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·Original Article·

Erectile potentials of a new phosphodiesterase type 5 inhibitor, DA-8159, in diet-induced obese rats

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Abstract

Aim: To examine the changes in the erectile function in diet-induced obese rats and investigate the oral efficacy of DA-8159, a new phosphodiesterase type 5 (PDE5) inhibitor, on penile erection in obese rats. **Methods:** The rats were fed a high-energy diet for 12 weeks and divided into three groups: an obesity-resistant (OR) control group, an obesity-prone (OP) control group, and an OP-DA-8159 treatment (DA-8159) group. The electrostimulation-induced erectile responses were measured in all groups. The body weight, plasma cholesterol, triglyceride and glucose levels were also measured. **Results:** In the OP control group, the maximum intracavernous pressure (ICP) and ICP/blood pressure (ICP/BP) ratio after electric stimulation were significantly lower than those in OR control group. The corresponding area under the curve (AUC) of the ICP/BP ratio, the detumescence time and the baseline cavernous pressure were also lower than those in the OR control group, but this difference was not significant. The body weight gain, plasma cholesterol and triglyceride level in the OP group were significantly higher than those in the OR group. After administering the DA-8159, a significant increase in the maximum ICP and the ICP/BP ratio were observed. The corresponding AUCs in the DA-8159 group were also higher than those in the two control groups. Furthermore, the detumescence time was significantly prolonged after treatment with DA-8159. **Conclusion:** These results demonstrate that diet-induced obesity affects the erectile function in rats and these erectile dysfunction (ED) can be improved by the treatment with DA-8159, indicating DA-8159 might be a treatment option for ED associated with obesity. *(Asian J Androl 2006 May; 8: 325–329)*

Keywords: phosphodiesterase type 5 inhibitor; penile erection; obesity; DA-8159; intracavernous pressure

1 Introduction

Erectile dysfunction (ED) is a common public health problem affecting millions of men worldwide [1]. Obesity is also one of the major public health problems, but there is little evidence available on the influence of obesity on sexual function. Some articles have reported that obesity in itself does not appear to be an underlying factor of ED [2]. On the other hand, obesity is known to be an independent predictor of ED and increases the incidence of ED [3, 4]. In addition, men who are overweight have an increased risk of developing ED regardless of whether they have lost weight [3]. Furthermore, it is well-known that obesity poses the risk of vasculogenic impotence due to associated chronic diseases, such as diabetes, hypertension, heart disease and hyperlipidemia [4, 5].

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Pharmacotherapy with phosphodiesterase type 5 (PDE5) inhibitors for treating ED has undergone dramatic advances since the successful introduction of sildenafil (Viagra, Pfizer,USA), the first PDE5 inhibitor. Another two PDE5 inhibitors, vardenafil (Levitra, Bayer, Germany) and tadalafil (Cialis, Eli Lilly, USA), are now available as potent and effective treatments with an approximately 80% response rate [6]. Therefore, the main target for the development of new ED drugs by several pharmaceutical companies is the PDE5 inhibitor. DA-8159 is a PDE5 inhibitor that has the most favorable pharmacokinetic profiles and fewer side effects compared to the other PDE5 inhibitors [7]. Its efficacy in ED has already been established in several animal models [8, 9].

With regard to the animal model of obesity, the diet-induced obese model, which was introduced by Levin *et al*. [10] and which has developed into a purified diet model [11–13], is one of the most commonly-used models. In this model, Sprague–Dawley (SD) rats fed a purified moderately high-fat, sucrose and energy diet (HE diet) exhibited a bimodal pattern in body weight gain, which is similar to that observed in human. Approximately half of the rats gained weight rapidly compared with the chow-fed rats (obesity prone, OP), whereas the other half gained body weight at a rate similar to or lower than that of the chow-fed animals (obesity resistant, OR).

The aim of the present study was to examine the changes in the intracavernosus pressure (ICP) responses after electrostimulation-induced penile erection in the diet-induced obese model and investigate the oral efficacy of DA-8159.

2 Materials and methods

2.1 Chemicals

DA-8159 (CAS No. 268203-93-6) was synthesized by the Dong-A Pharmaceutical Company (Kyunggi, Korea). DA-8159 was dissolved in a Titrisol buffer solution (citrate sodium hydroxide buffer, pH 5.0, Merck, USA) for administration.

2.2 Animals

This 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. Male SD rats (Charles River Japan, Yokohama, Japan) were used. The animals were acclimatized for one week and housed individually. Throughout the experiment, the animals were kept in a standard laboratory conditioned room (temperature 23 ± 2ºC, humidity range 40 –70%, 12 h light–dark cycle; lighting: 7:00–19:00).

2.3 Induction of obesity

The diet-induced obese model was prepared according to previous reports [10–13]. Fifty male SD rats (weighed approximately 270 g) were kept on Purina lab chow (No. 5001; Research Diets, USA) and water *ad libitum* for one week. Subsequently, all the rats were switched to the HE diet *ad libitum* for further 12 weeks. The HE diet was composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow (Research Diets, USA), and contained 18.718 kj/g, with 21% of the metabolizable energy content as protein, 31% as fat, and 48% as carbohydrates; 50% of which is sucrose. Purina rat chow (No. 5001) contains 13.819 kj/g, with 23.4% as protein, 4.5% as fat, and 72.1% as carbohydrates, which is mainly in the form of complex polysaccharides [14]. The body weight was measured weekly. After 12 weeks (week 13), the rats fed the HE diet diverged into distinct groups based on their body weight gains. The 16 rats with the highest body weight gain were designated as the OP group and the 8 with the lowest weight gain were designated as the OR group. The remaining 26 intermediate weight gainers were discarded.

2.4 Measurement of intracavernous pressure (ICP)

At the end of week 13, the animals were divided into three groups. The group 1 animals (n = 8) were the OR rats and served as age-matched control. The groups 2 and 3 animals were the OP rats, which were subdivided into the OP control (group 2, n = 8) and the DA-8159-treated OP rats (group 3, n = 8). The groups 1 and 2 rats were given vehicle orally and served as normal and obese control, respectively. The group 3 animals were given 10 mg/kg DA-8159 orally 1 h before the ICP measurement.

For the ICP measurement, after anesthetizing the rats with sodium pentobarbital (50 mg/kg, i.p.), the penile skin was incised and the prepuce was degloved to completely expose to the corpora cavernosa (CC). In order to measure the ICP, a 26 G needle connected to a polyethylene tube, which was filled with physiological saline containing 50 IU/mL heparin, was inserted into the CC on one side. The carotid artery was cannulated in a similar manner to the polyethylene tube in order to continu-

ously monitor the systemic arterial pressure. These parameters were recorded on a polygraph and the data acquisition and calculation of the derived parameters were performed using an on-line computer system (Signal processor, USA). The major pelvic ganglion was exposed through a midline abdominal incision. A stainless steel bipolar electrode (Type ON203-045, Unique Medical, Tokyo, Japan) was carefully positioned around the pelvic ganglion. Electric stimulation was performed at 10 Hz, for 60 s with a pulse duration of 5 ms and 3 V using a stimulator (Electrical Stimulator, HSE, Frankfurt, Germany). The maximum ICP (mmHg), the detumescence time, the ratio of the ICP to the blood pressure (BP) and the area under the curve (AUC) of the ratio of the ICP to blood pressure (ICP/BP ratio) was measured. The detumescence time was taken as the period from the termination of electric stimulation to the time when the ICP returned to its mean baseline value.

2.5 Biochemical analysis

Before the ICP measurement, 0.5 mL blood sample was harvested by a cannulated tube and placed into heparinized tubes. The plasma was immediately separated through centrifugation and used to measure the blood biochemistry (total cholesterol [TC], low-density lipoprotein [LDL] cholesterol, glucose and triglycerides) using an autoanalyzer (Spectrum, Abbott, USA).

2.6 Statistical analysis

All statistical analyses were performed using SigmaStat for Windows 2.0 (Jandel, CA, USA). An ANOVA test was used to make a comparison between the experimental groups and within each test group. All the results were expressed as mean ± SD. A Dunnett multiple range test was used to compare the group means at a significance level of $P = 0.05$.

3 Results

3.1 Changes in erectile response in diet-induced obese rats

In the OR control rats, the baseline cavernous pressure ranged from 9.9 to 19.2 mmHg (13.07 ± 3.95 mmHg) and the mean maximum ICP ranged from 44.9 to 62.8 mmHg (50.96 ± 6.97 mmHg; Figure 1). Electrostimulation of the pelvic ganglion resulted in an ICP increase of approximately 3- or 4-fold from the baseline. The maximum ICP/BP ratio also increased after electric stimulation (59.67 ± 7.45%) and the corresponding AUCs of the ICP/BP ratio was 12 419.59 ± 2 137.45. The detumescence time was 306.00 ± 75.37 s (Figure 2, Table 1).

In contrast to the OR control, the baseline ICP in the OP control rats ranged from 9.9 to 12.3 mmHg (10.94 ± 0.98 mmHg), which was lower but not statistically significantly different. However, the maximum ICP and ICP/BP ratio after electric stimulation were significantly reduced when compared to the OR control (Figures 1 and 2). The maximum ICP ranged from 32.50 to 48.88 mmHg (40.30 ± 6.93 mmHg) and the mean maximum ICP/BP ratio was 45.52 ± 7.75 %. The AUC of the ICP/BP ratio was 7 853.59 ± 2 059.41 and the detu-

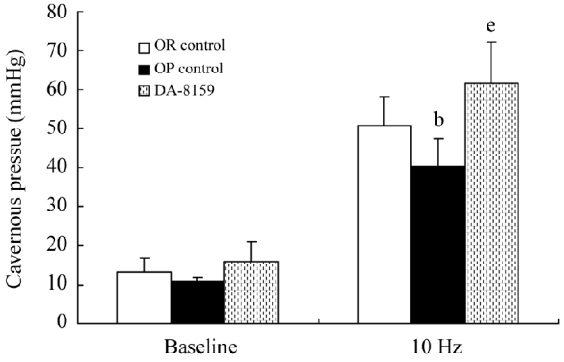

Figure 1. Comparison of the erectile function in obese rats treated with DA-8159 ($n = 8$). The data are expressed as mean ± SD. $^bP < 0.05$, compared with the OR control group, $^eP < 0.05$, compared with the OP control group. ICP, intracavernous pressure; OR, obesity-resistant rats; OP, obsesity-prone rats; DA-8159, OP rats treated with DA-8159.

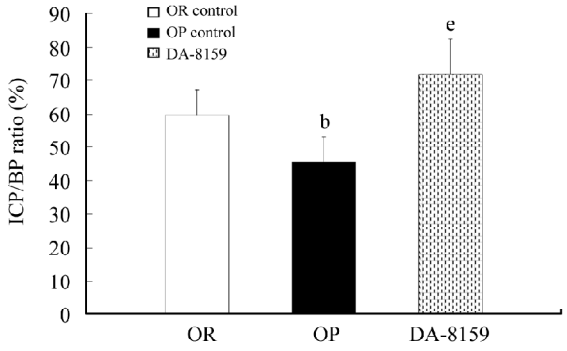

Figure 2. Effects of DA-8159 on the erectile responses in obese rats ($n = 8$). Maximum intracavernous pressure/blood pressure (ICP/BP) ratio (%) was obtained from the peak ICP/BP during the 60 s stimulation period. The data are expressed as mean ± SD. $^bP < 0.05$, compared with the OR control group, $^eP < 0.05$, compared with the OP control group. OR, obesity-resistant rats; OP, obesity-prone rats; DA-8159, OP rats treated with DA-8159.

Table 1. Effect of DA-8159 on detumescence time and AUC in diet-induced obese rats. $^{b}P < 0.05$, compared with the OP control group. AUC, area under the curve; DA-8159, OP rats treated with DA-8159; OP, obesity-prone rats; OR, obesity-resistant rats.

Groups	Detumescence time (s)	AUC (mmHg × s)
OR control (n = 8)	306.00 ± 75.37	12419.59 ± 2137.45
OP control (n = 8)	272.00 ± 89.13	7853.59 ± 2059.41
DA-8159 (n = 8)	423.33 ± 95.72[b]	22581.93 ± 2325.41[b]

Table 2. Body weight and blood biochemistry in OR and OP rats. $^{b}P < 0.05$, compared with the OR control group. LDL: low-density lipoprotein; OP: obesity-prone rats (n = 16); OR: obesity-resistant rats (n = 8).

		OR	OP
Body weight (g)	0 week	278.4 ± 5.0	277.8 ± 2.0
	12 weeks	545.2 ± 20.6	637.9 ± 25.5[b]
Glucose (mg/dL)		196.0 ± 27.4	224.3 ± 17.1
Total cholesterol (mg/dL)		91.2 ± 17.0	138.8 ± 16.7[b]
LDL cholesterol (mg/dL)		9.1 ± 2.4	16.5 ± 3.1[b]
Triglyceride (mg/dL)		151.5 ± 68.8	243.2 ± 53.3[b]

mescence time was 272.00 ± 89.13 s, which were decreased but not significantly different from the OR control (Table 1).

3.2 Effects of DA-8159 on penile erection in obese rats

Electrostimulation of the pelvic ganglion after the DA-8159 treatment in OP rats resulted in a significant increase in maximum ICP (61.5 ± 10.72 mmHg) and the ICP/BP ratio (71.72 ± 10.73 %) (Figures 1, 2). As shown in Table 1, the corresponding AUCs (22 581.93 ± 2 325.41) were also significantly increased ($P < 0.05$). Furthermore, the detumescence time was significantly prolonged after DA-8159 administration (423.33 ± 95.72 s).

3.3 Body weight and blood chemistry

The total cholesterol, LDL cholesterol and triglyceride in plasma in the OP rats were significantly higher than those of the OR control rats. However, the glucose level did not change. The body weights were also significantly increased in the OP rats when compared with those in the OR rats (Table 2).

4 Discussion

Obesity is not only a problem of aesthetics but also a risk factor with adverse effects on health. Obese men have a reduced penile rigidity in the spontaneous erection, some degree of impotence and a loss of libido [2, 15]. According to a population-based study that evaluated the effect of sociodemographic and lifestyle factors on the incidence of ED, it is reported that obesity increases the incidence of ED [4, 5]. Data from other surveys also indicated a higher prevalence of impotence in obese men [16]. Although obesity is highly correlated with the presence of ED, the exact mechanism of pathophysiology has not yet been elucidated. However, it is well-known that obesity is at least a contributing factor mediated by the increasing risk of vascular pathology in the penis or developing chronic diseases of diabetes, hypertension, heart disease and hyperlipidemia. In fact, obese men have a higher incidence of vascular impairment in their penile hemodynamics compared to non-obese men [2].

With regard to the animal obesity models, diet-induced obesity is of special interest because the system of these animals is the most relevant to the human system. They closely mimic some of the cardiovascular changes found in obese humans [17]. However, there is no report as to whether the erectile function in these obese rats becomes lower or not.

The present study demonstrates that diet-induced obesity moderately affected the erectile function in rats. The erectile functions were assessed using an established method, in which an erection was induced by electrostimulation of the pelvic ganglion. The baseline ICP value in the OP rats was lower than that of the OR rats, but this difference was not significant. This suggests that diet-induced obesity affects the erectile function to a lesser degree. However, the ICP/BP ratio is the most critical parameter for evaluating the penile erectile function. The ICP/BP ratio in the OP rats after electric stimulation was significantly lower than that of the OR rats. The maximum ICP in the OP rats was also significantly lower than that of the OR rats. In addition, the other parameters related to the erectile function were decreased without significance. Therefore, it is believed that obesity it itself affects the erectile function in the diet-induced rat model. However, there was also a significant increase in plasma lipid levels. It is well-known that hypercholesterolemia is one of the major risk factors associated with the development of ED and, in men, every mmol/L increase in the total cholesterol level results in a 1.32-fold increase in the risk of ED [18]. Taken together, ED

observed in this obese model was attributable to the obesity or hyperlipidemia or both, which would be elucidated by further study.

After confirming the decrease in the ICP responses in this obese model, the oral efficacy of DA-8159 on a penile erection was examined. The PDE5 inhibitors induced an increased penile erectile response in normal rats [19], a spinal cord-injured rabbit model [8], antidepressant-induced ED rats [20], and a diabetic animal model [9]. However, there is no report that has evaluated the erectile efficacy using an obesity model. After administration of DA-8159, a PDE5 inhibitor, a significant increase in the maximum ICP and the ICP/BP ratio were demonstrated when compared with the OP control. The corresponding AUCs were also higher than that of the control rats. Furthermore, the detumescence time was pronged after administration of the DA-8159. This indicates that DA-8159 can be a potential treatment option for an ED associated with obesity.

As reported previously, body weight, the serum cholesterol and triglyceride values for the OP group were significantly higher than those for the OR group, whereas there was no significant difference in the plasma glucose level [12, 17].

In conclusion, this study examined the ICP responses in diet-induced obese rats, and then assessed the oral efficacy of DA-8159 on penile erection in these obese rats. The results demonstrate that diet-induced obesity moderately affects the erectile function in rats, and these erectile functions were significantly improved by treatment with DA-8159. This indicates that DA-8159 can be a potential treatment option for an ED associated with obesity.

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