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Comparison of sildenafil with strontium fructose diphosphate in improving erectile dysfunction against upregulated cavernosal NADPH oxidase, protein kinase Cε, and endothelin system in diabetic rats

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Keywords
corpus cavernosum; erectile dysfunction; endothelin-1 (ED-1); strontium fructose diphosphate (FDP-Sr); NADPH oxidase

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Abstract

Objectives Phosphodiesterase type 5 inhibitors are potent in relieving erectile dysfunction (ED), however, they are less satisfactory in diabetic patients, probably due to the pro-inflammatory biomarkers caused by diabetes. Therefore, it was interesting to compare the effects of sildenafil with strontium fructose 1,6-diphosphate (FDP-Sr) on cavernosal vascular activity and expressions of pro-inflammatory biomarkers in diabetic rats.

Methods Male Sprague-Dawley rats were injected with streptozocin (60 mg/kg, i.p.) to develop diabetes. The animals were diabetic for eight weeks with sildenafil (12 mg/kg per day) or FDP-Sr (200 mg/kg per day) being administered for the last four of those eight weeks.

Key findings Sildenafil was more effective in relieving reduced vascular dilatation (relevant to ED), but less in attenuating over-expressions of NADPH oxidase p22, p47 and p67 subunits, and $ET_{A/B}R$ (endothelin receptor type A and type B) in the diabetic cavernosum. In contrast, FDP-Sr was less effective in improving ED, but more effective in normalizing the abnormal NADPH oxidase and $ET_{A/B}R$.

Conclusions The activated NADPH oxidase and upregulated ET_AR and ET_BR due to diabetic lesions played a minor or moderate role in ED. By offering extra ATP, FPD-Sr suppressed these abnormalities, however, sildenafil did not. A combined therapy of sildenafil with FDP-Sr may be more effective in relieving ED in diabetic patients through normalizing pro-inflammatory cytokines and improving the nitric oxide/cGMP pathway in the cavernosum.

Introduction

Patients with diabetes mellitus are at higher risk for erectile dysfunction (ED) than those age matched and nondiabetic subjects.[1,2] Even now, the mechanisms underlying ED are not fully elucidated. There are at least two main factors in ED, reduced nitric oxide (NO) and the activated NADPH oxidase–endothelin (ET) pathway contributing to the reduced vasorelaxation of the corpus cavernosum. Sildenafil, a phosphodiesterase type 5 inhibitor (PDE5i) remains the mainstay in treating ED based on positive data accumulated in the last 10 years. However, the efficacy of a PDE5i in alleviating ED in patients with diabetes mellitus is unsatisfied, less effective than those in nondiabetic persons.[3,4] The relaxation of human cavernosum strips to acetylcholine is impaired in diabetes mellitus due to lesions in the cavernosum.[5] It has been estimated that approximately 50% of patients over 10 years of diabetes suffers from ED. The increase in reactive oxygen species (ROS) and cytokines appears to account for the pathologies in diabetes mellitus-induced ED.[6,7] NADPH oxidase, consisting of the catalytic subunit gp91phox and regulatory subunits p22phox, p47phox, and p67phox, has been considered as the main source of ROS genesis, which may play a critical role in ED.[8] Enhanced NADPH oxidase activity is found in hypertension-associated ED.[9] In addition protein kinase, moreover the activation of NADPH oxidase, mainly depends upon phosphorylation of protein kinase C (PKC)-α in diabetic nephropathy.[10] An abnormal

endothelin (ET) system is implicated in pathologies of hyperadrenergic state in the myocardium caused by either isoproterenol or H_2O_2 medication, and upregulation of the endothelin A receptor (ET_AR) and the abnormal NADPH oxidase were dramatically suppressed by the endothelin receptor antagonist CPU0213.[11,12] An activation of the ET system which enhances vasoconstrictive responses and impairs vasodilatative responses is likely the underlying mechanism of diabetic complications.[13,14] It is uncertain if an abnormal ET system contributes to diabetic ED. Moreover, hyperglycaemia activates the protein expression of PKC isoforms and reduces NO, in association with an increase in ROS in cultured rat cavernosal smooth muscle cells, which were overcome by the antioxidant vitamin E.[15]

Diabetic injury is closely related to dysfunction of the mitochondria, at least in part, due to a shortage of the energy supply. An exogenous fructose diphosphate (FDP), which contains extra adenosine triphosphate (ATP), serves as a supplier of ATP to meet the need of the impaired energy metabolism in diabetes mellitus.[16] We previously reported that strontium fructose 1,6-diphosphate (FDP-Sr) possesses ROS scavenging activity and relieves the testopathy caused by either adenine or diabetes.[17,18] FDP-Sr alleviates early diabetic testopathy by suppressing abnormal NADPH oxidase and matrix metalloproteinase system in the testis of streptozocin-treated rats.[19] FDP-Sr has been proved to suppress the pro-inflammatory factors and biomarkers in the diabetic testis, which are likely attributed to its extra supply of ATP to meet the need of deficiency of the energy supply in mitochondria.

We hypothesized that FDP-Sr could improve dysfunction of the corpus cavernosum in diabetic rats, through normalizing the pro-inflammatory biomarkers, including NADPH oxidase, ET_AR and ET_BR in the cavernosal tissue. Sildenafil potently relaxes the cavernosal tissue by suppressing PDE5 and has been reported to suppress oxidative stress and inflammatory reactions in the renal tissue in streptozocin-injected rats.[20] However, the effectiveness of sildenafil in treating diabetic ED is reduced, which remains a concern.[5] Therefore, we have compared the effectiveness of sildenafil and FDP-Sr in relieving cavernosal vascular abnormality relevant to ED, and investigated if the compromised vasodilatation of the cavernosum could be related to an excess of pro-inflammatory bioactive molecules. Therefore, a comparison of effects of sildenafil with FDP-Sr was focused on the compromised vasodilatation against the pro-inflammatory molecules in the cavernosal strips in streptozocin-injected rats.

Materials and Methods

Experimental animals

All procedures were performed in accordance with the Animal Regulations of Jiangsu Province and were consistent with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85–23, revised 1996). Approval was granted by the Animal Ethics Committee of Jiangsu Province (NO.SYXK2007-0025). Male Sprague–Dawley rats (250–280 g) were supplied by the Animal Center of Nanjing Medical University.

Drugs

FDP-Sr was supplied by the Faculty of Life Science and Pharmaceutical Engineering, Nanjing University of Technology. Streptozocin was purchased from MP Biomedicals Znc Lot. no.2126F, Santa Ana, CA, USA.

Experimental protocol

Forty male rats were randomly assigned into four groups for this eight-week experiment: control; diabetes mellitus untreated; and diabetes mellitus with oral interventions in the last four weeks of the experiment with either sildenafil (12 mg/kg·per day) or FDP-Sr (200 mg/kg·per day). The effectiveness of relieving ED was assessed by an increase in relaxation of the diabetic cavernosal strips between sildenafil and FPD-Sr.

To induce diabetes mellitus, rats were administered a single injection of streptozocin (60 mg/kg, i.p., in citrate buffer, pH = 4.5). Blood was checked weekly after streptozocin administration and sustained blood glucose levels > 16.7 mmol/l was considered evidence of hyperglycaemia, indicating the establishment of diabetes. After the administration of streptozocin, rats experienced hyperglycaemia for eight weeks. The rats in the control group were given an equal volume of the vehicle (0.5% carmellose sodium). All rats were allowed free access to regular chow and tap water.

After eight weeks the animals were anaesthetized with urethane (1.5 g/kg, i.p.). Blood samples were collected via a catheter placed in the left common carotid. Serum was separated and stored for use after being centrifuged at 4000 rev/min (10 min, 4°C). Serum samples were used for the biochemical assays.

Biochemical measurements

Blood glucose, malondialdehyde (MDA) and superoxide dismutase (SOD) in the corpus cavernosum were measured according to the directions provided on the kits (Jiancheng Bio-engineering Company, Nanjing, China).

Vasoconstriction and relaxation of cavernosal strips

The penile tissue was harvested and placed in a dish containing Krebs solution (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, KH_2PO_4 1.2, glucose 11. pH 7.4. The glands of

penis and urethra were excised and the fibrous septum between two cavernosa was cut. Each corpus cavernosum was carefully dissected and cleared from the adherent tissues, keeping the tunica albuginea intact. Cavernosal strips (2×10 mm) were preloaded under 2 g tension and allowed to equilibrate for 90 min in a 3-ml organ bath with Krebs solution. The bath medium was maintained at 37°C and gassed with 95% O_2 mixed with 5% CO_2. During equilibration, the bath solution was replaced every 15 min. A cumulative concentration–response curve to phenylephrine (Phe, 10^{-10}–10^{-4} M) was recorded. To evaluate relaxant response, acetylcholine (ACh, 10^{-8}–10^{-4} M) was employed to relax cavernosal strips, which had been preconstricted with a submaximal concentration of Phe (10^{-6} M) to reach a stable plateau.

Reverse transcriptase polymerase chain reaction

Reverse transcriptase polymerase chain reaction (RT-PCR) was conducted as previously described.[13] In brief, the reagents used were from Promega Corporation, San Luis Obispo, CA, USA. Oligonucleotides for the primers were all synthesized by Invitrogen (Shanghai, China). Total ribonucleic acid (RNA) was extracted from the corpus cavernosum sample ($n = 5$) using Trizol reagent. RNA 5 μg was used to synthesize the first strand of cDNA as a template in the following PCR reactions (Eppendorf Mastercycler, Barkhausenweg, Hamburg, Germany) of the biomarkers: NADPH oxidase subunits p22phox, p47phox, p67phox, and ppET-1(prepro-endothelin-1), ET_AR and ET_BR (endothelin receptor A, B). Samples were then standardized by concomitant expression of 18 s. The primers for RT-PCR were as the follows: ppET-1 – sense: 5′- AGCAATAGCATCAAGGCATC –3′ and antisense: 5′- TCAGACACGAACACTCCCTA –3′; ETA – sense: 5′- ATCGCTGACAATGCTGAGAG-3′ and antisense: 5′- CCACGATGAAAATGGTACAG-3′; ETB – sense: 5′-CCGTATCCGATGACAATG-3′ and antisense: 5′-CCAG GCTCCAGGTAGTTT-3′; p22phox – sense: 5′-GCTCATCTG TCTGCTGGAGTA –3′ and antisense: 5′- ACGACCTCAT CTGTAACTGGA-3′; p47phox – sense: 5′-TCACCGAGATC TACGAGTTC –3′ and antisense: 5′-ATCCCATGAGGCTGT TGAAGT-3′; p67phox – sense: 5′-GAAAGCATGAAGGAT GCCTGG-3′ and antisense: 5′-ATAGCACCAAGATCACA TCT CC-3′; 18 s – sense: 5′-GCTGCTGGCACCAGACTT-3′ and antisense: 5′-CGGCTACCACATCCAAGG-3′. Finally, the density of bands was analysed with Labworks imaging acquisition and analysis software (GDS8000, Syngene, Cambridge, UK).

Western blotting

For the quantitative analysis of proteins of NADPH oxidase p22phox, p47phox, p67phox, ET_AR, and ET_BR, a portion of the corpus cavernosum tissue (ca. 50 mg) was homogenized in 4 vol extraction buffer and centrifuged at 10 000*g* for 10 min, at 4°C, as described previously.[13] Briefly, after determining the protein concentration, supernatants were stored at −20°C until used. A sample was heated to 95°C and size fractionated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Following transfer to nitrocellulose and blocking with nonfat milk (5% w/v), the incubation was conducted with the primary antibody (Wuha Boster Biological Technology, Wuhan, China for ET_AR and ET_BR; Uscnlife, Wuhan, China, for p22phox; Upstate, Charlottesville, VA, USA for p47phox; Affinity Bioreagents, Golden, CO, USA for p67phox). After three washes, the blot was incubated with the horseradish peroxidase conjugated goat secondary antibody immunoglobulin (Ig) G (1 : 1000; Santa Cruz Biotechnology, CA, USA) for an additional 1 h. Antigen was detected with a 3,3′-diaminobenzidine (DAB) kit (Wuha Boster Biological Technology, China). A linear relationship between blot density and protein load was observed when 20, 40, 60, 80, and 100 μg of membrane protein were used per lane.

Statistical analysis

SigmaPlot 9.0 (SPSS Inc, Chicago, IL, USA) was used to analyse the results. Data were expressed as mean ± SD. Student's *t*-test was used to determine the statistical differences between the two means, and the one-way analysis of variance Bonferroni test was followed to compare means between all experimental groups. The difference was considered statistically significant at $P < 0.05$.

Results

Blood glucose and malondialdehyde and superoxide dismutase in the corpus cavernosum

The blood glucose level was elevated significantly ($P < 0.01$) following streptozocin injection in rats (Table 1). However, no change in blood glucose was found by interventions with either FDP-Sr or sildenafil. In the corpus cavernosum of diabetic rats, MDA level was increased by 81% ($P < 0.01$), and the activity of SOD was decreased by 46% ($P < 0.01$) relative to control (Table 1), which indicated that these changes in the redox system were favourable to an overt oxidative stress. FDP-Sr markedly ameliorated the impaired redox system by recovering the altered parameters toward the normal ($P < 0.05$), respectively. However, sildenafil had no effect in attenuating these changes in the corpus cavernosum in streptozocin -injected rats (Table 1).

Impaired vascular activity of cavernosal strips

At concentrations of Phe ranging from 10^{-10}–10^{-4} M, the contractile responses of the cavernosal smooth muscle strips

Table 1 Changes in blood glucose, cavernosal malondialdehyde and superoxide dismutase, and mRNA expression of NADPH p22phox, p47phox, p67phox, ppET-1, ET_AR, ET_BR found eight weeks after streptozocin administration to rats, and the response to sildenafil and strontium fructose 1,6-diphosphate

	Control	Diabetes mellitus	Sildenafil	FDP-Sr
Blood glucose (mmol/l)	7.47 ± 1.6	29.3 ± 3.25**	25.44 ± 2.53	26.64 ± 2.09
MDA (mmol/mg protein)	5.82 ± 1.52	10.52 ± 1.75**	8.93 ± 1.04	6.51 ± 1.08##
SOD ((U/mg protein)	152.7 ± 21.94	83.15 ± 16.56**	97.58 ± 10.77	126.0 ± 14.60##
p22phox/18S mRNA	0.16 ± 0.075	0.63 ± 0.120**	0.52 ± 0.087	0.31 ± 0.071##
p47phox/18S mRNA	0.31 ± 0.082	0.64 ± 0.063**	0.58 ± 0.100	0.41 ± 0.071##
p67phox /18S mRNA	0.22 ± 0.076	0.68 ± 0.123**	0.64 ± 0.131	0.45 ± 0.133#
ppET-1 /18S RNA	0.35 ± 0.053	0.74 ± 0.086**	0.62 ± 0.121	0.54 ± 0.085##
ET_AR /18S mRNA	0.29 ± 0.035	0.69 ± 0.085**	0.63 ± 0.101	0.54 ± 0.110#
ET_BR /18S mRNA	0.29 ± 0.04	0.59 ± 0.104**	0.53 ± 0.107	0.42 ± 0.116#

Endothelin, ET; malondialdehyde, MDA; strontium fructose 1,6-diphosphate, FDP-Sr; superoxide dismutase, SOD. $n = 8$, $^{**}P < 0.01$ vs control; $^{\#}P < 0.05$, $^{\#\#}P < 0.05$ vs diabetes mellitus group.

were significantly enhanced in diabetic rats, with the maximum developed force of 1.42 ± 0.2 g compared with 0.73 ± 0.16 g ($P < 0.01$) in the control. (Figure 1a) FDP-Sr and sildenafil significantly decreased the tension to 1.19 ± 0.14 ($P < 0.05$) and 0.88 ± 0.09 g ($P < 0.05$), respectively. In addition, ACh produced a significant relaxation of the isolated cavernosal strips preconstricted by Phe. In normal rats, the relaxation rate was 61.5 ± 7.9%, which declined to 34.4 ± 5.75% in the diabetic group ($P < 0.01$) (Figure 1b). FDP-Sr and sildenafil were both effective in improving the impaired relaxation in streptozocin-injected rats, but the relaxation was greater with sildenafil than with FDP-Sr.

NADPH oxidase in the corporal cavernosum

Activated NADPH oxidase is an important source of ROS generation resulting in oxidative stress in the diabetic corporal cavernosum. The combination of upregulated NADPH oxidase subunits at the membrane is prerequisite for the full activation of NADPH oxidase. Thus, the expressions of three subunits were assessed, and mRNA abundances of p22phox, p47phox and p67phox were extremely upregulated by 271.4%, 202%, and 94.4%, respectively, compared with the control ($P < 0.01$) (Table 1). Furthermore, the protein levels of p22phox, p47phox and p67phox were obviously elevated in the diabetic corpus cavernosum ($P < 0.01$) (Figure 2a, b, c). Administration of FDP-Sr for four weeks completely abolished the abnormal expression of NADPH oxidase. In contrast, no benefit of sildenafil was found in suppressing activated NADPH oxidase expressions in the cavernosum.

The endothelin-1 system in the corporal cavernosum

The expression of ET_AR and ET_BR may be importantly implicated in reduced vasorelaxation of the cavernosal smooth muscle strips. Therefore, the expressions of the ET system in the diabetic penile structure were conducted. Consistent with the upregulation of NADPH oxidase, a marked upregulation in the abundance of mRNA for ppET-1, ET_AR and ET_BR mRNA was found (Table 1). Consistently, the upregulation of protein levels of ET_AR and ET_BR was significant ($P < 0.01$) relative to control (Figure 2d, e). FDP-Sr significantly inhibited the upregulated ppET-1, ET_AR and ET_BR ($P < 0.05$), relative to the untreated group. Sildenafil was less effective in this regards (Table 1 and Figure 2d, e).

Discussion

In diabetes, ED is a syndrome caused by multifactors, consisting of vascular disease, metabolic syndrome, endocrine disorders, oxygen free radicals, pro-inflammatory cytokines, neuropathic and psychogenic factors.[21] Numerous therapeutic options have been applied in the treatment of diabetic ED. Besides PDE5 inhibitors, insulin, androgen, surgical therapy and gene therapy are employed to relieve ED.[22]

ROS have been increasingly focused on the pathophysiology of many diseases and enhanced ROS production contributes significantly to the pathogeneses of ED. In diabetes mellitus, excessive ROS are produced in mitochondria, while the antioxidant system including SOD, glutathione peroxidase and other free radical scavenging molecules are damaged. Pro-inflammatory cytokines are increased due to an increase in ROS, which is relevant to an impairment of mitochondrial energy metabolism in diabetes. Diabetic complications are associated with an upregulation of endoplasmic reticulum (ER) stress chaperone such as PERK (PKR-like ER kinase), which indicates that chronic inflammation is present in diabetic tissue.[23] All these pro-inflammatory factors impair the erectile function of cavernosal tissue. Therefore, FDP-Sr offers extra ATP to meet the need of the reduced energy metabolism in the mitochondria, resulting in an improvement in the diabetic testopathy and partly relieves dysfunction of the cavernosal vascular activity in this study.[17,19]

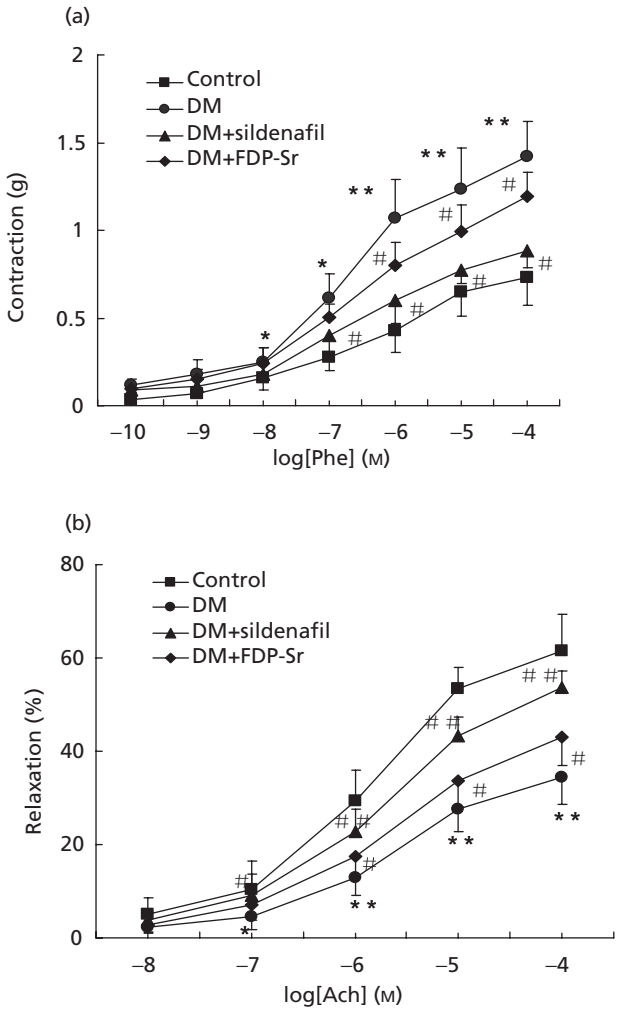

Figure 1 Enhanced phenylephrine-induced contraction–concentration curves (a) and reduced relaxation to acetylcholine (b) in isolated cavernosal strips from streptozocin-induced diabetic rats and streptozocin-induced diabetic rats administered strontium fructose 1,6-diphosphate or sildenafil. Phe, phenylephrine, 10^{-10}–10^{-4} M; ACh, acetylcholine 10^{-8}–10^{-4} M. Rats had been diabetic for eight-weeks and were administrated strontium fructose diphosphate (FDP-Sr; 200 mg/kg per day) or sildenafil (12 mg/kg per day) for the final four weeks. $n = 8$. Means ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.05$ compared with diabetes mellitus (DM) group.

The sources of ROS in the penis remain largely unknown and given the emergent evidence that a definite role of NADPH oxidase in inducing ROS and ED may be suggested.[9] In this study, a decreased activity of SOD and an increased level of MDA were in association with an activation of NADPH oxidase, responsible for lesions caused by diabetes in the corporal cavernosum. ROS genesis is sourced from an activation of NADPH oxidase and the ET system.[13,24] Moreover, oxidative stress is more predominant with longer-term diabetes, and the longer the term of diabetes, the more severe ED in rats.[6] ROS is at the centre of pathological changes in diabetes-associated ED and an excess of ROS activates matrix metalloproteinases (MMPs) which induces endothelial dysfunction of the corpus cavernosum, and the causal factors to induce ED.[25]

The source of ROS leading to ED is likely in part from circulating monocytes.[26] NADPH oxidase-derived ROS are available at low levels in the normal penis. However, more ROS are produced in the cavernosal tissue while NADPH oxidase is activated, which suggests that NADPH oxidase plays an important role to cause ED in hypercholesterolaemic rabbits and hypertensive patients.[9,27] In this study, three NADPH oxidase subunits were upregulated in the corporal cavernosum in diabetic rats. It indicated that an activation of NADPH oxidase was linked to dysfunction of the corporal cavernosum. Interestingly, the abnormal expressions of NADPH oxidase and ED in patients could be improved by participating in regular physical training.[28]

ET-1 is produced by the vascular endothelium in the penis.[25] Plasma ET-1 levels are not regularly elevated in diabetes mellitus, due to the fact that ET-1 is a parakine exerting its action within the local region. However, penile ET-1 levels are significantly increased in diabetic rats, consistent with the findings in this study that upregulated ET_AR/ET_BR were significant in the corporal cavernosum. ET-1 causes an increase in vasoconstriction of the corpus cavernosum to Phe, counteracting its vasodilative response.[29] The activation of both ET_AR and ET_BR mediated the constriction of cavernosal smooth muscle, which may be largely dependent upon Rho-kinase in the penis. ET_AR mediates vasoconstriction and cellular proliferation of cavernosal smooth muscle. Apart from vasodilatation through the production of NO, ET_B has a vasoconstriction of the cavernosal tissue.[29] Thus, an elevation of ET_BR may be a contributory factor to the dysfunction of cavernosal vasculature. Furthermore, the promitogenic effect of the ET_BR may facilitate the formation of atherosclerotic-like lesions implicated in diabetic lesions, allowing the occurrence of ED through the veno-occlusive events in the diabetic penile tissue.[25] By suppressing the upregulated ET receptors, ET antagonists alone or in combination with established drugs could be beneficial in the treatment and prevention of ED.[30] Given data collected in this study, the pro-inflammatory factors in the diabetic cavernosal tissue are sensitive to FDP-Sr, rather than sildenafil. However, an increase in the vasodilatation of the cavernosal smooth muscles is mainly responded to by PDE5i related to the NO/cGMP pathway. The reduced responsiveness to PDE5i in diabetic patients is increased due to these inflammatory biomarkers in the cavernosal tissue.[30] Sildenafil could be beneficial in combination with FDP-Sr to enhance the effectiveness in treating ED in diabetic patients. It has been confirmed that a combined therapy of sildenafil with an antioxidant was better than sildenafil alone in treating diabetes mellitus-related ED.[31,32]

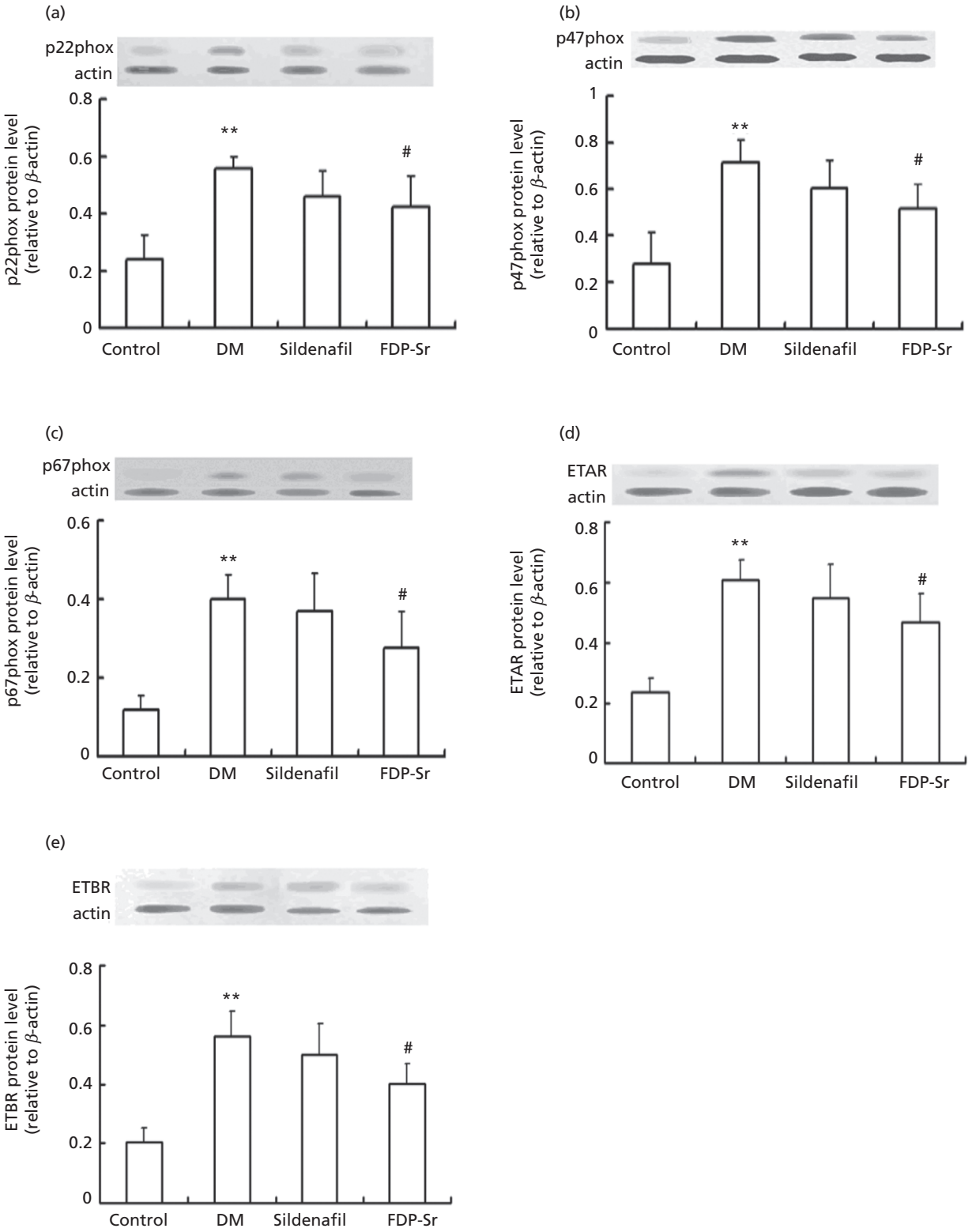

Figure 2 The effect of strontium fructose diphosphate or sildenafil on the protein levels of some inflammatory factors in the cavernosum of streptozocin-induced diabetic rats. (a) p22phox; (b) p47phox; (c) p67phox; (d) ET_AB; and (e) ET_BR. Rats were diabetic for eight-weeks and were administrated strontium fructose diphosphate (FDP-Sr; 200 mg/kg per day) or sildenafil (12 mg/kg per day) for the final four weeks. $n = 5$, means ± SD; $^{**}P < 0.01$ compared with control; $^{\#}P < 0.05$ compared with diabetes mellitus (DM) group.

Conclusions

Pro-inflammatory factors including an activation of NADPH oxidase and the ET-1 system, which does not respond to sildenafil, may contribute in part to ED in diabetes mellitus. An excess of pro-inflammatory cytokines in the diabetic cavernosal strips are likely to be relevant to the insufficient energy supply in the mitochondria, and act as the causal factors limiting the effectiveness of sildenafil, a PDE5i, in alleviating diabetes-induced ED. FDP-Sr suppressed these pro-inflammatory cytokines by providing an extra ATP supply, but it only afforded a moderate effect in attenuating ED. Suppression on these abnormal biomarkers by FDP-Sr may provide a supplement to the activity of PDE5i. Therefore, a combined therapy of PDE5i with FDP-Sr may be promising to achieve satisfactory effects in attenuating ED in diabetic patients.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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