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Effects of a New Selective Phosphodiesterase Type 5 Inhibitor, KJH-1002, on the Relaxation of Rabbit Corpus Cavernosum Tissue

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Abstract – The present study examined functional effects of a new selective phosphodiesterase type 5 inhibitor, 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-thioxo-3-propyl-1H-pyrazolo[4,3]pyrimidin-5-yl)phenylsulphonyl]-4-methyl piperazine (KJH-1002), in the isolated rabbit corpus cavernosum (RCC). Relaxing effects of KJH-1002 were also compared with those of sildenafil, which is currently used as an oral therapy for penile erectile dysfunction. In the isolated RCC precontracted with phenylephrine, both KJH-1002 and sildenafil in the concentration range of 1 to 1000 nM, produced a comparable potentiation of the electrical field stimulation-induced relaxation in a concentration-dependent manner. In the sodium nitroprusside (SNP)-induced relaxation, the IC_{50} values, concentrations of SNP required to produce a 50% relaxation of the phenylephrine-induced contraction, were significantly decreased to the similar extent by treatments with KJH-1002 and sildenafil. The results suggest that a new selective phosphodiesterase type 5 inhibitor, KJH-1002, has an augmentative effect on penile erection comparable to that of sildenafil and can be useful for the treatment of erectile dysfunction.

Key words □ Penile erection, Rabbit corpus cavernosum, Relaxation, Phosphodiesterase type 5 inhibitor, KJH-1002, Sildenafil

INTRODUCTION

Penile erection is a hemodynamic event involving relaxation of smooth muscle of the corpus cavernosum and its associated arterioles resulting in increased blood flow into the trabecular spaces (Andersson *et al.*, 1995). Although corpus cavernosum relaxation may be mediated by various mechanisms, a major physiological event is the formation and actions of nitric oxide (Burnett, 1997). Nitric oxide (NO), during sexual stimulation, is synthesized in the nerve terminals of parasympathetic non-adrenergic, non-cholinergic (NANC) neurons in the penis and also by the endothelial cells lining blood vessels and the lacunar spaces of the corpora cavernosa (Burnett *et al.*, 1992; Burnett, 1995; Trigo-Rocha *et al.*, 1993). Nitric oxide activates guanylate cyclase resulting in an increased conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which provides the signal that leads to relaxation of smooth muscle of the corpus cavernosum and penile arterioles (Burnett, 1995). The level of cGMP is regulated by a balance between the rate of synthesis by guanylate cyclase and the rate of hydrolytic breakdown to guanosine 5-monophosphate by cyclic nucleotide phosphodiesterase (PDE) isozymes (Beavo, 1995). Therefore, agents that inhibit cGMP phosphodiesterase increase the cGMP levels in the corpus cavernosum and thereby facilitate the erectile response.

Human phosphodiesterases have been classified into 10 families according to their chromatographic properties, substrate specificity and susceptibility to inactivation by inhibitors (Beavo, 1995; Fisher *et al.*, 1998a and b). Of the PDE isozyme families, only PDE5 and PDE6 are specific for cGMP as a substrate. PDE3 and PDE4 are specific for cyclic adenosine monophosphate (cAMP), and PDE1 and PDE2 hydrolyze both cGMP and cAMP (Beavo *et al.*, 1994).

Three isozymes of cyclic nucleotide phosphodiesterase, namely, PDE2, PDE3 and PDE5, have been identified in extracts of human penis (Robert *et al.*, 1999). However, PDE5 is the predominant isozyme responsible for the metabolism of cGMP in the corpus cavernosum. A compound with phosphodiesterase isozyme selectivity is required because insufficient

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selectivity for the target phosphodiesterase leads to undesirable effects.

A new selective phosphodiesterase type 5 inhibitor, 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-thioxo-3-propyl-1H-pyrazolo [4,3]pyrimidin-5-yl)phenylsulphonyl]-4-methylpiperazine (KJH-1002) synthesized at the Korea Institute of Science and Technology (KIST), is being developed as a potential drug candidate for the treatment of erectile dysfunction. In the PDE inhibition study done in our laboratory, KJH-1002 was shown to inhibit PDE5 more potently and selectively than sildenafil. Therefore, the purpose of the present study was to evaluate and compare functional effects of KJH-1002 on corpus cavernosum isolated from rabbit in comparison with sildenafil.

MATERIALS AND METHODS

Tissue preparation

Corpus cavernosum tissue was excised from the penis of male New Zealand White rabbits (3.5-4.0 kg) purchased from Hallym Experimental Animal Center (Korea) under anesthesia with sodium pentobarbital, and immediately placed in oxygenated ice-cold Krebs buffer. Two strips (0.3 × 0.3 × 0.7 cm) of rabbit corpus cavernosum (RCC) smooth muscle were dissected from one penis. Each strip was mounted between two parallel platinum electrodes in an organ bath chamber containing 15 ml of Krebs-bicarbonate solution maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2. The upper end of each strip was suspended from an isometric force transducer, which was in turn linked to an amplifier and chart recorder. The initial resting tension of each strip was set at 2 gm and the tissues were allowed to equilibrate for 1.5 hours. The tension was recorded on a polygraph (Model 7D, Grass Instruments). The tissue samples were then washed with 120 mM KCl in Krebs-bicarbonate solution for 15 minutes to depolarize the tissue and induce a contractile response. Depolarization with KCl increases and stabilizes subsequent submaximal contractile responses to phenylephrine. Following this step, the tissues were allowed to return to baseline tension by repeatedly washing for 30 minutes with Krebs solution modified to contain 1 mM atropine and 5 mM guanethidine to block muscarinic receptors and prevent the release of norepinephrine, respectively, during subsequent electrical field stimulation (EFS) or sodium nitroprusside (SNP) stimulation. This modified Krebs solution was used throughout subsequent experiments. Tissue preparation and following *in vitro* functional bioassays were performed according to the methods described by Ballard *et al.* (1998) and Bush *et al.* (1992).

Effects of KJH-1002 and sildenafil on EFS-induced relaxation of rabbit corpus cavernosum

Each tissue strip was submaximally contracted to approximately 70% of the KCl-induced contraction by adding phenylephrine hydrochloride to each organ chamber to a final concentration of 10 mM. After the phenylephrine contractile responses had stabilized (10 minutes), the tissues were subjected to EFS-induced relaxation at 10 volts using sequential frequencies of 2, 4, 8, 16 Hz in the form of square wave pulses (0.2 msec) delivered as 10-second trains (Grass S48 Stimulator).

Tissue strips were washed, the organ baths were refilled with 15 ml of Krebs buffer and then 50 μl of sildenafil solution in 0.6% lactic acid vehicle or 50 μl of KJH-1002 solution in 0.6% lactic acid vehicle or 50 μl of 0.6% lactic acid as time-matched control (TMC) was added. The final concentrations of sildenafil and KJH-1002 used in these studies were ranged from 1 nM to 1000 nM. The tissues were allowed to equilibrate with sildenafil, KJH-1002 or vehicle for 15 minutes and then reset to the original baseline tension before again being contracted with phenylephrine. After the contractile responses stabilized, the tissues were subjected to sequential frequency EFS as described above. The tissues were washed for 15 minutes before adding vehicle or the next concentration of sildenafil or KJH-1002 and then repeating the EFS profile.

Effects of KJH-1002 and sildenafil on sodium nitroprusside-induced relaxation of rabbit corpus cavernosum

Each tissue strip was submaximally contracted to approximately 70% of the KCl-induced contraction by adding phenylephrine hydrochloride to each organ chamber to a final concentration of 10 mM. After the phenylephrine contractile responses had stabilized (10 minutes), the tissues were subjected to sodium nitroprusside (SNP)-induced relaxation. Tissue strips were washed, the organ baths were refilled with 15 ml of Krebs buffer and then 50 μl of sildenafil solution in 0.6% lactic acid vehicle or 50 μl of KJH-1002 solution in 0.6% lactic acid vehicle or 50 μl of 0.6% lactic acid as TMC was added. The final concentrations of sildenafil and KJH-1002 used in these studies were ranged from 10 nM to 1000 nM. The tissues were allowed to equilibrate with sildenafil, KJH-1002 or vehicle for 15 minutes and then reset to the original baseline tension before again being contracted with phenylephrine. After the contractile responses stabilized, the tissues were subjected to

sequential concentrations of SNP. The tissues were washed for 15 minutes before adding vehicle or the next concentrations of sildenafil or KJH-1002.

Chemicals and solutions

Sildenafil was synthesized at the KIST according to the method described by Pfizer (1999). KJH-1002 was also synthesized at the KIST with the modification of sildenafil synthesis.

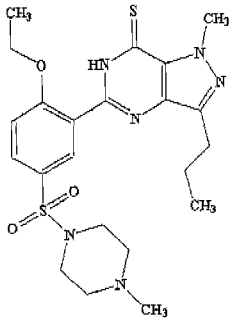

Fig. 1. Chemical structure of 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-thioxo-3-propyl-1H-pyrazolo[4,3]pyrimidin-5-yl)phenylsulphonyl]-4-methylpiperazine, KJH-1002.

Chemical purity of both compounds was above 99% as judged by HPLC analysis. Phenylephrine hydrochloride, atropine sulfate, guanethidine sulfate and sodium nitroprusside were purchased from Sigma (St. Louis, MO, USA). Sodium nitroprusside was prepared as serial dilutions. Krebs solution consisted of: NaCl, 118 mM; $NaHCO_3$, 25 mM; KCl, 4 mM; NaH_2PO_4, 0.5 mM; $CaCl_2$, 0.7 mM. Krebs-bicarbonate solution consisted of: NaCl, 118 mM; $NaHCO_3$, 25 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; glucose, 11 mM; $CaCl_2$, 1.5 mM; EDTA, 0.023 mM.

Statistics

All data are expressed as mean ± S.E.M. Statistical significance was tested by Wilcoxon two-sample test (Table I and II), Student's *t*-test for grouped data (Fig. 2 and 3) or Duncan's multiple range test (Fig. 4).

RESULTS AND DISCUSSION

Our previous study showed that KJH-1002 possessed a high selectivity for PDE5 relative to the other phosphodiesterases.

Table I. Comparison of IC_{50} values for inhibition of phosphodiesterase isozymes between KJH-1002 and sildenafil.

Compound	IC_{50} value				
	PDE1 (μM)	PDE3 (μM)	PDE4 (μM)	PDE5 (nM)	PDE6 (μM)
KJH-1002	2.15	1.16	0.92	0.59	0.014
Sildenafil	0.22	10.58	2.05	3.43	0.017

IC_{50} values were determined from sigmoidal curves fitted to plots of enzyme activity versus log compound concentration using a curve fitting program.
The activities were measured with 0.15 μM of [^{3}H]cGMP or cAMP as a substrate.

Table II. Effects of KJH-1002 on the relaxation of phenylephrine (10 mM)-contracted rabbit isolated corpus cavernosum induced by sodium nitroprusside (SNP).

Treatment	n	IC_{50} for SNP (mM) Mean (95% CI)	Treatment	n	IC_{50} for SNP (mM) Mean (95% CI)
Pre-treatment	5	0.3732 (0.1886-0.7388)			
TMC for 10 nM	7	0.5949 (0.2900-1.2210)	KJH-1002 10 nM	7	0.3555 (0.1818-0.6955)
TMC for 100 nM	7	0.8971 (0.4177-1.9270)	KJH-1002 100 nM	7	0.1140*,** (0.0308-0.4220)
TMC for 1000 nM	4	0.3067 (0.1726-0.5453)	KJH-1002 1000 nM	4	0.1615** (0.0582-0.4480)

Pre-treatment indicates responses prior to addition of KJH-1002 or vehicle.
IC_{50} means concentration required to produce a 50% relaxation of the phenylephrine-induced contraction.
n: number of tissues, CI: confidence interval, TMC: time-matched control.
Significance of difference from TMC $^{**}P<0.01$, $^{***}P<0.001$ (Wilcoxon two-sample test).

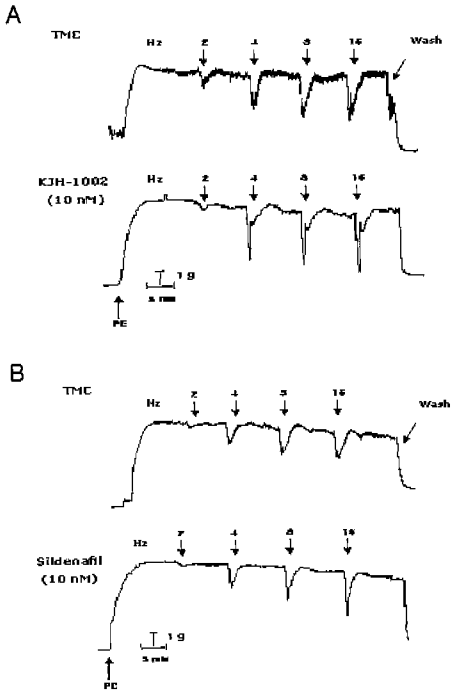

Fig. 2. Typical chart tracings showing effects of KJH-1002 (A) and sildenafil (B) on the relaxation of phenylephrine (10 mM)-contracted rabbit corpus cavernosum tissue strips induced by electrical field stimulation (EFS). Relaxation responses to EFS were determined at frequencies of 2, 4, 8 and 16 Hz.

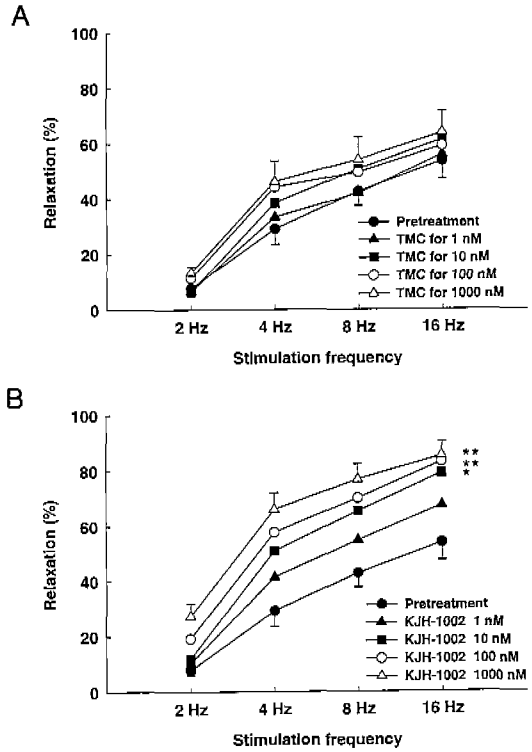

Fig. 3. Effect of KJH-1002 on EFS-induced relaxation of phenylephrine-contracted rabbit corpus cavernosum tissue strips. Tissues were incubated for 15 min with KJH-1002 (1, 10, 100 and 1000 nM) or vehicle (0.6% lactic acid) and then precontracted with phenylephrine before EFS at frequencies of 2, 4, 8 and 16 Hz. A, responses for time-matched control (TMC) tissues; B, effects of KJH-1002 on tissue responses. Significance of differences in intercepts at 4 Hz between pretreatment and treatment responses was examined using Student's *t*-test for grouped data. $^{*}P<0.05$; $^{**}P<0.01$.

For example, the inhibitory activity of KJH-1002 for PDE5 was approximately 23-fold more potent than that for PDE6. In addition, KJH-1002 showed more potent and selective PDE5 inhibitory activity than sildenafil (Table I). However, no evidence has been obtained whether KJH-1002 exerts pharmacological function as a specific phosphodiesterase type 5 inhibitor in mammalian tissues. Cavernous smooth muscle cells play a key role in the control of penile erection. In the present study, therefore, the properties of KJH-1002 as a candidate for the treatment of erectile dysfunction in the isolated RCC were investigated. Sildenafil was used as a reference drug, which is currently used as an oral therapy for penile erectile dysfunction (Boolell *et al.*, 1996; Stephen *et al.*, 1998).

In the penile corpus cavernosum tissue, NO released from nitrergic nerves in the walls of arteries and sinusoids appears to play a major role in the relaxation of the corpus cavernosal smooth muscle by elevation of cGMP levels (Andersson *et al.*, 1995; Bush *et al.*, 1992; Holmqist *et al.*, 1992). It has been reported that the main phosphodiesterase that hydrolyzes cGMP in the corpus cavernosum is the PDE5 (Wallis *et al.*, 1999). This physiological process is the reason why specific phosphodiesterase type 5 inhibitors cause a potentiation of penile erection during sexual stimulation (Corbin *et al.*, 1999; Langtry *et al.*, 1999). Many previous reports (Ballard *et al.*, 1998; Hayashide *et al.*, 1996) suggested that EFS-induced relaxation was associated with NO released from nitrergic nerves, that is, EFS of isolated tissue is the most representative and suitable method for evaluating agents that modulate the NO-cGMP pathway (Ignarro *et al.*, 1990). Under these conditions, typical tracings of the influence of KJH-1002 and sildenafil on EFS-induced relaxation in the isolated rabbit corpus cavernosum are shown in Fig. 2. Electrical field stimulation (2 to 16 Hz) caused a frequency-dependent relaxation in the corpus cavernosum precontracted with phenylephrine. The relaxation was enhanced by pretreatment with KJH-1002 or sildenafil in a concentration-dependent manner. The increases observed at concentrations of 10, 100 and 1000 nM of KJH-1002 and sildenafil were statistically significant over the 2 to 16

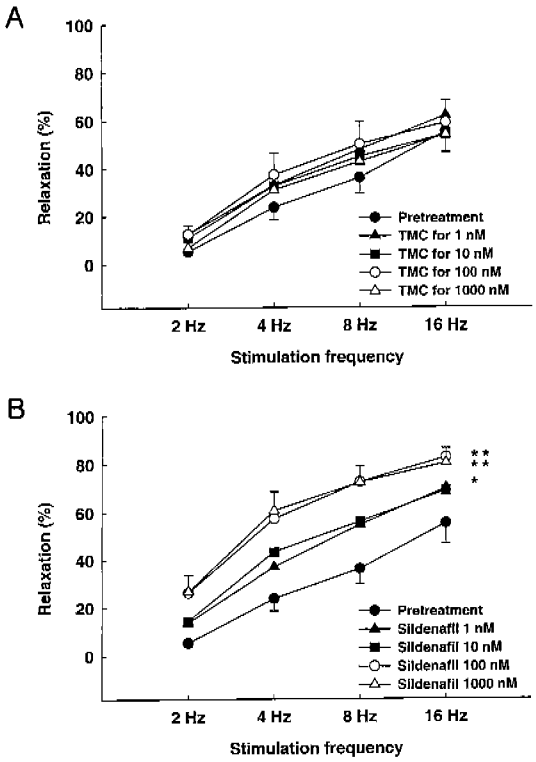

Fig. 4. Effect of sildenafil on EFS-induced relaxation of phenylephrine-contracted rabbit corpus cavernosum tissue strips. Tissues were incubated for 15 min with sildenafil (1, 10, 100 and 1000 nM) or vehicle (0.6% lactic acid) and then precontracted with phenylephrine before EFS at 2, 4, 8 and 16 Hz. A, responses for time-matched control (TMC) tissues; B, effects of sildenafil on tissue responses. Significance of differences in intercepts at 4 Hz between pretreatment and treatment responses were examined using Student's *t*-test for grouped data. $^{*}P < 0.05$; $^{**}P < 0.01$.

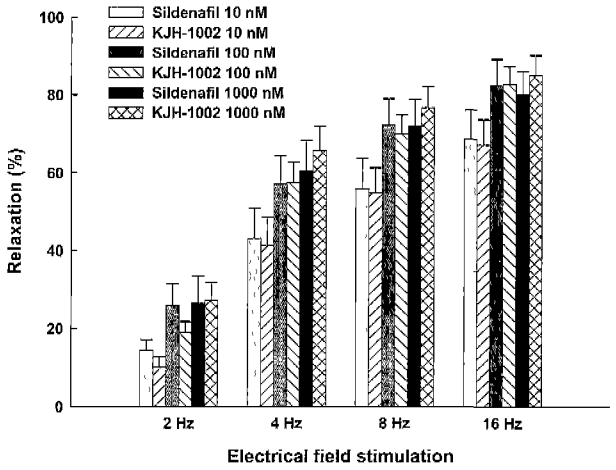

Fig. 5. Comparisons of effects of KJH-1002 and sildenafil on EFS-induced relaxation in phenylephrine-contracted rabbit corpus cavernosum. There were no significant differences in EFS-induced corpus cavernosal relaxation between KJH-1002 and sildenafil.

Hz EFS range when compared with the relaxation responses observed for time-matched control tissues (Fig. 3 and Fig. 4). There were no significant differences in EFS-induced corpus cavernosal relaxation between KJH-1002 and sildenafil (Fig. 5).

Sodium nitroprusside dissociates in solution to generate NO, which in turn directly activates guanylate cyclase in vascular smooth muscle and causes the resulting vasorelaxation (Lincoln and Fisher-Simpson, 1984; Michino *et al.*, 2001; Sobey and Faraci, 1997; Van der Zypp and Majewski, 1998). In the SNP-stimulated relaxation, the IC_{50} values, concentrations of SNP required to produce a 50% relaxation of the phenylephrine-induced contraction, were significantly reduced approximately 8 and 2 times by treatments with 100 and 1000 nM of KJH-1002, respectively, when compared with the time-

Table III. Effects of sildenafil on the relaxation of phenylephrine (10 mM)-contracted rabbit isolated corpus cavernosum induced by sodium nitroprusside (SNP).

Treatment	n	IC_{50} for SNP (mM) Mean (95% CI)	Treatment	n	IC_{50} for SNP (mM) Mean (95% CI)
Pre-treatment	5	0.3732 (0.1886-0.7388)			
TMC for 10 nM	7	0.8158 (0.3460-1.9240)	Sildenafil 10 nM	7	0.4378 (0.2076-0.9230)
TMC for 100 nM	7	0.9366 (0.3833-2.2890)	Sildenafil 100 nM	7	0.1642^{**} (0.0743-0.3628)
TMC for 1000 nM	4	0.5771 (0.2434-1.3690)	Sildenafil 1000 nM	4	0.1247^{**} (0.0462-0.3361)

Pre-treatment indicates responses prior to addition of sildenafil or vehicle.
IC_{50} means concentration required to produce a 50% relaxation of the phenylephrine-induced contraction
n: number of tissues, CI: confidence interval, TMC: time-matched control.
Significance of difference from TMC $^{**}P<0.01$, $^{***}P<0.001$ (Wilcoxon two-sample test).

matched controls (Table II). Similarly, treatments with 100 and 1000 nM of sildenafil also significantly reduced the IC_{50} values approximately 6 and 5 times, respectively, when compared with the time-matched controls (Table III).

Although KJH-1002 showed approximately 6-fold more potent PDE5 inhibitory activity than sildenafil, it produced relaxing effects on RCC with potency similar to that of sildenafil. The observed difference in the relationship of potencies between PDE5 inhibition and RCC relaxation may suggest that KJH-1002 has less RCC tissue/cell permeability or faster disposal rate than sildenafil, which remains to be elucidated.

The present study indicates that KJH-1002, a specific phosphodiesterase type 5 inhibitor, produces relaxing effects on RCC comparable to those of sildenafil. In conclusion, the KJH-1002 possesses an augmentative effect on penile erection and may be useful for the treatment of erectile dysfunction.

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