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The effect of DA-8159 on corpus cavernosal smooth muscle relaxation and penile erection in diabetic rabbits

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Abstract A previous study showed that DA-8159, a potent type 5 phosphodiesterase inhibitor, enhanced the relaxation of the smooth muscles in the normal rabbit corpus cavernosum. In this study, we investigated the in vitro effects of DA-8159 on cavernosal smooth muscle relaxation and the in vivo erectogenic potential in diabetic rabbits, since erectile dysfunction is a well-known sequela of diabetes mellitus. Diabetes mellitus was induced in male New Zealand White rabbits with alloxan monohydrate. Cavernosal strips from age-matched control and 8-week diabetic animals were mounted in organ baths. The relaxation responses to sodium nitroprusside ($10^{-9} \backsim 10^{-5}$ M), a nitric oxide donor, were assessed in the presence or absence of DA-8159 ($10^{-9} \backsim 10^{-6}$ M). For the penile erection test, DA-8159 was given orally (1∼10 mg/kg) to diabetic rabbits and the length of the uncovered penile shaft was measured in a time-course manner in the presence or absence of intravenous sodium nitroprusside. The sodium nitroprusside-stimulated relaxations were significantly impaired in the corpus cavernosum from the diabetic group ($IC_{50} = 1.07 \times 10^{-6}$ M following 8 weeks of diabetes mellitus; compared with 0.48×10^{-6} M for age-matched controls). DA-8159 significantly and dose-dependently enhanced the sodium nitroprusside-stimulated relaxation in the diabetic groups. In addition, DA-8159 induced a dose-dependent penile erection in diabetic rabbits, which was potentiated by intravenous sodium nitroprusside. These results suggest that DA-8159 is an effective treatment for diabetic erectile dysfunction but further evaluation of the efficacy on human needs to be performed.

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Introduction

Diabetes mellitus is a major risk factor for erectile dysfunction (ED) [1] and the prevalence of ED in diabetic men has been estimated to range from 35 to 75% [2]. Although the pathophysiology of erectile dysfunction in diabetic men is multifactorial, the main mechanism is known to have a vascular and neuronal origin [3, 4]. In addition, there is increasing evidence that ED in diabetic men is the result of a decrease in nitric oxide synthase (NOS) activity [5] and an alteration in the endothelial cell-mediated physiological mechanism that controls penile smooth muscle tone [1].

Pharmacotherapy for treating ED has undergone dramatic advances over the past decade since the successful introduction of phosphodiesterase type 5 (PDE5) inhibitors [6]. One drug, sildenafil (Viagra) is a potent and an effective treatment, with an approximately 80% response rate in patients with an unknown etiology. However, the response rate in diabetic subjects is only 50% [7, 8]. Since neuropathy and arteriopathy can be a complication of diabetes mellitus, it can be argued that the treatment agent for ED may be less effective in diabetic patients. Other selective PDE5 inhibitors have been recently developed and are currently under development [6, 9, 10, 11]. Among them, Vardenafil (Levitra) and Tadalafil (Cialis) appear to be the most promising orally active agents for treating ED.

DA-8159 is a selective type 5 cyclic guanosine monophosphate (cGMP) inhibitor which inhibits the degradation of cGMP, resulting in a significant relaxation of the corpus cavernosum [12]. It has also been reported that DA-8159 induces a penile erection in a dose-dependent manner in anesthetized dogs and the relaxation of the normal rabbit corpus cavernosal smooth muscles in vitro [13]. The aim of this study was to investigate the effect of DA-8159 on the relaxation of

the corpus cavernosum in diabetic rabbits. In addition, we examined the oral efficacy of DA-8159 for inducing a penile erection in diabetic rabbits.

Materials and methods

Test materials

DA-8159 (CAS No. 268203-93-6) is a pyrazolopyrimidinone derivative with a molecular weight of 516.66, which was synthesized by Dong-A Pharmaceutical Company (Kyunggi, Korea) with a >99.8% purity as determined by both HPLC and potentiometric titrations in glacial acetic acid. Phenylephrine (PE), sodium nitroprusside (SNP) and alloxan monohydrate were purchased from Sigma Chemical Co. (USA). DA-8159 was dissolved in a Titrisol buffer solution (citrate sodium hydroxide buffer, pH 5.0, Merck) prior to administration.

Animals

The study was performed in accordance with the institutional Standard Procedure for Animal Care and Experiments (SOP-ANC) of the Dong-A Pharmaceutical Company and with the *Guide for the Care and Use of Laboratory Animals* from the National Institutes of Health. New Zealand white rabbits (Kwangsan Laboratory Animals, Korea) weighing 3–4 kg were used in this study. The animals were acclimatized for at least a week and housed individually. Throughout the experiments, the animals were kept in a standard laboratory conditioned room (temperature 23 ± 2°C, humidity range 40∼70%, 12-h light/dark cycle [lighting: 7:00–19:00]). The rabbits were given regular rabbit chow and water ad libitum.

Induction of diabetes and blood glucose measurement

Sexually mature male rabbits were randomly assigned to either the control (n = 6) or diabetes (n = 54) group. Diabetes was induced in the animals by a single injection of alloxan monohydrate (120 mg/kg of body weight) via the ear vein. The control animals were injected with the vehicle (0.9% NaCl) alone. The blood glucose levels were monitored (One Touch Basic, Lifescan, USA) each week after the alloxan treatment. The rabbits with blood glucose concentrations greater than 300 mg/dl each after the injection for 8 weeks were accepted as being diabetic. At the end of the study, eight of the 54 rabbits treated with alloxan had died and 17 remained euglycemic.

Organ chamber experiments

Strips of the rabbit corpus cavernosum were obtained from age-matched controls (n = 6) and diabetic rabbits (n = 10). Rabbits were sacrificed by cervical dislocation and a penectomy was performed immediately. The whole penis was placed in chilled Krebs buffer solution (NaCl 118.3 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25.0 mM, Ca-EDTA 0.016 mM and glucose 11.1 mM) and the corpus spongium and urethra were excised. The corpus cavernosal smooth muscle (CCSM) was then isolated from the enveloping tunica albuginea by a careful dissection. Four strips of the CCSM (about 3×3×7 mm) were obtained from each corpus cavernosum. Each strip was then mounted in an organ-bath chamber containing 10 ml of the Krebs-Hanseleit solution. The solution was aerated with 95% O_2, and 5% CO_2, at a pH of 7.4 and maintained at 37°C. The bath solution was replaced every 10∽15 min during equilibration. After 60-min equilibration, the CCSM strips were loaded with a resting tension of 2 g. The changes in the isometric tension were measured using a Grass F-60 transducer (Narco Bio-system, USA), and recorded on a physiograph (Trace 80, Narco Bio-system, USA). The strips were pre-contracted with PE (3×10^{-5} M). Strips that did not reach a steady tension were excluded from the study. The relaxation of the CCSM strips as a result of DA-8159 (1×10^{-9}∽1×10^{-6} M) was then assessed by adding SNP (1×10^{-9}∽1×10^{-5} M) cumulatively to the bathing medium. The DA-8159-induced relaxation of the CCSM strips was calculated as a percentage of the PE-induced active muscle tone; all the values reported are expressed as a mean value ± the standard deviation (S.D.). All the reported data were generated from at least eight CCSM samples from six animals. The SNP IC_{50} values at various DA-8159 concentrations were determined by linear interpolation. The significance of any differences among the IC_{50} values groups was determined using both a Bonferroni test and ANOVA. A p value < 0.05 was considered significant.

Penile erection test in diabetic rabbits

Transparent plastic observation chambers were made and all rabbits were acclimated individually in the chambers for 2 h/day for at least 3 days prior to the experiments. After an oral dose of DA-8159, at a dose range of 1–10 mg/kg, the length of the uncovered penile shaft was measured using sliding digital calipers. The controls were treated with the corresponding vehicle. The length of the exposed penile shaft was measured every minute for 60 min. The SNP was then dissolved in saline (0.2 mg/1 ml/kg) and injected into the ear veins with a volume of 1 ml/kg after 60 min of drug administration. After the intravenous injection of SNP, the length of the exposed penile shaft was measured for a further 30 min. The mean length of the exposed penile shaft from the five animals was calculated at each time point. The mean length was plotted against time and the area under the curve was calculated using an integration program (WinNolin Standard, Pharsight Corporation, California). The effect of the sodium nitroprusside injection alone (area under the curve) was subtracted from the results.

Results

Animal weights and blood glucose concentrations

The initial body weight in the control and diabetic animals was similar. However, the body weights of diabetic rabbits at 8 weeks after alloxan treatment were significantly ($p < 0.05$) lower than those of the age-matched controls (Table 1). At 8 weeks, the average blood glucose concentrations were significantly greater in the diabetic group than in the control group ($p < 0.05$) (Table 1).

Organ chamber studies

In the isolated corpus cavernosal strips pre-contracted with phenylephrine (3×10^{-5}M), the administration of

Table 1 Body weight and blood glucose level in the control and diabetic groups

	Body weight (kg)		Blood glucose (mg/dl)
	Initial	At 8 weeks	At 8 weeks
Control (n = 6)	3.21 ± 0.20	4.84 ± 0.34	112.3 ± 11.1
Diabetic (n = 29)	3.24 ± 0.19	3.90 ± 0.24*	452.5 ± 89.7*

*$p < 0.05$ statistically different from the controls. n number of animals

SNP ($1\times10^{-9}\backsim1\times10^{-5}$ M) resulted in a concentration-dependent relaxation response both in the normal and diabetic rabbits (Fig. 1). However, the SNP-stimulated relaxation was impaired in the corpus cavernosum from the diabetic rabbits ($IC_{50}=1.07\times10^{-6}$ M) compared with the age-matched controls ($IC_{50}=0.48\times10^{-6}$ M) (Fig. 1). DA-8159 administration ($1\times10^{-9}\backsim1\times10^{-6}$ M) significantly enhanced the SNP-stimulated corpus cavernosal relaxation in the diabetic rabbits in a concentration-dependent manner (Fig. 2). The IC_{50} values were 0.71×10^{-6} M (DA-8159 1 nM), 0.40×10^{-6} M (DA-8159 10 nM), 0.16×10^{-6} M (DA-8159 100 nM) and 0.06×10^{-6} M (DA-8159 1,000 nM), respectively. A significant difference was observed from a dose level of 10 nM of DA-8159 compared with the diabetic control ($p<0.05$).

Penile erection test in diabetic rabbits

A single oral administration of DA-8159 showed no response for the first 60 min at a dose of 1 mg/kg. However, there was a weak response of exposing the uncovered penile shaft at 3 mg/kg and the maximum effect was observed at 10 mg/kg, with a much stronger and long-lasting erection (Fig. 3). In contrast, no exposure of the penile shaft was observed in the vehicle-treated animals. However, when SNP was given intravenously, the erectogenic effect of DA-8159 was markedly potentiated (Fig. 3). A dose-dependent increase in the mean length of the uncovered penile shaft was observed between 1 and 10 mg/kg, peaking at 10 mg/kg. The effect was much stronger and long lasting compared with that of SNP alone. The maximum mean length of the uncovered shaft induced by DA-8159 was 9.5 mm (±2.2 mm) at a 10 mg/kg dose. The time course of the penile erection expressed as an area under the curve after the oral administration of DA-8159, followed by an intravenous injection of SNP, is shown in Table 2. The table shows that the erectogenic effect of DA-8159 was dose dependent and was potentiated by co-administration with SNP, which was two to three times more effective than that of DA-8159 alone (Table 2).

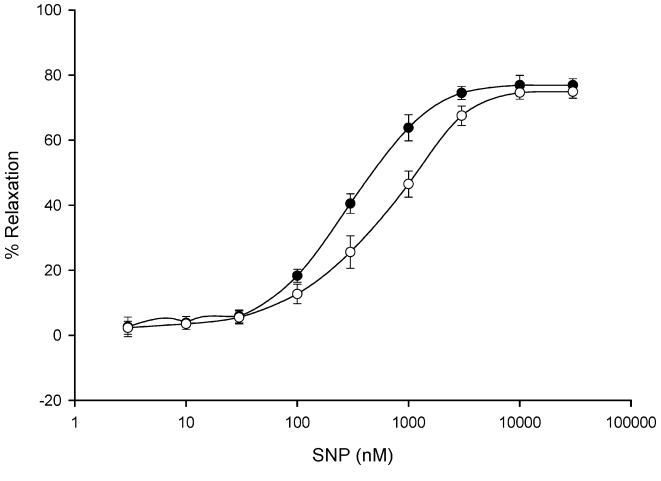

Fig. 1 Sodium nitroprusside-mediated relaxation of the corpus cavernosal strips from age-matched control (*closed circle*) and diabetic rabbits (*open circle*). The strips were pre-contracted with phenylephrine (3×10^{-5} M) and cumulative response curves were constructed for SNP($1\times10^{-9}\backsim1\times10^{-5}$ M). The results are expressed as the % relaxation of the phenylephrine-induced tone. The number of animals was six in the control and ten in the diabetic group

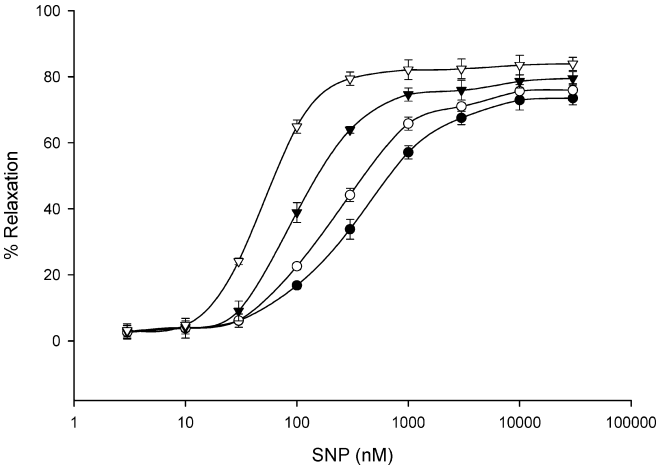

Fig. 2 The effect of DA-8159 on SNP-mediated relaxation of corpus cavernosal strips taken from diabetic rabbits. The strips were pre-contracted with phenylephrine (3×10^{-5} M) and cumulative response curve was constructed for SNP ($1\times10^{-9}\backsim1\times10^{-5}$ M) in the presence of DA-8159. The results are expressed as % relaxation of the phenylephrine-induced tone (*closed circle,* DA-8159 1 nM; *open circle,* DA-8159 10 nM; *closed triangle,* DA-8159 100 nM; *open triangle,* DA-8159 1,000 nM) for diabetic cavernosal strips

Discussion

The results presented in this study demonstrate that DA-8159, a specific PDE5 inhibitor, relaxed the corpus

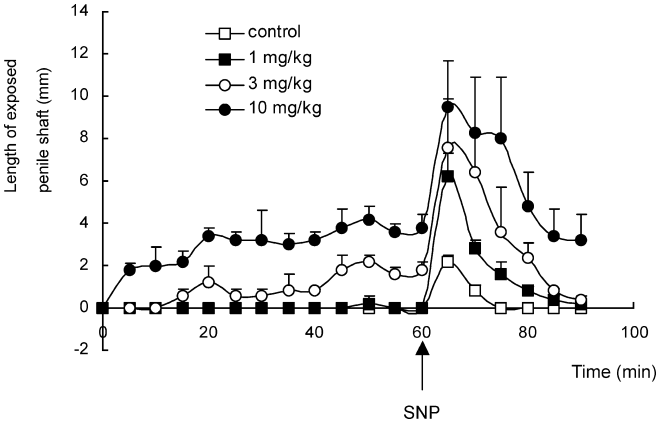

Fig. 3 Effects of DA-8159 on the length of the exposed penile shaft in diabetic rabbits (DA-8159 groups (n = 5); control group (n = 4)). The length of the exposed penile shaft was measured after the oral administration of DA-8159, followed 60 min later by an intravenous sodium nitroprusside (*SNP,* 0.2 mg/kg) injection. The SNP was administered alone for a comparison

Table 2 Time course of a penile erection after the oral administration of DA-8159 followed by an intravenous injection of sodium nitroprusside

Dose (mg/kg)	Area under the curve[a]	
	−SNP (mm × min)	+ SNP (mm × min)
1	1	45.5
3	55.5	150.0
10	177.5	350.0

AUC was calculated as Time (min) × Length of the uncovered penile shaft (millimeters). DA-8159 was orally administered and followed by an injection of SNP (0.2 mg/kg) at 60 min.
[a]Cumulative AUC from 0 to 90 min

cavernosum smooth muscle and induced a penile erection in diabetic rabbits, which was potentiated by sodium nitroprusside, a nitric oxide donor.

It has been established that a relatively brief period of alloxan-induced diabetes can induce changes in the physiological mechanisms that modulate the trabecular smooth muscle in rabbits [1, 14]. The similarity of these changes to those in the tissues from diabetic impotent men make the alloxan-induced diabetic rabbits a suitable model to investigate the effects of diabetes on the penile smooth muscle [15]. This study also showed that there was an impairment of corporal smooth muscle relaxation in the cavernosal tissue strips from the alloxan-induced diabetic rabbits. The SNP-stimulated corpus cavernosal relaxation was significantly reduced in the diabetic groups compared with the age-matched control animals. A significant decrease in body weight and an increase in the blood glucose concentration were also noted, which indicated that the alloxan-induced diabetic model is a suitable model to investigate the effects of DA-8159 on diabetic erectile dysfunction.

Diabetic men have an approximately threefold increased prevalence of ED compared with non-diabetic men. However, oral medications acting via phosphodiesterase inhibition in the penile vasculature have revolutionized the treatment of impotence in diabetic men [16]. The PDEs comprise eleven distinct families of enzymes that catalyse the termination of second messenger activity in cells by hydrolyzing the phosphodiester bond of cyclic adenosine monophosphate (cAMP) or cGMP to its corresponding monophosphate [17, 18, 19, 20]. Inhibitors of PDEs can elevate the intracellular level of cAMP, cGMP or both, depending on the substrate specificity of the particular PDE that is blocked. PDE5 is cGMP specific and present in high concentration in the smooth muscle of the corporal cavernosum of the penis [21, 22, 23]. PDE5 inhibitors enhance erectile function during sexual stimulation by maintaining sufficient cellular levels of cGMP in the corpus cavernosum, thus increasing dilatation of the corporal sinusoids and allowing more blood flow, which induces a penile erection [24, 25]. This approach has been validated by the clinical efficacy and safety of sildenafil citrate, the first orally administered selective PDE5 inhibitor developed for the treatment of ED. DA-8159 is also a potent and selective PDE5 inhibitor that can inhibit the cGMP hydrolysis, thereby elevating the levels of this cyclic nucleotide [12]. While the efficacy of DA-8159 in healthy animals has been demonstrated [13], the efficacy in diabetic animals has not yet been established. In this study, the SNP-stimulated relaxation of corpus cavernosal tissue and the erectogenic potentials in diabetic rabbits were measured to investigate the efficacy of DA-8159 on diabetic ED.

In the in vitro study, DA-8159 significantly enhanced the SNP-stimulated corpus cavernosal relaxation from the diabetic rabbits in a concentration-dependent manner. A significant difference was observed from the dose level of 10 nM of DA-8159 compared with the diabetic control. These findings concur with a previous in vitro study showing that DA-8159 enhances the corpus cavernosal relaxation in healthy rabbits. On the other hand, the primary mechanism for the relaxation of the corpus cavernosum smooth muscle and penile erection depends upon the NO-induced elevation of the cGMP levels [26]. It was reported that the main phosphodiesterase that hydrolyzes cGMP in the corpus cavernosum is the PDE type 5, and that the minor isozyme is PDE type 2 [27]. This means that agents such as phosphodiesterase type 5 inhibitors, which enhance the NO–cGMP signal transduction pathway, might prove beneficial in treating erectile dysfunction. Based on these results and references, it was believed that DA-8159 can elicit the relaxation response through the NO-cGMP phosphodiesterase pathway in diabetic animals, although the cGMP levels were not measured in this study.

Besides the in vitro study, the in vivo efficacy of DA-8159 for inducing penile erection was investigated using diabetic rabbits. These results show that DA-8159 is effective and induces a dose-dependent penile erection after oral administration. The results showed that no exposure of the penile shaft was observed in lower doses in the absence of SNP. However, more than 3 mg/kg of DA-8159 induced an erection prior to the injection of the NO donor. It is well known that the erectogenic effect of a PDE5 inhibitor is dependent on NO being present in response to sexual stimulation. Although there was no sexual stimulation or exogenous NO injection, it may be that there is a basal release of NO, which was the reason why DA-8159 alone induced penile erection prior to SNP, but it was not conclusive. On the other hand, after administering a nitric oxide donor, the erectogenic effect of DA-8159 was potentiated and the minimal effective dose was decreased to 1.0 mg/kg. These results demonstrate the efficacy of DA-8159 in diabetic rabbits and its efficacy was potentiated in the presence of NO.

In conclusion, the in vitro study demonstrated a concentration-dependent relaxant response of DA-8159 on the cavernosal tissue of diabetic rabbits. In addition, DA-8159 induced a dose-dependent penile erection in diabetic rabbits, which was potentiated by intravenous sodium nitroprusside. These results demonstrate that DA-8159 is a promising treatment for diabetic erectile

dysfunction, but further evaluation of the efficacy on humans needs to be performed.

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