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Erectogenic Effect of the Selective Phosphodiesterase Type 5 Inhibitor, DA-8159

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DA-8159, a new phosphodiesterase 5 inhibitor, was assessed for its erectogenic potential by a penile erection test in rats, the relaxation of isolated rabbit corpus cavernosum (CC), and estimation of the intracavernous pressure (ICP) in the anesthetized dog. Oral administration of DA-8159 (0.3 to 1 mg/kg) increased the number of erections in rats with increasing dosage, with the highest penile erection index at 10 mg/kg. DA-8159 induced the relaxation of phenylephrine (PHE)-induced contractions in the rabbit CC and decreased the IC_{50} of the nitric oxide donor sodium nitroprusside (SNP) in a dose-dependent fashion. In pentobarbital-anesthetized dogs, the intravenous administration of DA-8159 (1~300 μg/kg) potentiated the increase in ICP induced by the intracavernosal SNP in a dose-related manner. These findings suggest that DA-8159 has significant therapeutic potential in the treatment of erectile dysfunction.

Key words: DA-8159, Phosphodiesterase 5 inhibitor, Rabbit corpus cavernosum, Intracavernous pressure (ICP), Penile erection index (PEI), Erectile dysfunction (ED)

INTRODUCTION

Erectile dysfunction(ED), formerly referred somewhat imprecisely to as impotence, is defined as the inability to achieve or maintain a hard, erect penis sufficient for a sexual intercourse. Its prevalence is strongly related to age, with an estimated occurrence of 2% at age 40 rising to 25~30% by age 65 (Furlow, 1985). Although no data is available showing the prevalence of ED in men over 75, the number probably exceeds 50% (Furlow, 1985). According to the guidelines from the American Urological Association, several safe and effective treatment options for physiologically caused ED are available (American Urological Association Press Release, 1996). Methods including vacuum constriction devices, drug injection therapy and prosthesis implants are often cited, as are venous and arterial surgery for impotent men with arteriolosclerotic disease, although only as an exploratory method.

Among these therapeutic options, a drug injection therapy of vasoactive agents has been widely investigated. The most frequently used substances are alprostadil, phentolamine, and papaverine. These drugs activate the vascular component of the penile erection, inhibiting the sympathetic tone and stimulating the relaxation of the corporal smooth muscle. In as much as 90% of patients, a combination of two- or three-drug therapies provide pharmacological synergy with few side effects (Butler *et al.*, 1994; Lipshultz, 1996; Montague *et al.*, 1996; Montorsi *et al.*, 1995). However, there may be several adverse effects as a result of intracavernosal injection therapy. These include pariapism, fibrosis, urethral damage, pain after injection, local infection and hematomas (Koeneman *et al.*, 1997; Montorsi *et al.*, 1995). To avoid these limitations, a transurethral administration device for alprostadil (MUSE) was developed. However, it's efficacy and side effects are comparable to intracavernous alprostadil (Padma-Nathan *et al.*, 1997; Werthman and Rajfer, 1997). Therefore a great deal of research effort has been directed at developing new, and simple-to-use treatments, in particular oral medication. Oral apomorphine and phentolamine are currently in the final stages of development (Leland, 1997).

More recently, a great deal of interest has been focused on compounds capable of elevating the cyclic guanosine monophosphate (cGMP) levels. cGMP is an ubiquitous second messenger for G-protein-coupled receptors, which are involved in regulation of the vascular tone mediated by endogenous substances such as nitric oxide (NO) and the atrial natriuretic factor. Recent studies have indicated

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that relaxation of the corpus cavernosal smooth muscle cells is triggered by NO and cGMP (Bush *et al.*, 1993; Kim *et al.*, 1991). One such drug, sildenafil, the active ingredient of Viagra, was discovered as a potent and competitive inhibitor of the type 5 cGMP-specific phosphodiesterase (PDE5), the predominant isoenzyme in the human corpus cavernosum. Since its first introduction in 1998, its potential use in the treatment of ED is now well-accepted after a successful global marketing campaign. Sildenafil has shown an approximately 90% response rate in ED patients with unknown etiology and a 50% response rate in diabetic subjects (Boolell *et al.*, 1996; Wagner and Saenz de Tejada, 1998). The adverse effects of sildenafil include headache (16%), flushing (10%) and dyspepsia (7%). In addition, mild, transient vision disturbance has also been reported in approximately 3% of patients. Moreover, sildenafil is contraindicated in persons who are currently using organic nitrates in any form, as the agent potentiates the hypotensive effects of nitrates (Viagra product information, 1998).

The Dong-A Pharmaceutical Company has recently developed a novel selective PDE5 inhibitor, DA-8159, for use as a potential oral drug for the treatment of ED. According to the results obtained from preliminary studies, DA-8159 has superior isozyme selectivity and a longer half-life with less hepatic metabolism when compared to sildenafil (data not shown). The purpose of present study was (1) to examine the effect of DA-8159 on penile erection using rats as a model, (2) to study the relaxation effect on the rabbit corpus cavernosum smooth muscle *in vitro* and (3) to evaluate the erectogenic potential by measuring the intracavernous pressure in anesthetized dogs.

MATERIALS AND METHODS

Animals

The studies were 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 National Institutes of Health. Male Sprague-Dawley rats (230~250 g; Charles River Japan, Kanagawa, Japan), male New Zealand White rabbits (2.8~3.2 kg; Kwangsan, Kyunggi, Korea), and male mixed-breed dogs (9.0~11.0 kg; Kwangsan, Kyunggi, Korea) were used in this study. During the experiments, the animals were kept under standard laboratory conditions and allowed free access to food and UV-sterilized tap water.

Materials

DA-8159, a pyrazolopyrimidinone derivative with the molecular weight of 516.66, was synthesized by the Dong-A Pharmaceutical Co. (Kyunggi, Korea) with a > 99.8% purity as determined by both HPLC and potentiometric titrations in glacial acetic acid. DA-8159 was stable for at least 12 weeks both in the ambient temperature and the accelerated condition (20°C, 75% RH). Sildenafil citrate was obtained from the Dong-A Pharm. Co., and sodium nitroprusside (SNP), phenylephrine (PHE) and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO). DA-8159 was dissolved in 10% DMSO and suspended in 1% hydroxypropylmethyl-cellulose (HPMC, Aldrich) for either intravenous or oral administration.

Penile Erection test in the rat model

The penile erection test was performed using the methods reported by Benassi-Benelli *et al.* (1979) and described elsewhere in detail (Islam *et al.*, 1990; Taha *et al.*, 1995). Adult male Sprague-Dawley rats, were maintained in a quiet conditioned (temperature 23 ± 2°C, humidity range 40~70%) room under a reversed light-dark cycle (lighting from 19:00 07:00) for 1 week prior to the experiment. After acclimatization, either DA-8159 or sildenafil citrate was freshly suspended in 1% HPMC and orally administered at doses of 0.3, 1, 3 or 10 mg/kg. 10 ml/kg of 1% HPMC was gavaged for the control group. At least 6 rats were used for each test condition.

After drug administration, the rats were placed individually in the observation cage (40 × 30 × 30 cm) with a clear acrylic bottom and attached mirror, and observed continuously for the frequency of genital grooming and number of penile erections for 2 h. The penile erectile response was defined as the period of pelvic thrusting followed by genital grooming and the display of an engorged penis. The erectile response was expressed as the penile erection index (PEI) and was obtained by multiplying the percentage of rats exhibiting at least one episode of penile erection during the observation time by the mean number of penile erections. The behavioral tests were performed between 10:00 a.m. and 2:00 p.m. The statistical significance for the differences between groups was calculated using *Duncan's multiple comparison* method.

Effect on the isolated rabbit corpus cavernosum

Rabbit corpus cavernosum smooth muscle (CCSM) strips were obtained from sexually matured male New Zealand White rabbits (2.8~3.2 kg). Penectomies were performed on euthanized animals and the whole penis was dissected free in 0~-4°C. The Krebs-Hanseleit solution consisted of 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. 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. During equilibration, the bath solution was replaced every 10~15 min. After equilibration for 60 min, the CCSM strips were loaded with a resting tension of 2 g. 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 always precontracted with PHE (1×10^{-5} M). Strips that did not reach a maximal tension or did not show stable contraction were excluded from the study. The relaxation of the CCSM strips by 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. DA-8159-induced relaxation of the CCSM strips was calculated as a percentage of the PHE-induced active muscle tone; all values reported are presented as the mean value ± the standard error of the mean (S.E.M.). All the reported data was generated from at least 6 experiments in different 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 were determined using both a t-test and ANOVA, with a p value set at 0.05.

Erectile function in the anesthetized dog

Male mixed-breed dogs, with body weights ranging from 9.0 to 11.0 Kg, were used. After overnight fasting, animals were anesthetized with pentobarbital (35 mg/kg) administered intravenously *via* an angiocatheter (sp 55, Natsume, Japan) in the left cephalic vein. The anesthesia was maintained throughout the experiment by the continual infusion of pentobarbital (0.7 mg/kg/hr). A catheter was introduced into the left femoral vein for the administration of the drug. The left femoral artery was cannulated for measuring both the blood pressure and heart rate. The penis was carefully denuded to the base without damaging the prepuce, and the left corpus cavernosum was exposed. A 23-gauge scalp needle, which was attached by flexible catheter to a pressure transducer, was inserted into the left corpus cavernosum for measuring the intracavernous pressure (ICP). The system was filled with heparinized saline (25 U/ml), which was flushed through prior to each measurement cycle. The parameters were recorded on a polygraph and all data acquisition and calculation of the derived parameters were performed using an on-line computer system (Signal Processor, USA). Following a period of equilibration, DA-8159 was administered intravenously at doses of 0, 1, 3, 10, 30, 100 or 300 μg/kg. SNP (10 μg/0.1 ml/head), a NO donor, was dissolved in saline and injected into the right corporal carvenosum for 5 min after administration of the test compound. Any changes in all parameters measured were recorded for 10 min. At the completion of each experiment, 0.5 μl of methylene blue solution was injected intracavernously to verify the position of the recording needle. The data obtained is presented as the mean ± S.E.M.. Any differences among the treatment groups were evaluated by ANOVA, followed by the Dunnett test for the post hoc comparison of the means against the control. Values were considered significant when $p<0.05$.

RESULTS

Penile erection test in the rat model

It was found that oral administration of DA-8159 increased the number of penile erections (Table I) in the rats. Dose related increases in the erection response were observed between 0.3 and 10 mg/kg DA-8159, peaking at 10 mg/kg. Similarly, sildenafil citrate increased penile erection in a dose range of 0.3~10 mg/kg. The highest penile erection index (PEI) for DA-8159 and sildenafil citrate were 255.0 ± 95.7 and 225.7 ± 92.7, respectively. During the observation, there was no difference in the occurrences of genital grooming between the control and treated groups (Table I).

Effects on the isolated rabbit corpus cavernosum

DA-8159 alone produced an inhibition of the stretch-evoked tension in the rabbit corpus cavernosum with the effect increasing with increasing DA-8159 concentrations between 1×10^{-9} and 1×10^{-6} M. When the corporal smooth muscle strips were pre-contracted by PHE (1×10^{-5} M), SNP applied at the plateau phase, caused a concentration-dependent relaxation (1×10^{-8} to 3×10^{-5} M). Cumulative SNP addition to the PHE- contracted strips exhibited concentration-dependent and rapid relaxant responses. The IC_{50} value for the SNP ranged from 306.0~640.2 nM. Pretreatment with DA-8159 induced a significant decrease in the SNP IC_{50} value over the concentrations ranging from 1 nM to 1000 nM, in a concentration-dependent manner (Table II). The IC_{50} values for

Table I. The effects of DA-8159 on penile erection response in male rats

Test substances	n	Dose (mg/kg)	Penile Erection Index	No. of Genital Groomings
Control	14	-	62.2 ± 29.7	1.9 ± 1.0
DA-8159	14	0.3	137.5 ± 47.9	1.8 ± 1.3
	18	1.0	132.5 ± 23.5*	1.7 ± 0.9
	14	3.0	193.8 ± 41.8*	1.8 ± 0.5
	13	10.0	255.0 ± 45.7*	2.0 ± 1.2
Sildenafil citrate	12	0.3	162.5 ± 95.7	1.6 ± 0.6
	10	1.0	145.9 ± 38.3*	1.1 ± 0.8
	10	3.0	163.2 ± 31.2*	2.3 ± 1.3
	18	10.0	225.7 ± 32.7*	3.1 ± 1.5

*Significantly different from control ($p<0.05$)

Table II. The effect of DA-8159 on sodium nitropurusside-induced relaxation of the rabbit corpus cavernosum strip.

Treatment	n	IC_{50} for SNP (nM)	Treatment	n	IC_{50} for SNP (nM)
Time matched control	7	417.6	DA-8159 1 nM	7	390.2
Time matched control	7	640.2	DA-8159 10 nM	6	251.2*
Time matched control	8	435.6	DA-8159 100 nM	8	116.5*
Time matched control	7	306.0	DA-8159 1000 nM	7	107.7*

*: Significantly different from each time matched control ($p<0.05$).

the SNP were 390.2, 251.2, 116.5, and 107.7 nM at DA-8159 concentrations of 1, 10, 100, and 1000 nM, respectively.

Erectile function in the anesthetized dogs

The baseline intracavernous pressure (ICP) in the anesthetized dogs was 6.0 ± 1.05 mmHg (n=16). Intravenous DA-8159 administration alone did not change the ICP. However with SNP, DA-8159 significantly increased the ICP with increasing dosage (Fig. 1). The peak ICP values achieved by intravenous DA-8159 were 11.1 ± 1.36, 20.4 ± 2.51, 32.6 ± 4.74, 51.7 ± 4.84, 66.0 ± 6.74, and 74.4 ± 8.51 mmHg at doses of 1, 3, 10, 30, 100, and 300 μg/kg, respectively. The ratio of the ICP to the mean blood pressure (MBP) increased from 5.3 ± 0.36% (0 μg/kg) to 63.7 ± 2.04% (300 μg/kg of DA-8159). The duration of maximal ICP also increased with increasing dosage (Fig. 1). During the experiment, the MBP was not changed (Fig. 1).

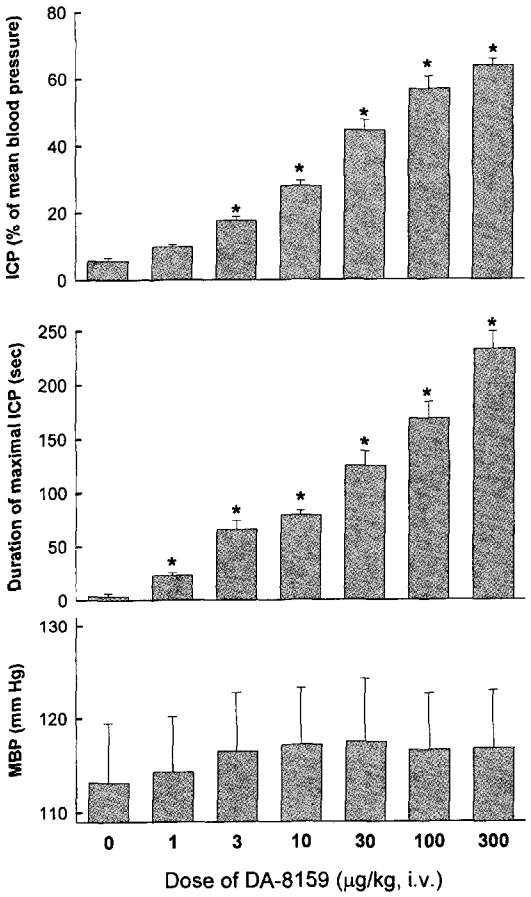

Fig. 1. Effect of intravenous DA-8159 on intracavernous pressure (ICP), duration of increased ICP and mean blood pressure (MBP) in the anesthetized dog. Values are mean ± S.E.M., n=8. *P< 0.05 *vs.* control in the Dunnett test.

DISCUSSION

The present study demonstrates the erectogenic potential of DA-8159, a new phosphodiesterase type 5 inhibitor, using the rat erection counting, relaxation of the rabbit corpus cavernosum smooth muscle strip, and the intracavernous pressure (ICP) in anesthetized dogs. In preclinical evaluation of candidates for erectile dysfunction (ED) treatment, counting the erection episodes (Heaton *et al.*, 1990) and ICP monitoring (Chen *et al.*, 1992; Carter *et al.*, 1998) should be conducted concurrently to assess erectogenic activity. The rat erection tests adopted in this study are a convenient and cheap assay system, especially for oral agents. However, skilled techniques are required in order to obtain reproducible results as penis engorgement during a rat erection is not remarkable and the erectile angle is not prominant from its standing position. In contrast, estimating the ICP in the dog as an index offers a more objective, accurate, and quantitative assessment of penile erection (Carter *et al.*, 1998). Chen *et al.* (1992) suggested a rat model for ICP monitoring. However, because the most important and difficult task in recording the ICPs of laboratory animals is how accurately and reproducibly the recording needle can be inserted into the corpus cavernosum, the dog model was used in this study. The mean oblique anteroposterior and transverse dimension of each corpus cavernosum in the rat are 1.9 and 0.9 mm, respectively (Chen *et al.*, 1992). Thus, accurate insertion and fixation of the recording needle in the rat corpus cavernosum is not easy. As in the present study, when the intracavernous SNP is used as a stimulus, stable fixation of the recording needle (0.5 mm in diameter) with increased ICP is not feasible. Moreover, the volumic effect of the intracavernous SNP also interferes with the accurate interpretation of any ICP change. Because of

these reasons, the dog model is more widely used for ICP recording (Ayajiki *et al.*, 1997). In this study, an intravenous injection of DA-8159 induced a dose-dependent elevation of the ICP in the dog. Though the data is not shown, sildenafil also increased the ICP in the same test system, and the erectogenic potential and duration of the increased ICP as a result of sildenafil were comparable with those of DA-8159.

Penile erection and yawning are two different behavioral patterns that often occur concurrently under physiological and experimental conditions (Holmgren *et al.*, 1985). In the current rat erection test, there was no difference in the frequency of yawning (data not shown). This result suggests that the erectogenic action of DA-8159 is not associated with an increase in NO production. NO acts as an activator of guanylate cyclase, which catalyzes the conversion of guanosine 5'-triphosphate (GTP) to cGMP, thus increasing the intracellular cGMP level and promoting erectile tissue relaxation, which is the essential mechanism for a rigid erection (Trigo-Rocha *et al.*, 1993).

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes responsible for the hydrolysis of cGMP and cAMP to monophosphates, which can no longer participate in penile relaxation (Thompson, 1991). PDEs were first detected by Sutherland and coworkers (Butcher *et al.*, 1962). The superfamily of PDEs is subdivided into two major classes, class I and II, which have no recognizable sequence similarity. Class includes all known mammalian PDEs and is comprised of at least 10 identified families that are products of separate genes (Conti *et al.*, 1995; Degerman *et al.*, 1997; Houslay, 1995). Some PDEs are highly specific for cAMP (PDE4, PDE7, PDE8) hydrolysis, some are highly cGMP-specific (PDE5, PDE6, PDE9), and some have mixed specificity (PDE1, PDE2, PDE3, PDE10). Arterial blood flows into the sinusoidal spaces of the corpus cavernosum and corpus spongiosum in the penis and exits *via* the postcavernous venules (Wagner and Saenz de Tejada, 1998). The arteries supplying the sinusoids, as well as the sinusoids themselves, contain a layer of smooth muscle cells in their walls. Relaxation of these smooth muscle cells reduces the resistance to blood flow into the penis, thereby increasing both the amount of blood flow into the sinusoids and the volume within these compartments. This causes a state of increased penile tumescence. The expanded corporal cavernosa presses against the inflexible tunica albuginea, and reduces venous blood outflow; the pressure in the corpora cavernosa increases until it approaches the systolic blood pressure, thus resulting in a penile erection.

The mean resting ICP in the dogs recorded in this study was approximately 6 mmHg, which is similar to that in humans (Goldstein *et al.*, 1989). However, the ICP at maximal erection in response to 300 μg/kg of DA-8159 is lower than that in humans (Goldstein *et al.*, 1989). Chen *et al.* (1992) suggested that this difference might be due to a thicker tunica albuginea in humans than in other animals, which might result in a higher venous resistance and higher ICP surrounded by the tunica albuginea.

In this study, oral administration of DA-8159 increased the number of penile erections in rats and potentiated the ICP elevation induced by nitric oxide bonor SNP in anesthetized dogs. These results clearly demonstrate DA-8159 has the potential to be a significant advance in ED treatment options.

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