

## Preparation and *in vivo* Evaluation of a Sildenafil Lactate-loaded Solid Self-emulsifying Drug Delivery System

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**ABSTRACT** – To develop a sildenafil lactate-loaded solid self-emulsifying drug delivery system (SEDDS) with a fast onset of action and immediate action of erection, sildenafil lactate (0.3 g), which was prepared using a spray dryer, was dissolved in 4.7 g of the mixture of glyceryl monooleate/Transcutol/ Tween 20 (3/0.5/1, g). Its emulsion droplet size and pharmacokinetics in rabbits were evaluated compared with sildenafil citrate-loaded commercial tablet. The sildenafil lactate-loaded SEDDS showed an emulsion droplet size of about 300 nm. In pharmacokinetics study, it gave significantly faster  $T_{max}$  than did the commercial tablet. Thus, the sildenafil lactate-loaded SEDDS at the one-third drug dose compared to sildenafil citrate-loaded conventional tablet might induce a fast onset of action and immediate erection without enhanced bioavailability compared with the sildenafil citrate-loaded commercial tablet.

**Key words** – Sildenafil lactate, SEDDS, Immediate erection, Pharmacokinetics

Sildenafil (1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine) is used to treat male erectile dysfunction. It is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) (Boolell et al., 1996). However, this drug has low aqueous solubility and high membrane permeability, and is included in class 2 of the Biopharmaceutical Drug Classification system (Amidon et al., 1995). Its bioavailability is relatively low after oral administration since it is practically insoluble in water (Elshafeey et al., 2009; Wang et al., 2008). Thus, a commercial product has been developed with a salt form such as sildenafil citrate, which improves the drug's solubility. This commercial product (Viagra<sup>®</sup>; Pfizer) is a tablet form containing sildenafil citrate equivalent to doses of 25, 50 and 100 mg of sildenafil base. It is rapidly absorbed after oral administration but gives a relatively low absolute bioavailability of about 40% (Nichols et al., 2002). However, its onset of action is relatively late, taking over 60 minutes in the presence of sexual stimulation (Eardly et al., 2002). Thus, a novel commercial product with a fast onset of drug effect and immediate erection must be needed.

In recent years much attention has been focused on lipid-

microemulsion formulation with particular emphasis on self-emulsifying or self-micro emulsifying drug delivery systems (SEDDS) to improve oral bioavailability of poorly water-soluble drugs (Balakrishnan et al., 2009). The clinical usefulness of the SEDDS are comprised of mixture of drug, oil, surfactants and/or cosolvents which form fine oil in water and/or water in oil emulsions upon dilution with aqueous medium or *in vivo* administration. Factors controlling the *in vivo* performance of SEDDS include their ability to form small droplets of oil and the polarity of the oil droplets to promote faster drug release into aqueous phase (Shah et al., 1994). The smaller oil droplets provide a large interfacial area for pancreatic lipase to hydrolyze triglycerides and thereby promote the rapid release of the drug and/or formation of mixed micelles of the bile salts containing the drug.

In this study, to develop a sildenafil lactate-loaded solid self-emulsifying drug delivery system (SEDDS) with a fast onset of action and immediate action of erection, it was prepared with sildenafil lactate, a salt form of sildenafil with improved solubility and bioavailability of poorly water-soluble sildenafil (Jung et al., 2011). Its emulsion droplet size and pharmacokinetics in rabbits were evaluated compared with sildenafil citrate-loaded commercial tablet.

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## Materials and methods

### Materials

Sildenafil base, sildenafil citrate and glyceryl monooleate were obtained from Hanmi Pharm. Co. (Hwasung, South Korea). Transcutol P was supplied by Gattefosse (Saint-Priest Cedex, France). Polysorbate 20 (Tween 20) and polyethylene glycol 4000 were purchased from Duksan Chemical Co. (Ansan, South Korea). Ethanol was of USP grade. The commercial product (Viagra®; in a tablet form) was purchased from Pfizer Korea Pharm. Co. (Seoul, South Korea).

### Preparation of sildenafil lactate

A Buchi 190 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of sildenafil lactate. Sildenafil base and lactic acid (1:1, molar ratio) were dissolved in ethanol and delivered to the nozzle (0.7 mm diameter) at a flow rate of 5 mL/min using a peristaltic pump, and spray-dried at 100°C (inlet temperature) and 65-70°C outlet temperature. The pressure of spray air was 4 kg/cm<sup>2</sup>. The flow rate of the drying air was maintained at the aspirator setting of 10, which indicated the pressure of the aspirator filter vessel (-25 mbar). The direction of the air flow was the same as that of the sprayed products (Li et al., 2008).

### Solubility

An excess of sildenafil base, sildenafil citrate and sildenafil lactate (about 100 mg) was added to 10 mL solvents as shown in Table I. They were shaken in a water bath at 25°C for 24 h, centrifuged at 3000 g for 10 min (Eppendorf, USA) and filtered through a membrane filter (0.45 mm) (Choi et al., 2008). The concentration of sildenafil in the resulting solution was analysed by HPLC as described below.

### Preparation of Sildenafil lactate-loaded SEDDS

Sildenafil lactate (0.3 g) was dissolved in 4.7 g of the mixture of glyceryl monooleate /Transcutol/Tween 20 (3/0.5/1, g).

**Table I.** Solubility in various solvents

Solvent	Solubility (mg/mL)		
	Sildenafil base	Sildenafil citrate	Sildenafil lactate
Distilled water	0.0±0.0	4.1±1.3	92.5±10.5
Transcutol	24.7±4.7	3.4±0.7	38.3±2.6
Glyceryl monooleate	10.2±3.1	2.5±0.4	20.5±3.6
Tween 20	12.0±3.8	5.6±0.9	19.8±3.2

Each value represents the mean ± S.D. (n=3)

The final mixture was vortexed until a clear solution was obtained. The final drug content of the liquid SEDDS was 6% w/w ratio. The formulation was examined for signs of turbidity or phase separation.

Furthermore, the emulsion particle size of the emulsion was determined using a Zetasizer Nano ZS (Malvern Instruments, U.K.) dynamic light scattering particle size analyser at a wavelength of 635 nm and a scattering angle of 90° at 25°C. Liquid SEDDS were added to 25 mL of distilled water and shaken gently to form a fine emulsion and kept for 12 h at room temperature. All studies were repeated three times and the values of z-average diameters were used. The z-average diameter, also referred to as the harmonic intensity-weighted average hydrodynamic diameter, of each emulsion was derived from cumulated analysis by Automeasure software (Malvern Instruments, Malvern, UK).

### Pharmacokinetics

Animals – all animal care and procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989, revised in 1999 and amended in 2008 by the Society of Toxicology (SOT, 2008). Furthermore, the protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Yeungnam University. Twelve New Zealand albino male rabbits weighing 2.5-3.5 kg were fasted for 24 h prior to the experiments but allowed free access to water at a temperature of 20-24°C and a relative humidity (RH) of 55 ± 10% with a normal 12 h light/dark cycle starting one week before the experiment.

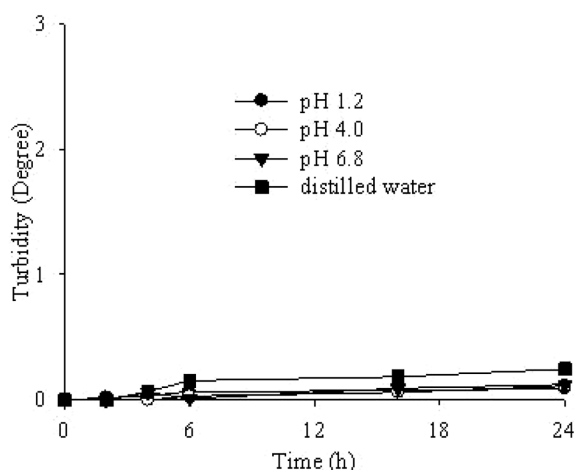
Oral administration and blood collecting – Ten rabbits were divided into two groups. The sildenafil citrate-loaded conventional tablet was orally administered at the equivalent dose of 35 mg/kg sildenafil citrate (25 mg/kg sildenafil) in one group. The sildenafil lactate-loaded SEDDS was orally administered at the equivalent dose of 9.93 mg/kg sildenafil lactate (8.33 mg/kg sildenafil). Then, 1 mL of blood samples were obtained at various intervals from the left or right ear vein into heparinised glass tubes, centrifuged at 3,000 g for 10 min using a 5415C centrifuge (Eppendorf, USA) and stored at -70°C prior to analysis.

Blood sample analysis – Plasma (0.1 mL) was thoroughly mixed with 0.05 mL 0.1 N borax, 1 mL ether and 0.05 mL of acetonitrile solution containing nifedipine (200 µg/mL) as an internal standard. This mixture was vortexed for 2 min and centrifuged at 10,000 g for 10 min to precipitate the proteins. The supernatant layer (0.5 mL) was evaporated under N<sub>2</sub> (g). The residue was reconstituted in 50 µL for the mobile phase. The resulting solution (20 µL) was analysed by HPLC (Jasco

UV-975, Japan) equipped with an Inertsil ODS-3 C<sub>18</sub> column (GL science, 0.5  $\mu\text{m}$ , 25 cm  $\times$  0.46 cm i.d.) and UV detector (Model L-7450). The mobile phase consisted of 20 mM KH<sub>2</sub>PO<sub>4</sub> and acetonitrile (70:30, v/v). The eluent was monitored at 292 nm with a flow rate of 1.0 mL/min (Gratz et al., 2004; Shin et al., 2006; Quintero et al., 2009).

## Result and Discussion

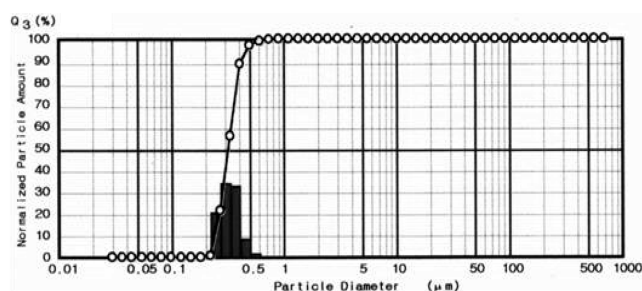
Sildenafil lactate was prepared easily by spray-drying sildenafil base and lactic acid (1:1, molar ratio). The aqueous solubility of sildenafil base was about 14.5  $\mu\text{g/mL}$ , which indicated that this drug was poorly water-soluble (Elshafeey et al., 2009; Wang et al., 2008). As shown in Table I, the aqueous solubility of sildenafil salts was in the order of sildenafil base < sildenafil citrate < sildenafil lactate. In particular, the aqueous solubility of sildenafil lactate was about 25-fold higher than sildenafil citrate, the commercial drug ( $92.5 \pm 10.5$  vs.  $4.1 \pm 1.3$  mg/mL). Compared with sildenafil base, sildenafil citrate reduced the drug solubility in other solvents such as glyceryl monooleate, Transcutol and Tween 20, but sildenafil lactate increased it further. Thus, sildenafil lactate was a salt form that provided an improved solubility of sildenafil (Jung et al., 2011). Furthermore, glyceryl monooleate, Transcutol and Tween 20 were selected as an oily vehicle, surfactant and co-surfactant, respectively, because they could solubilize sildenafil lactate and well mix together (Jung et al., 2011). In particular, the sildenafil lactate-loaded SEDDS composed of [sildenafil lactate/glyceryl monooleate/Transcutol/ Tween 20 (3/30/5/10, g)] showed no turbidity in water, pH 1.2, pH 4.0



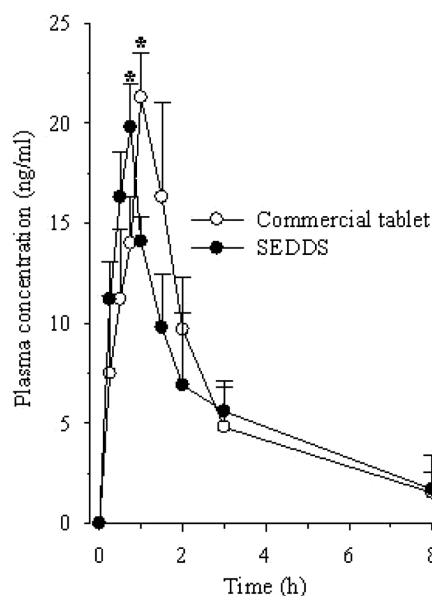
**Figure 1.** Turbidity of sildenafil lactate-loaded SEDDS in various solutions. Each value represents the mean  $\pm$  S.D. ( $n=3$ ). The sildenafil lactate-loaded SEDDS was composed of [sildenafil lactate /glyceryl monooleate/Transcutol/ Tween 20 (3/30/5/10, g)].

and pH 6.8 solution for 24 h (Figure 1), and showed an emulsion droplet size of about 300 nm (Figure 2) (Balakrishnan et al., 2009). Thus, this SEDDS formulation was selected for pharmacokinetic study.

Figure 3 shows the change in mean plasma concentration of sildenafil after the oral administration of two sildenafil preparations at the different dose. The sildenafil citrate-loaded conventional tablet and sildenafil lactate-loaded SEDDS were orally administered at the equivalent dose of 35 mg/kg sildenafil citrate (25 mg/kg sildenafil) and 9.93 mg/kg sildenafil lactate (8.33 mg/kg sildenafil; one-third drug dose compared to sildenafil citrate-loaded conventional tablet), respectively. To



**Figure 2.** Emulsion droplet size of sildenafil lactate-loaded SEDDS in water. Each value represents the mean  $\pm$  S.D. ( $n=3$ ). The sildenafil lactate-loaded SEDDS was composed of [sildenafil lactate /glyceryl monooleate/Transcutol/ Tween 20 (3/30/5/10, g)].



**Figure 3.** Plasma concentration–time profiles of the drug after the oral administration of the sildenafil citrate-loaded commercial tablet and sildenafil lactate-loaded SEDDS to rabbits. Each value represents the mean  $\pm$  S.D. ( $n=5$ ). \* $P<0.05$  compared with the sildenafil citrate-loaded commercial tablet. The sildenafil lactate-loaded SEDDS was composed of [sildenafil lactate /glyceryl monooleate/Transcutol/ Tween 20 (3/30/5/10, g)].

**Table II. Pharmacokinetic parameters**

Parameters	Commercial tablet	SEDDS
AUC (h·ng/mL)	35.97±6.61	35.51±5.18
T <sub>max</sub> (h)	1.02±0.14	0.58±0.12*
C <sub>max</sub> (ng/mL)	18.69±2.58	17.77±2.41
t <sub>1/2</sub> (h)	1.43±0.48	1.02±.42
K <sub>el</sub> (h <sup>-1</sup> )	0.49±0.38	1.68±0.62

Each value represents the mean ± S.D. (n=5).

\*P<0.05 compared with the commercial tablet.

The sildenafil lactate-loaded SEDDS was composed of [sildenafil lactate/glyceryl monooleate /Transcutol/Tween 20 (3/30/5/10, g)].

45 min, the SEDDS gave higher initial plasma concentrations, followed by lower plasma concentrations compared with the commercial tablet. In particular, the initial plasma concentration of sildenafil from the SEDDS at 45 min was significantly higher than that of the commercial tablet (P<0.05). In the self-emulsifying systems, the amount of free energy required to form an emulsion is very low, thereby allowing the spontaneous formation of an interface between oil droplets and the water (Balakrishnan et al., 2009). This suggests that the oil/surfactant/co-surfactant and water phases effectively swell, decreasing the size of the oil droplets and eventually increasing the initial absorption.

The pharmacokinetic parameters are shown in Table II. The AUC, C<sub>max</sub>, K<sub>el</sub> and t<sub>1/2</sub> values of the solid SEDDS formulation were not significantly different from those of the commercial tablet in the rabbits. However, it gave significantly faster T<sub>max</sub> than did the commercial tablet. In particular, the T<sub>max</sub> value of the SEDDS was about 0.5-fold lower than that of the commercial tablet (Jung et al., 2011). Thus, the sildenafil lactate-loaded SEDDS at the one-third drug dose compared to sildenafil citrate-loaded conventional tablet might induce a fast onset of action and immediate erection without enhanced bioavailability compared with the sildenafil citrate-loaded commercial tablet.

### Conclusion

The sildenafil lactate-loaded SEDDS composed of [sildenafil lactate/glyceryl monooleate /Transcutol/Tween 20 (3/30/5/10, g)] showed an emulsion droplet size of about 300 nm and gave faster about 0.5-fold T<sub>max</sub> than did the commercial tablet. Thus, the sildenafil lactate-loaded SEDDS at the one-third drug dose compared to sildenafil citrate-loaded conventional tablet might induce a fast onset of action and immediate erection without enhanced bioavailability compared with the sildenafil citrate-loaded commercial tablet.

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