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ORIGINAL ARTICLE

Erectile Dysfunction

Pancreatic kininogenase improves erectile function in streptozotocin-induced type 2 diabetic rats with erectile dysfunction

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Erectile dysfunction (ED) associated with type 2 diabetes is a severe problem that requires effective treatment. Pancreatic kininogenase (PK) has the potential to improve the erectile function of ED patients. This study aims to investigate the effect of PK on erectile function in streptozotocin-induced type 2 diabetic ED rats. To achieve this goal, we divided male Sprague–Dawley rats into five groups. One group was not treated, and the other four groups were treated with saline, sildenafil, PK or sildenafil, and PK, respectively, for 4 weeks after the induction of type 2 diabetic ED. Then, intracavernous pressure under cavernous nerve stimulation was measured, and penile tissue was collected for further study. Endothelial nitric oxide synthase levels, smooth muscle content, endothelium content, cyclic guanosine monophosphate (cGMP) levels in the corpus cavernosum, and neuronal nitric oxide synthase levels in the dorsal penile nerve were measured. Improved erectile function and endothelium and smooth muscle content in the corpus cavernosum were observed in diabetic ED rats. When treating diabetic ED rats with PK and sildenafil at the same time, a better therapeutic effect was achieved. These data demonstrate that intraperitoneal injection of PK can improve erectile function in a rat model of type 2 diabetic ED. With further research on specific mechanisms of erectile function improvement, PK may become a novel treatment for diabetic ED.

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Keywords: cyclic guanosine monophosphate; endothelial nitric oxide synthase; erectile dysfunction; intracavernous pressure; pancreatic kininogenase; type 2 diabetes

INTRODUCTION

Erectile dysfunction (ED) is a common complication of men with diabetes mellitus (35%–90% of diabetic men are reported to suffer from ED).[1] ED among type 2 diabetic men is a severe problem, considering the similar risks of developing ED among men with type 1 and type 2 diabetes and the high prevalence of type 2 diabetes (90%–95% of diabetic patients have type 2 diabetes).[2,3] The current first-line therapy for diabetic ED is phosphodiesterase type 5 inhibitors (PDE5 inhibitors).[4] However, the effect of PDE5 inhibitors on diabetic ED is lower than that on nondiabetic ED.[5] Therefore, new therapeutic strategies are required for diabetic ED.

Pancreatic kininogenase (PK) has been reported to be the main treatment for diabetic nephropathy.[6–8] Its therapeutic effect is achieved by activating the kallikrein–kinin system, which includes kallikrein, kininases, and the kinin receptor. PK, a tissue kallikrein extracted from pig pancreas, can convert kininogen into bradykinin and kallidin-like peptide (in rodents) or kallidin (in humans).[9] After binding to type I or II bradykinin receptors, bradykinin stimulates the production of nitric oxide (NO) and prostaglandins, which have protective effects.[10] Bradykinin has been reported to provide renal protection in various ways, including NO release, endothelium-dependent vasodilation, protection against fibrosis, protection against hypertrophy, and stimulation of glucose uptake.[11]

The diabetic condition impairs the endothelium and smooth muscle in corpus cavernosum, which are fundamental for normal erections.[12] NO production, a key process in normal erection, is suppressed by diabetes-induced endothelial dysfunction.[13] The effect of PK on NO release may be helpful for the treatment of diabetic ED. However, it remains unknown whether PK has the same effect on corpus cavernosum as on the kidney. In the present study, we investigated the effect of PK on the erectile function of streptozotocin-induced type 2 diabetic ED rats. The effect of PK on the endothelial function and endothelium and smooth muscle content in corpus cavernosum was also studied. As PK and the PDE5 inhibitor target different aspects of the erection process, the combined effect of PK and the PDE5 inhibitor on diabetic ED rats was investigated to determine whether they achieved a better therapeutic effect than the use of PK or PDE5 inhibitor alone.

MATERIALS AND METHODS

Animals and study design

All animal experiments were approved by the Experimental Animals Welfare and Ethics Committee of Nanjing Drum Tower

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Hospital in Nanjing, China. A total of 35 male Sprague–Dawley rats (12 weeks old, Nanjing Medical University, Nanjing, China) were used to induce type 2 diabetes mellitus (DM2 group). The treatment method was adopted from Reed *et al*.[14] and Albersen *et al*.[15] Five age-matched rats were used as nondiabetic controls (control group). The daily light/dark cycle was 12/12 h, and animals had access to water *ad libitum*. The DM2 group was fed high-fat chow containing (as a percentage of total kcals) 40% fat, 40% carbohydrate, and 20% protein, while the control group was fed normal chow. All animals were maintained on their original diet until the end of this study.

After 2 weeks of the high-fat diet, rats in the DM2 group were intraperitoneally injected with streptozotocin (STZ, 30 mg kg^{-1}, Sigma-Aldrich, St. Louis, MO, USA, dissolved in citrate buffer). Three days later, the same dose was injected. The rats in the control group received the same injection of citrate buffer at the same time. Three days after the second injection of STZ, blood glucose levels were measured by tail prick. Blood glucose was measured weekly until the end of this study. Rats with blood glucose levels higher than 16.7 mmol l^{-1} were considered as diabetic. Seven weeks after the first injection of STZ, an insulin challenge test was conducted to confirm insulin sensitivity. Typically, bovine insulin (1 IU ml^{-1} kg^{-1}, dissolved in phosphate-buffered solution) was intraperitoneally injected, and blood glucose levels were measured by tail prick at 0 (before insulin injection), 15, 30, 45, 60, 90, and 120 min after injection. The glucose levels were presented as percentages of the glucose level at 0 min. The apomorphine (100 μg kg^{-1}; Medchemexpress, Shanghai, China) test was used to select rats with erectile dysfunction.[16]

Then, rats from the DM2 group with ED (rats with diabetes mellitus-associated erectile dysfunction [DMED]) were divided into four groups and received vehicle (saline) or treated with sildenafil (Pfizer, 10 mg kg^{-1}, homogenized and dissolved in saline) and/or PK (14.4 U kg^{-1}, in saline; Qianhong Bio-pharma Company, Changzhou, China). The daily treatments were as follows: (1) saline by gavage and by intraperitoneal (IP) injection (DMED group); (2) sildenafil by gavage and saline by IP injection (DMED + Sil group); (3) saline by gavage and PK by IP injection (DMED + PK group); and (4) sildenafil by gavage and PK by IP injection (DMED + Sil + PK group). The dose of PK was adopted from a previous research study on its therapeutic effect in rats.[7,17] The dosage of sildenafil was adopted from a previous research study on diabetic ED.[18,19] Rats tend to struggle after repeated oral administration of drugs. For the safety of the rats and researchers, we used higher dosages and a shorter treatment period. After 4 weeks of treatment and a washout period of 2 days, intracavernous pressure (ICP) was measured by electrical field stimulation (EFS). Then, the rats were sacrificed, and their penises were harvested for further study. A 2 to 3-mm sample from the middle part of the penile shaft was used for histology. The remaining tissue from the middle part of the penile shaft (from 2 mm below the glans to 1 mm above the crus of the penis) was collected, and the inner layer of the penile shaft was harvested for western blotting and cyclic guanosine monophosphate (cGMP) measurement.

Evaluation of erectile function

Maximal intracavernous pressure (MIP)/mean arterial pressure (MAP) was used to evaluate erectile function after the 4-week treatment. ICP was measured under cavernous nerve EFS as previously described.[20] After anesthesia was induced by ketamine (30 mg kg^{-1}, Jiangsu Hengrui Medicine Co., Ltd., Lianyungang, China) and xylazine (4 mg kg^{-1}, Aladdin, Shanghai, China) administration, a low abdominal incision was performed to expose the major pelvic ganglion and cavernous nerve (CN). A 23G needle connected to a PE-50 tube filled with 200 U ml^{-1} heparinized saline was inserted into the right crus penis to measure the ICP. Then, the right carotid artery was exposed and cannulated with another PE-50 tube to record the MAP. The ICP and MAP were recorded with an RM6042B/C multichannel signal collection processing system (Chengdu Instrument Factory, Chengdu, China). The right CN was stimulated with continual monophasic pulses (5 V, 15 Hz, pulse width 5 ms, 60-s duration) from a bipolar hook electrode connected to a signal generator (Chengdu Instrument Factory). EFS was performed at least 3 times for each rat with 5 min of rest between stimulations. For each male, the average of the maximal ICP during each EFS was used for data analysis.

Histology

For immunohistochemistry, the middle part of the penile shaft was fixed in 4% paraformaldehyde in PBS overnight and subsequently stored in 70% ethanol at 4°C until sectioning. Tissue slices (5 μm) were deparaffinized in xylene and rehydrated in 100%, 95%, 70%, and 50% ethanol and water. A solution of 3% H_2O_2 was used to inhibit endogenous peroxidase. After blocking with 5% BSA (KeyGEN BioTECH, Nanjing, China) for 30 min, the tissue was incubated with rabbit anti-α-smooth muscle actin (anti-α-SMA, 1:100, ab5694; Abcam, Cambridge, MA, USA) or anti-von Willebrand factor (anti-vWF, 1:200, ab6994; Abcam) antibodies at 4°C overnight. Subsequently, the tissue was incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:1000, ab6721; Abcam) for 2 h at room temperature. 3,3'-diaminobenzidine and hematoxylin were used for target protein and nuclear staining, respectively. The primary antibody was replaced with 5% BSA for the negative control. Images acquired from five fields of each section at ×400 magnification (five sections from each subject) were used for analysis. Images were captured using a Nikon microscope (Eclipse Ti-S, Nikon Instruments Co., Ltd., Tokyo, Japan), and the results are presented as the percentage of stained area versus the total area.

For immunofluorescence staining, the storage, deparaffinizing, and rehydrating processes were similar. Then, after 30 min of blocking with 5% BSA, the tissue was incubated with rabbit anti-neuronal nitric oxide synthase (anti-nNOS, 1:100, ab5586; Abcam) or mouse anti-endothelial nitric oxide synthase (anti-eNOS, 1:200, ab76198; Abcam) at 4°C overnight. Subsequently, the tissue was incubated with CY3-conjugated goat anti-rabbit (for nNOS) secondary antibody (1:200, 111-165-003; Jackson, Bar Harbor, ME, USA) or goat anti-mouse (for eNOS) secondary antibody (1:200, 115-165-003; Jackson) for 1 h at room temperature. 4,6-diamidino-2-phenylindole was used for nuclear staining. Primary antibody was replaced with 5% BSA for the negative control. For statistical analysis of nNOS content, the percentage of nNOS-positive area in the dorsal nerve area (in pixel) was calculated at ×400 magnification. All branches of the dorsal nerve on each slide were included in the analysis. Images acquired from five fields of each section at ×400 magnification were used for eNOS analysis. Cells expressing eNOS were counted and presented as the percentage of positive cells/total cells for data analysis.

Western blotting

The middle part of the penile shaft was stored at −70°C until western blotting. Protein was prepared as previously described.[21] After protein extraction and determination of protein concentration, the protein samples (5 μg) were separated on 10% sodium dodecyl sulfate polyacrylamide gel (P0012A; Beyotime, Nanjing, China) and were transferred to a polyvinylidene difluoride membrane (IPVH00010; Millipore, Darmstadt, Germany). Rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH,

1:2500, ab9485; Abcam), anti-vWF (1:500, ab6994; Abcam), and anti-α-SMA (1:100, ab5694; Abcam) were used as primary antibodies. HRP-conjugated goat anti-rabbit antibody (1:10000, ab6721; Abcam) was used as the secondary antibody. GAPDH was used as the loading control. Bands were detected and analyzed on a Bio-imaging System (Tanon, Shanghai, China).

Measurement of cyclic guanosine monophosphate levels

The middle part of the penile shaft was stored at −70°C until cGMP determination. For the measurement of cGMP levels, penile tissue samples from all groups were assayed using an enzyme-linked immunosorbent assay (ELISA) kit (Jiancheng Biotechnology Institute, Nanjing, China, H163). This assay was based on the competition between free cGMP and HRP-conjugated cGMP for a limited amount of cGMP-specific antibody sites. cGMP levels are presented as nmol per gram of protein.

Statistical analyses

Data are expressed as the mean ± standard deviation. One-way analysis of variance with multiple comparisons followed by Bonferroni's *t*-test was performed using SPSS 14 (SPSS Inc., Chicago, IL, USA) and used to compare differences among multiple groups. $P < 0.05$ was considered statistically significant.

RESULTS

Establishment of type 2 diabetes

After the second injection of STZ, thirty rats with blood glucose levels higher than 16.7 mmol l^{-1} were used in the experiment. Seven weeks after the first injection of STZ, the body weights of the STZ-treated rats were lower than those of control rats, and the blood glucose levels of the STZ-treated rats were higher than those of the control rats. The insulin challenge test confirmed that all subjects had developed insulin resistance (**Figure 1**); these rats were then considered to be type 2 diabetic. After the apomorphine test, 29 rats with erectile dysfunction and type 2 diabetes were considered to be DMED rats. After 4 weeks of treatment, body weights and blood glucose levels did not change (**Table 1**). DMED rats had significantly lower body weights ($P < 0.0005$) and significantly higher blood glucose levels ($P < 0.0005$) than control rats. During the treatment, one rat died in each of the DMED, DMED + Sil, and DMED + PK groups. The remaining number of rats in the control group, DMED group, DEMD + Sil group, DMED + PK group, and DMED + Sil + PK group was 5, 7, 6, 6, and 7, respectively.

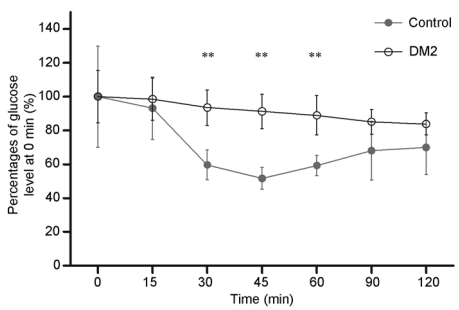

Figure 1: Insulin challenge test at 7 weeks after the first injection of STZ. $^{**}P < 0.005$. STZ: streptozotocin; DM2: type 2 diabetes mellitus.

ICP and MIP/MAP

The ICP in response to cavernous nerve electric stimulation is shown in **Figure 2**. The MIP/MAP ratio of the DMED group was significantly lower than that of the control group ($P < 0.0005$). Treatment with sildenafil ($P < 0.0005$) or PK ($P = 0.029$) significantly improved the MIP/MAP ratio. The simultaneous treatment of sildenafil and PK significantly increased the MIP/MAP ratio compared to treatment with sildenafil ($P = 0.038$) or PK ($P < 0.0005$) alone. The MIP/MAP ratio of all the treated groups was lower than that of the control group (all $P < 0.0005$).

Histology

Endothelium (indicated by vWF, $P < 0.0005$) and smooth muscle (indicated by α-SMA, $P < 0.0005$) content in the corpus cavernosum decreased significantly in diabetic rats (**Figure 3** and **4**). Treatment with PK (vWF: $P = 0.034$, α-SMA: $P = 0.014$) or sildenafil (vWF: $P < 0.0005$, α-SMA: $P < 0.0005$) significantly increased endothelium and smooth muscle content. Simultaneous treatment with sildenafil and PK led to a significant increase in endothelium and smooth muscle content compared with treatments with either sildenafil (vWF: $P < 0.0005$, α-SMA: $P = 0.009$) or PK (vWF: $P < 0.0005$, α-SMA: $P < 0.0005$) alone. The endothelium and smooth muscle content in all of the treated groups was lower than that in the control group (all $P < 0.0005$ for vWF and α-SMA).

There was no significant difference in the number of nNOS-containing fibers among treated groups and diabetic controls; therefore, these results are not presented. eNOS expression also increased significantly after treatment with either PK ($P = 0.035$) or sildenafil ($P < 0.0005$) (**Figure 5**). Simultaneous treatment with PK and sildenafil significantly increased eNOS expression compared with either PK ($P < 0.0005$) or sildenafil treatment alone ($P = 0.009$). The eNOS expression levels of all the treated groups were lower than those of the control group (all $P < 0.0005$).

Western blotting

The expression levels of vWF, α-SMA, and GAPDH in all groups were measured by western blotting (**Figure 6a–6c**). vWF and α-SMA expression levels were lower in the DMED group than in the control group (all $P < 0.0005$). Treatment with either PK (vWF: $P = 0.003$, α-SMA: $P = 0.005$) or sildenafil (vWF: $P < 0.0005$, α-SMA: $P < 0.0005$) increased vWF and α-SMA expression levels significantly, while treatment with both sildenafil and PK exhibited a better effect than treatment with sildenafil (vWF: $P = 0.020$, α-SMA: $P = 0.041$) or PK (vWF: $P < 0.0005$, α-SMA: $P < 0.0005$) separately. The vWF expression levels of all the treated groups were lower than those of the control group (all $P < 0.0005$). The α-SMA expression levels of the DMED + Sil group ($P = 0.001$) and the DMED + PK group ($P < 0.0005$) were lower than those of the control group. There was no significant difference between the DMED + Sil + PK group and the control group (α-SMA, $P = 0.833$).

cGMP levels

The cavernous cGMP concentrations of all groups were measured by ELISA (**Figure 6d**). The cGMP concentrations of DMED rats

Table 1: Body weights and blood glucose levels (after 4 weeks of treatments)

	Control	*DMED*	*DMED + Sil*	*DMED + PK*	*DMED + Sil + PK*
Body weight (g)	561.6±28.7	403.7±39.8**	400.8±38.0**	407.7±41.9**	404.7±43.1**
Blood glucose (mmol l^{-1})	5.3±1.6	24.4±2.8**	23.8±3.4**	23.2±3.0**	21.9±3.7**

$^{**}P<0.005$ different from control. DMED: rats with diabetes mellitus-associated erectile dysfunction; Sil: sildenafil; PK: pancreatic kininogenase

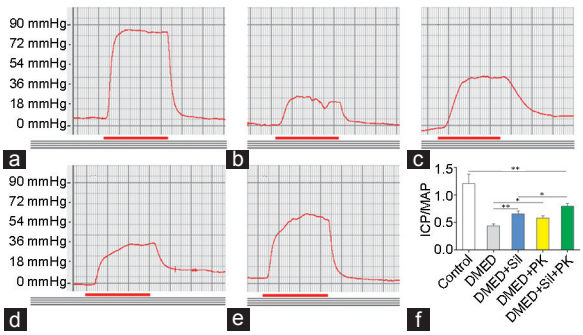

Figure 2: Evaluation of erectile function after 4 weeks of treatment. Representative ICP curve in response to cavernous nerve electric stimulation in (**a**) control, (**b**) DMED, (**c**) DMED + Sil, (**d**) DMED + PK, and (**e**) DMED + Sil + PK groups. (**f**) MIP/MAP ratio of each group. $^{*}P < 0.05$; $^{**}P < 0.005$. DMED: rats with diabetes mellitus-associated erectile dysfunction; Sil: sildenafil; PK: pancreatic kininogenase; MIP: maximal intracavernous pressure; MAP: mean arterial pressure.

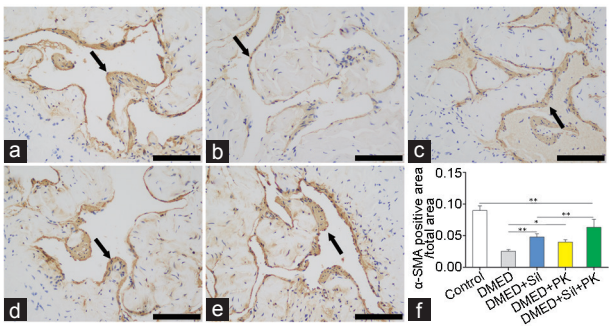

Figure 4: α-SMA-positive smooth muscle in corpus cavernosum. Representative immunohistochemical staining of α-SMA-positive smooth muscle (indicated by arrows) in (**a**) control, (**b**) DMED, (**c**) DMED + Sil, (**d**) DMED + PK, and (**e**) DMED + Sil + PK groups. Scale bars = 100 μm. (**f**) Semi-quantification results of α-SMA (presented as α-SMA-positive area/total area ± s.d.) in each group. $^{*}P < 0.05$; $^{**}P < 0.005$. DMED: rats with diabetes mellitus-associated erectile dysfunction; Sil: sildenafil; PK: pancreatic kininogenase; α-SMA: α-smooth muscle actin; s.d.: standard deviation.

were significantly lower than those of the control rats ($P < 0.0005$). Treatment with either PK ($P = 0.010$) or sildenafil ($P < 0.0005$) significantly increased cGMP levels. The cGMP levels of rats in the DMED + Sil + PK group were significantly higher than those of the DMED + Sil ($P = 0.031$) and DMED + PK groups ($P < 0.0005$). The cGMP expression levels of all the treated groups were lower than those of the control group (DMED + Sil, $P < 0.0005$; DMED + PK, $P < 0.0005$; and DMED + Sil + PK, $P = 0.007$).

DISCUSSION

Penile erection requires the normal functions of nitrergic nerves, endothelium, and smooth muscles in the penis.[12] The NO released from endothelium and nerve terminals increases the level of cGMP, which triggers a series of cellular events. These cellular events result in the relaxation of smooth muscle and increase intracavernous pressure. Therefore, endothelium and smooth muscle play vital roles in the process of erection.

DMED is a type of organogenic ED that mainly involves neural impairment, vascular dysfunction, metabolic disturbance, and local factors (Peyronie's disease, fibrosis, and balanitis).[22] Diabetes can induce neuronal degradation, which can result in decreased nNOS activity and NO production.[23] This neuronal degradation may be

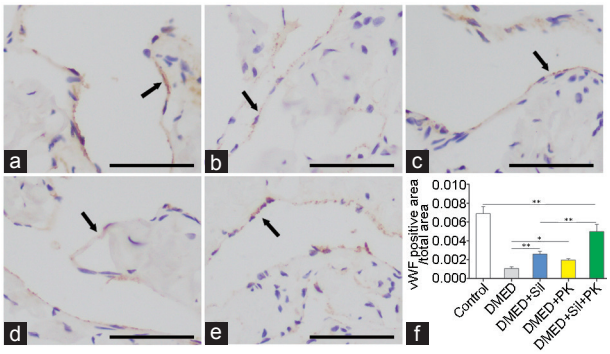

Figure 3: vWF-positive endothelium in corpus cavernosum. Representative immunohistochemical staining of vWF-positive endothelium (indicated by arrows) in (**a**) control, (**b**) DMED, (**c**) DMED + Sil, (**d**) DMED + PK, and (**e**) DMED + Sil + PK groups. Scale bars = 50 μm. (**f**) Semi-quantification results of vWF (presented as vWF-positive area/total area ± s.d.) in each group. $^{*}P < 0.05$; $^{**}P < 0.005$. DMED: rats with diabetes mellitus-associated erectile dysfunction; Sil: sildenafil; PK: pancreatic kininogenase; vWF: von Willebrand factor; s.d.: standard deviation.

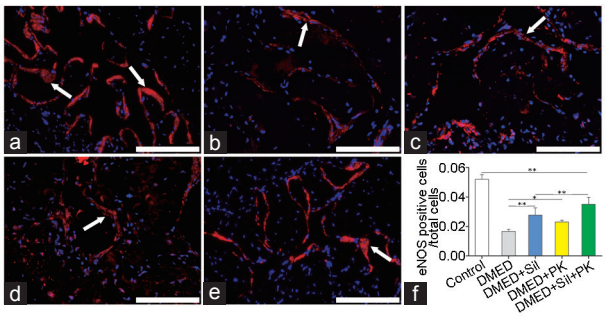

Figure 5: eNOS-positive smooth muscle in corpus cavernosum. Representative immunofluorescence staining of eNOS (red, indicated by arrows) expression in (**a**) control, (**b**) DMED, (**c**) DMED + Sil, (**d**) DMED + PK, and (**e**) DMED + Sil + PK groups. Scale bars = 100 μm. (**f**) Semi-quantification results of eNOS expression (presented as eNOS-positive cells/total cells ± s.d.) in each group. $^{*}P < 0.05$; $^{**}P < 0.005$. DMED: rats with diabetes mellitus-associated erectile dysfunction; Sil: sildenafil; PK: pancreatic kininogenase; eNOS: endothelial nitric oxide synthase; s.d.: standard deviation.

reflected by the loss of nNOS in the dorsal nerves of DM rats.[24,25] It has been reported that chronic high blood glucose leads to the production of free oxygen radicals and glycosylation end products, thereby impairing endothelial and smooth muscle function in penile cavernosum.[26] Reduced endothelium and smooth muscle content has been demonstrated in diabetic rats.[21]

PK can convert kininogen into bradykinin, which stimulates the production of NO and prostaglandins.[10] This process has a protective effect on diabetic nephropathy. The regulation of endothelial NO synthase by PK indicates its potential in improving erectile function and enhancing the therapeutic effects of PDE5 inhibitors on diabetic erectile dysfunction.

In the present study, we treated DMED rats with PK and found that it restored erectile dysfunction. In diabetic ED rats, MIP/MAP, vWF, α-SMA, eNOS, and cGMP levels were lower than those in control rats, which was consistent with findings from previous studies on diabetic animals and patients.[20,27] After 28 days of treatment with PK, significant increases in all of these parameters were observed. Treatment with sildenafil and PK together resulted in a better effect than treatment with either PK or sildenafil alone. The increased levels of vWF and α-SMA suggest an increase in the number of endothelial

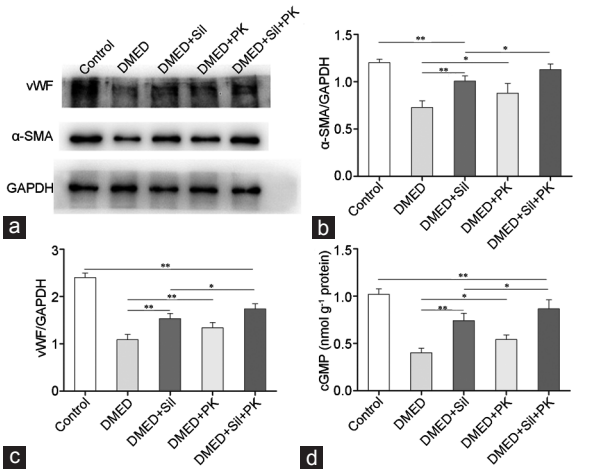

Figure 6: Cavernous protein and cGMP levels. (**a**) Representative western blot results of vWF, α-SMA, and GAPDH. (**b**) α-SMA protein expression level examined by western blot. (**c**) vWF protein expression level examined by western blot. (**d**) cGMP levels were measured by ELISA. $^{*}P < 0.05$; $^{**}P < 0.005$. DMED: rats with diabetes mellitus-associated erectile dysfunction; Sil: sildenafil; PK: pancreatic kininogenase; cGMP: cyclic guanosine monophosphate; α-SMA: α-smooth muscle actin; vWF: von Willebrand factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; ELISA: enzyme-linked immunosorbent assay.

cells and smooth muscle, whose relaxation causes erection.[12] Although the smooth muscle cell/myofibroblast ratio is high in a healthy penis, in a diabetic penis, smooth muscle loss is usually accompanied with the proliferation of myofibroblasts, which also express α-SMA. This may be the reason why the level of α-SMA expression in the DMED + Sil + PK group was close to that in the healthy control group. As the endothelium is a major source of NO production, eNOS plays a critical role in the process of erection.[21,27] The increases in vWF and eNOS suggest that endothelial cells are protected, leading to increases in NO release and subsequently in cGMP levels.[28] This protective effect on endothelial cells eventually contributes to the restoration of erectile function. Therefore, PK restores erectile function in diabetic rats by increasing endothelium and smooth muscle content and protecting endothelial cells. However, after treatment with sildenafil and PK, none of the parameters we measured returned to the levels of healthy controls. Our treatment achieved only partial recovery. The persistent damage caused by diabetes, which began before treatment, and the fact that our treatment was not able to address all the damage caused by diabetes (such as neuropathy) may be reasons for the partial recovery.

PK increases the release of NO by activating the kallikrein-kinin system,[10] whereas sildenafil blocks the degradative action of cGMP-specific phosphodiesterase type 5.[12] Both drugs target the NO-cGMP pathway, which may explain why PK had similar effects on endothelium and smooth muscle as sildenafil. When treating rats with these two drugs together, PK leads to an increase in cGMP levels, while sildenafil leads to a reduction in cGMP degradation. The fact that these two drugs target different points in the NO-cGMP pathway may explain why PK and sildenafil together had a better therapeutic effect than the use of PK or sildenafil alone.

The present study has several limitations. Due to the limited amount of research on the therapeutic effect of PK on diabetic rats, we used a low dosage over a short period of time for safety considerations. In previous studies of rats, the dosages varied from 7.2 U kg^{-1} to 14.4 U kg^{-1}.[7,17] However, in a study on mice, the dosage was 60 U kg^{-1}.[6] This dosage limit may explain why PK exhibited lower effects than sildenafil in all parameters in the present study. Research on varying doses for an extended period of time in diabetic rats should be performed to facilitate further tests on the therapeutic effect of PK. In the present study, the NO-cGMP pathway was demonstrated to be involved in the therapeutic effect of PK on penile tissue. However, the correlation between the increase in NO release and the activation of the kallikrein-kinin system triggered by PK was not shown. Therefore, the mechanism underlying the effect of PK on DMED remains to be explored. In our experiment, we used PK (a pancreatic enzyme) to treat DMED rats that had damaged pancreases. These rats might have defects in PK synthesis. We are unable to determine whether the therapeutic effect will still be present in models with normal PK synthesis. Therefore, further study on animal models with normal pancreatic function (such as aging models) is required for a better understanding of the therapeutic effect of PK on ED.

CONCLUSION

This report was the first to describe the effect of PK on a DMED in a rat model from the aspects of morphology, structure, and protein expression. The present study demonstrated that in DMED rats, PK improved erectile function and the content of endothelium and smooth muscle in the corpus cavernosum. When treating DMED rats with PK and sildenafil at the same time, a better therapeutic effect was achieved. With further research on the specific mechanism by which PK improves erectile function, PK could represent a potential novel treatment for DMED.

AUTHOR CONTRIBUTIONS

GTC, BBY, YC, and YTD conceived and designed the experiments. GTC, BBY, JHC, LLZ, HSJ, and WY performed the animal experiments. ZZ, GTC, and BBY analyzed the data. GTC and BBY wrote the paper. YC and YTD revised the draft. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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