extraction. After mixing and centrifugation, 630 μl of the organic layer was taken and dried under a stream of nitrogen gas. The residue was reconstituted with 50 μl of 50% MeOH in 0.1% formic acid and injected into an HPLC column.

For the determination of mirodenafil and sildenafil in the corpus cavernosum samples, 1 volume of the thawed tissue was diluted with 2 volumes of 50 mM NaOH and 6 volumes of distilled water. The mixture was homogenized, centrifuged, and 800 μl of the supernatant liquid was mixed with 10 μl of 1 μg/ml of KJH-1045 and 3 ml ethylacetate. After mixing and centrifugation, 2.4 ml of the organic layer was taken and dried under a stream of nitrogen gas. The residue was reconstituted with 100 μl of 50% MeOH with 0.1% formic acid and injected into an HPLC column.

2.4. LC–MS/MS analysis

The HPLC–MS system consisted of a surveyor HPLC system (Thermo Finnigan, San Jose, CA, USA) and triple quadruple mass spectrometry (TSQ Quantum Discovery MAX, Thermo Scientific) equipped with a electrospray ionization (ESI) source. The column used for the separation was a CapcellPak phenyl (2.1 mm × 150 mm, 5 μm) [3]. Column temperature was maintained constant at 40 °C using a thermostatically controlled column oven. The HPLC mobile phases consisted of 5 mM ammonium formate at pH 6.0 (A) and 90% acetonitrile in 5 mM ammonium formate at pH 6.0 (B). A gradient program was used for the HPLC separation with a flow rate of 0.2 ml/min. The initial composition was 20% B, programmed linearly to 80% B after 0.5 min, and maintained for 2.5 min.

Nitrogen was used both as a sheath gas at a pressure of 30 arb and an ion sweep gas at 10 arb. Capillary temperature was set at 320 °C, vaporizer temperature 270 °C, and the spray voltage 3.2 kV. Multiple reaction monitoring (MRM) detection in positive ion mode was employed; each transition monitored were 532.4 → 296.2 for mirodenafil, 475.3 → 283.1 for sildenafil, and 505.2 → 299.1 for KJH-1045 (IS) [4,5].

2.5. Method validation

2.5.1. Linearity and calibration curve

The calibration curves for mirodenafil and sildenafil in rat plasma and corpus cavernosum were generated by plotting the peak-area ratios of analytes to the internal standard versus the concentrations of analytes by least-square linear regression. Calibration curves were constructed with the following ranges: 10–5000 ng/ml for plasma and 5–500 ng/ml for tissue, respectively.

Table 1
Calibration curves parameters of mirodenafil and sildenafil in plasma and corpus cavernosum.

Analytes	Range	Slope	Intercept	*R*-Squared	Range (ng/ml)	
					LLOQ	ULOQ
Plasma						
Mirodenafil	Low	0.0925	0.326	0.9906	10	200
	High	0.0808	5.367	0.9844	200	5000
Sildenafil	Low	0.0499	0.102	0.9956	10	200
	High	0.0276	5.369	0.9855	200	5000
Tissue						
Mirodenafil	Low	0.4261	2.551	0.9823	5	100
	High	0.3037	10.169	0.9909	50	500
Sildenafil	Low	0.1328	0.612	0.9836	5	100
	High	0.0907	2.460	0.9919	50	500

LLOQ, lower limit of quantification; ULOQ, upper limit of quantification. Values are the mean of three calibration curves.

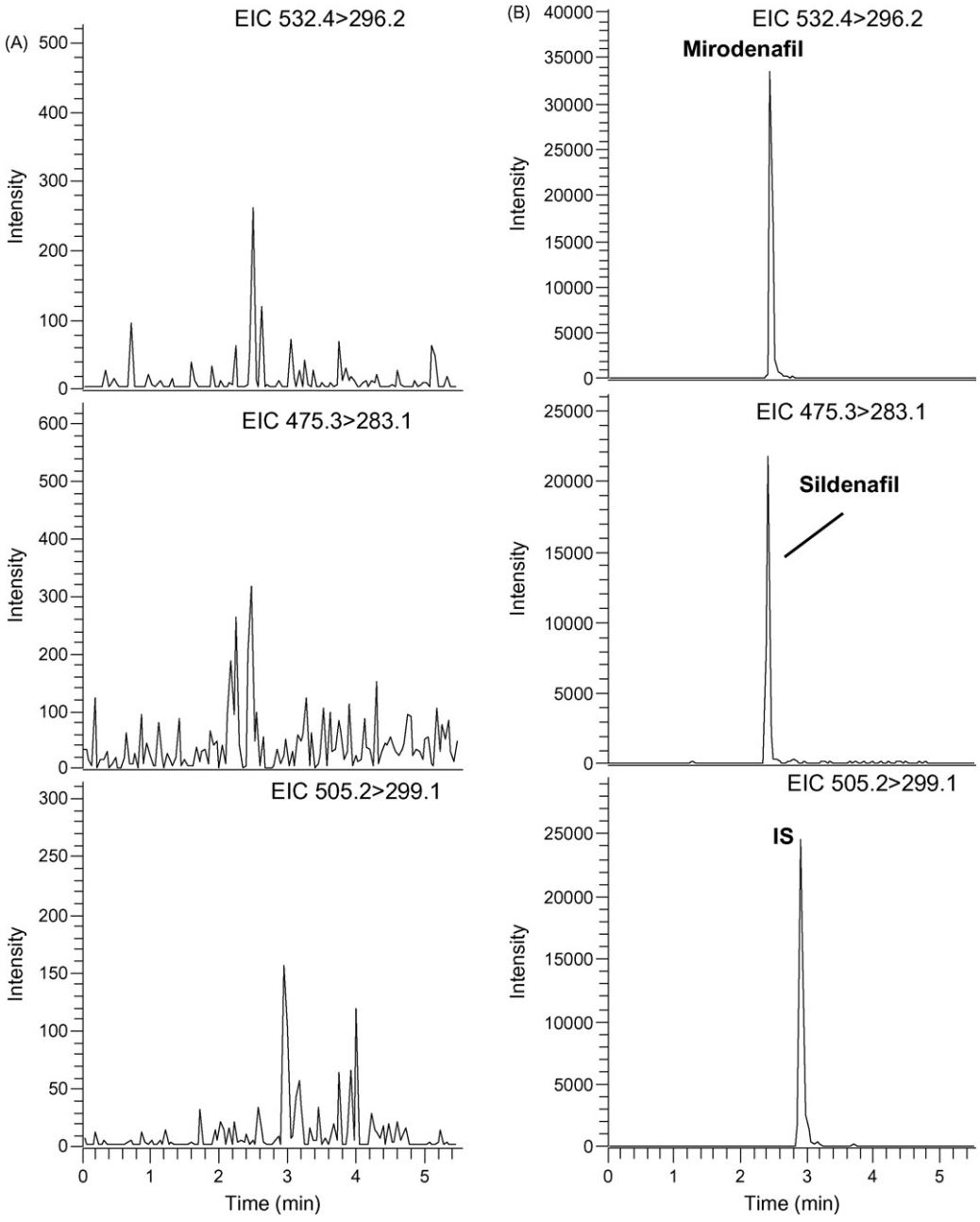

Fig. 2. Multiple reaction monitoring chromatograms of blank rat corpus cavernosum (A) and corpus cavernosum tissue spiked with 50 ng/ml of mirodenafil and sildenafil and IS (1 μg/ml) (B).

2.5.2. Accuracy and precision

Quality control (QC) samples at three different concentration (10, 200 and 5000 ng/ml) for plasma and four concentration (5, 50, 100 and 500 ng/ml) for corpus cavernosum ($n = 5$) were prepared and assayed by replicated analysis ($n = 5$) to determine the intra-day accuracy expressed as the relative error and precision as the relative standard deviation (R.S.D.). The same method was used over 5 days for the inter-day validation. The lower limit of quantification (LLOQ) was determined as the lowest concentration on the standard calibration curve which was measured with a precision within 20% and accuracy between 80 and 120%.

2.6. Pharmacokinetic parameters

The pharmacokinetic parameters were determined using the standard non-compartmental method. Area under curves (AUCs) in the plasma and tissue were calculated using WinNonlin (version 2.1, Scientific Consulting, KY, USA) by a log-linear trapezoidal method.

2.7. Statistics

The results obtained were expressed as the mean ± S.E. and the statistical significance of the results were analyzed at $P < 0.05$ (*) and $P < 0.01$ (**) using the *t*-test.

3. Results and discussion

Representative MRM chromatograms of blank, standard-spiked and dosed tissue samples are shown in Figs. 2 and 3. No endogenous sources of interference were observed at the retention time of analytes. Mirodenafil, sildenafil and IS were observed in the

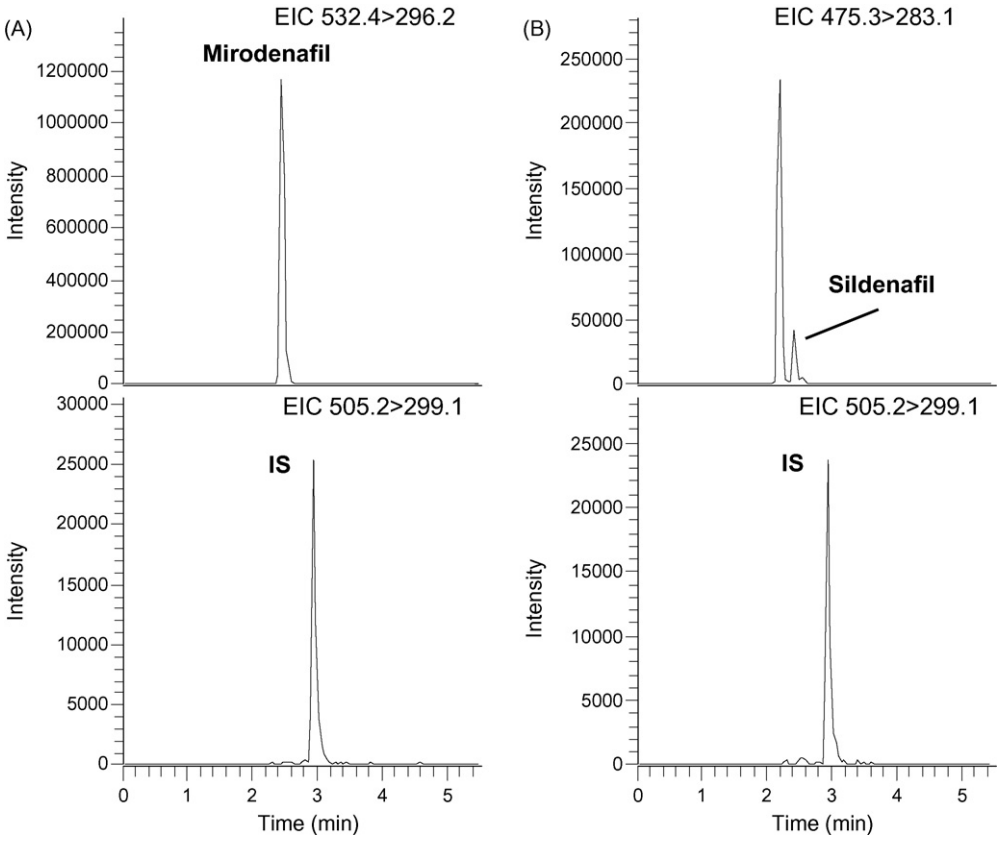

Fig. 3. Multiple reaction monitoring chromatograms of corpus cavernosum collected from rats treated with mirodenafil (A) and sildenafil (B).

MRM-chromatogram at retention times of 2.5, 2.4 and 2.9 min, respectively. In the sildenafil-dosed tissue sample, another peak was observed with the sildenafil peak. This peak was considered to be a peak of a metabolite of sildenafil. The MRM chromatograms of mirodenafil, sildenafil and IS in plasma samples were similar to those in corpus cavernosum (data not shown).

Linearity of calibration curve for determination of mirodenafil and sildenafil was over 0.9844 and 0.9823 in the range of 10–5000 ng/ml in plasma and 5–500 ng/ml in corpus cavernosum, respectively (Table 1). The limit of quantification (LOQ) of mirodenafil and sildenafil were 10 and 5 ng/ml in plasma and tissue, respectively. These were the lowest concentration of the analytes that can be measured with a coefficient of variation with less than 20% and accuracy between 80 and 120%. The intra- and inter-day precision and accuracy for validation are shown in Table 2. The inter- and intra-day precision in plasma and tissue were less than 14.4 and 14.7%, except the values at LOQ, respectively. The accuracy of the method in plasma and tissue was from 86.1 to 114.6% and 85.0 to 103.8% except the values at LOQ, respectively.

The validated method was applied to determine mirodenafil and sildenafil concentration in rat plasma and corpus cavernosum tissue after single oral administration at 40 mg/kg. Blood and tissue

Table 2
Intra- and inter-day coefficient of variation and accuracy for the determination of mirodenafil and sildenafil in plasma and corpus cavernosum ($n = 5$).

Analytes	Range	Theoretical concentration (ng/ml)	Intra-day			Inter-day		
			Concentration found	CV (%)	Accuracy (%)	Concentration found	CV (%)	Accuracy (%)
Plasma								
Mirodenafil	Low	10	11.8 ± 0.7	10.6	118.2	11.26 ± 0.6	9.7	112.6
		200	221.9 ± 5.0	4.9	110.9	204.9 ± 12.9	14.4	102.5
	High	200	191.7 ± 5.7	4.9	95.8	172.2 ± 14.7	14.4	86.1
		5000	4760.6 ± 220.4	10.1	95.2	4979.0 ± 137.8	6.5	99.6
Sildenafil	Low	10	11.6 ± 0.8	12.2	116.1	10.2 ± 0.9	17.6	101.9
		200	229.2 ± 10.8	10.4	114.6	206.1 ± 6.8	9.5	103.0
	High	200	223.9 ± 19.5	10.4	111.9	182.1 ± 12.3	9.5	91.0
		5000	5531.7 ± 171.3	6.7	110.6	4823.7 ± 130.1	9.6	96.5
Tissue								
Mirodenafil	Low	5	5.0 ± 0.7	13.6	100.6	5.9 ± 0.9	16.6	118.1
		100	90.6 ± 6.4	14.7	90.6	103.8 ± 5.9	11.9	103.8
	High	50	45.2 ± 5.1	14.5	90.3	46.2 ± 3.5	9.9	92.4
		500	441.9 ± 30.9	14.5	88.4	429.1 ± 14.2	6.8	85.8
Sildenafil	Low	5	4.2 ± 0.6	13.1	83.0	4.5 ± 0.7	17.1	90.3
		100	85.3 ± 5.6	10.9	85.3	87.03 ± 1.6	3.9	87.0
	High	50	51.5 ± 5.2	14.6	103.1	47.7 ± 3.1	9.2	95.4
		500	422.7 ± 25.4	13.8	85.0	423.1 ± 7.7	3.8	85.0

Table 3
Pharmacokinetic parameters in plasma and corpus cavernosum of mirodenafil and sildenafil after single p.o. administration of SK-3530 and sildenafil at a dose of 40 mg/kg to rats.

Parameters	Plasma		Corpus cavernosum	
	Mirodenafil	Sildenafil	Mirodenafil	Sildenafil
AUC (ng h/ml)	5702.7 ± 1481.5*	368.3 ± 63.4	8425.6 ± 1194.4[a,**]	2460.5 ± 857.5[a]
T_{max} (h)	1.0 ± 0.0	1.6 ± 0.6	1.4 ± 0.2	1.4 ± 0.2
C_{max} (ng/ml)	2728.0 ± 714.6*	171.3 ± 38.0	2812.5 ± 212.8[b,**]	1116.6 ± 208.3[b]
$T\lambda_{1/2}$ (h)	1.5 ± 0.3	1.4 ± 0.3	1.3 ± 0.1	0.9 ± 0.2
MRT (h)	1.9 ± 0.1	2.1 ± 0.3	2.2 ± 0.1	1.9 ± 0.3

Each value represents mean ± S.E. of five animals.
[a] Units of AUC was represented by ng h/g.
[b] Units of C_{max} was represented by ng/g.
* Values significantly different between two groups at $P < 0.05$.
** Values significantly different between two groups at $P < 0.01$.

samples were collected at 1, 2, 4, 8, 10 and 24 h post-dose. The mean plasma and tissue concentration–time curves of mirodenafil and sildenafil were shown in Fig. 4. The pharmacokinetic parameters of mirodenafil and sildenafil in the plasma and corpus cavernosum were summarized in Table 3. Mirodenafil could be detected in both plasma and corpus cavernosum tissue until 10 h after dosing, whereas sildenafil was detected only until 6 h after dosing in the present analytical system. Statistically significant differences in the $T\lambda_{1/2}$ and MRT were not shown between mirodenafil and sildenafil in both plasma and corpus cavernosum. The C_{max} and AUC of mirodenafil in the corpus cavernosum were notably higher than those of sildenafil. Although the C_{max} and AUC of mirodenafil in plasma were also found to be higher than those of sildenafil, the actual C_{max} of sildenafil in plasma is supposed to be comparable to that of mirodenafil; Shin et al. [2] demonstrated that T_{max} values of oral administration of sildenafil were about 20 min in rats and C_{max} of sildenafil in plasma at a dose of 30 mg/kg was between 1000 and 2000 ng/ml. Taken collectively, mirodenafil seems to circulate in plasma as well as distributes in the tissue longer than sildenafil after oral administration at the same dose.

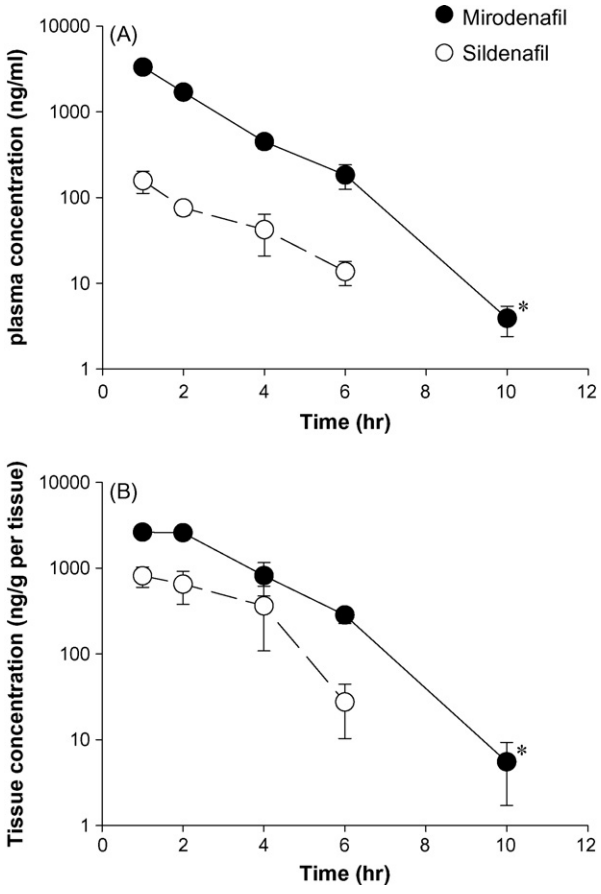

Fig. 4. The mean concentration-time profile of mirodenafil and sildenafil in the plasma (A) and corpus cavernosum (B) after oral administration of mirodenafil or sildenafil at a dose of 40 mg/kg ($n = 5$). Vertical bars represent mean ± S.E. *Means beyond the calibration range but over the LOD value.

According to the previous reports [2,3,6], oral bioavailability (BA) of both drugs were less or more than 20% and obvious differences in oral BA were not shown between two drugs. Therefore, differences in the drug concentration in the plasma or tissue seem not to be related to the oral bioavailability.

Another important factor which can affect the distribution of drug is metabolism. Both sildenafil and mirodenafil are known to be extensively metabolized by CYPs [5–7]. In case of sildenafil, it has been reported that a considerable amount of parent drug is metabolized to form the piperazine *N*-desmethyl metabolite (UK-103,320) in rats [6]. Mirodenafil is also known to be biotransformed to the piperazine *N*-desmethyl metabolite (SK-3541), mainly [5]. Especially, the metabolic clearance rate of sildenafil is thought to be higher compared with mirodenafil [2,3]. This can partly support our data showing that the concentrations of mirodenafil in the plasma and tissue were higher that those of sildenafil and also explain the reason for the relatively high tissue/plasma ratio of sildenafil.

Furthermore, UK-103,320 and SK-3541 are known to be active metabolites [7,8]. Therefore, further pharmacokinetic study for SK-3541 and UK-103,320 can provide an explanation for the present result in view of metabolic clearance and evidences for a comparison of the in vivo drug efficacy based on pharmacokinetic approach. In addition, further study with multiple doses and more time points including earlier time points would be followed.

In conclusion, mirodenafil was found in the plasma and corpus cavernosum longer than sildenafil after oral administration. This suggests that mirodenafil might exist longer in the target tissue above the minimum effective therapeutic concentration than sildenafil. Considering the difficulties of tissue distribution study in human subjects, the finding of this investigation provided meaningful evidence to compare the in vivo drug efficacy between sildenafil and mirodenafil.

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