JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 943–951
© 2011 The Authors
JPP © 2011 Royal Pharmaceutical Society
Received October 4, 2010
Accepted January 31, 2011
DOI
10.1111/j.2042-7158.2011.01268.x
ISSN 0022-3573

Correspondence: De-Zai Dai, Research Division of Pharmacology, China Pharmaceutical University, Nanjing 210009, China.
E-mail: dezaidai@vip.sina.com

Research Paper

Sildenafil improves diabetic vascular activity through suppressing endothelin receptor A, iNOS and NADPH oxidase which is comparable with the endothelin receptor antagonist CPU0213 in STZ-injected rats

Lu Luo[a], De-Zai Dai[a], Yu-Si Cheng[a], Qi Zhang[b], Wen-Jun Yuan[c,d] and Yin Dai[a]

[a]Faculty of Pharmacy, China Pharmaceutical University, [b]College of Life Science and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing, [c]Department of Physiology and Key Lab of Ministry of Education in Fertility Preservation and Maintenance (Ministry-Region Sponsored), Ningxia Medical University, Ningxia and [d]Department of Physiology, Second Military Medical University, Shanghai, China

Abstract

Objectives Abnormal vascular activity in diabetes is related not only to impaired nitric oxide bioavailability but also to inflammatory cytokines, endothelin A receptor (ET_A) activation and NADPH oxidase in the vasculature. The potential role of sildenafil in improving vascular function was investigated. Its action was likely blocking upregulated ET_A and NADPH oxidase, and was compared with the endothelin receptor antagonist CPU0213.
Methods Diabetes was induced by single-dose administration of streptozotocin (65 mg/kg, i.p.) to rats and the vascular activity of the thoracic aorta was measured.
Key findings An increase in contractile tone to phenylephrine and a decrease in relaxant tone to acetylcholine was found in the thoracic aorta. Oxidative stress was evident by increased malondialdehyde and reduced glutathione peroxidase levels in serum and upregulation of ET_A, MMP-9 (matrix metalloproteinase-9), inducible nitric oxide synthase and NADPH oxidase $p67^{phox}$ were found in the vascular wall. The vascular abnormalities and abnormal biomarkers were attenuated significantly by either sildenafil or CPU0213 along with an improvement of nitric oxide bioavailability and vascular activity.
Conclusions Improvement of diabetic vascular abnormal activity by sildenafil results from its suppression of activation of ET_A and NADPH oxidase in the vasculature, and these actions are comparable with those of the endothelin receptor antagonist CPU0213.
Keywords diabetes; endothelin receptor antagonists; inflammatory cytokines; sildenafil; vascular relaxation

Introduction

Vascular abnormality due to endothelial dysfunction contributes to cardiovascular complications covering about 80% of morbidity and mortality in diabetes mellitus.[1] Concerning the vascular abnormality, there are at least two basic events implicated: reduced nitric oxide (NO) bioavailability and increased inflammatory factors in the vasculature. NO bioavailability, as an indicator for endothelial function, is greatly reduced in the diabetic vascular bed and sildenafil, an inhibitor for phosphodiesterase type 5 (PDE-5), elevates vasodilator activity by an increase in NO bioavailability through upregulation of NO-cGMP signalling.[2] Inflammatory reactions in the vascular beds caused by hyperglycaemia greatly impair the vascular activity in diabetes. Endothelin-1, as a major vasoconstrictive substance interacting with NO, contributes to vascular abnormality while overactivation of endothelin receptor A (ET_A) occurs in diabetes. Inhibition of ET_A produces an enhanced vasorelaxation resulting from elevated NO bioavailability.[3,4] Either selective or non-selective, ET receptor antagonists blunt the impairment in diabetic vascular activity.

An increase in advanced glycation end products by hyperglycaemia plays a critical role in inducing abnormal vascular activity by activating inflammatory factors, including cytokines (ET-1) and reactive oxygen species (ROS). Accordingly, blood-sugar-lowering agents appear to be the drugs of choice in reducing vascular complications by lowering HbA1c.

However, data from clinical trials do not encourage the idea.[5,6] Intensive control of hyperglycaemia, even reducing HbA1c levels to 6%, does not relieve the vascular complications or increase life span.[7] This indicates that the reduced survival rate of diabetic patients appears to be related to the vascular complications due to endothelial dysfunction, rather than hyperglycaemia *per se*.

Compromised vascular activity is initiated by either hypercholesterolaemia or diabetes.[8] The impaired vascular activity in diabetes is multifaceted, mainly due to inflammatory cytokines, such as elevated ET_A, NADPH oxidase, ROS, matrix metalloproteinase-9 (MMP-9) and protein kinase ε (PKCε). These changes may be caused hyperglycaemia or hypercholesterolaemia alone, with decreased NO availability and impaired renal function.[9]

A reduction in NO availability, associated with increased levels of ROS in diabetic tissues, is sourced from an impaired mitochondrial electron-transport chain[10] and an activation of NADPH oxidase mediated by activated ET_A[11,12] NADPH oxidase is considered to be the main source of ROS, not only in non-phagocytic cells but also in vascular smooth muscle cells, endothelial, tubular and glomerular cells and cardiac fibroblasts.[13] ET-1, growth factors, cytokines and high sugar serve as activators to NADPH oxidase, leading to excessive production of ROS linked to insulin resistance and diabetic complications.[14] Upregulated NADPH oxidase in diabetic blood vessels is relieved dramatically by a dual endothelin receptor antagonist, CPU0213.[4] Inducible nitric oxide synthase (iNOS) generates non-physiological NO as much as 1000 times the physiological level[15] and is suppressed by CPU0213.[16] The therapeutic effects of endothelin receptor antagonists are predominant in blunting the upregulated pro-inflammatory changes and abnormal vascular activity in diabetes, without a blood-glucose-lowering effect.[12,17]

Sildenafil, a PDE-5 inhibitor, improves vasodilatation by accumulation of NO in the vasculature, associated with a relief of diabetic vascular abnormalities.[18] However, the sensitivity to sildenafil of the cavernosal vascular structure in diabetic patients with erectile dysfunction (ED) is reduced as compared with that in non-diabetic subjects.[19,20] It is interesting to investigate whether the responsiveness of diabetic vascular activity to sildenafil is reduced due to the inflammatory cytokines in the vascular structure. We hypothesized that sildenafil could mitigate upregulated inflammatory cytokines in vascular wall, including iNOS, MMP-9, NADPH oxidase, p67phox and ET_A, caused by hyperglycaemia, in the same way as CPU0213. In this study, we investigated whether sildenafil is as potent an ET antagonist as CPU0213 in suppressing the pro-inflammatory factors in the vasculature contributing to a recovery of vascular activity in streptozotocin-injected rats.

Materials and Methods

Drugs and reagents

CPU0213 (Figure 1) was provided by the Laboratory of Medical Chemistry at China Pharmaceutical University. Streptozotocin (STZ, Lot No. LotB56981) was purchased from Sigma Co. (St Louis, MO, USA), preserved at −20°C and dissolved in citric acid buffer before use. Sildenafil hydrochloride (sildenafil) was purchased from Sigma Co. Isoprot-

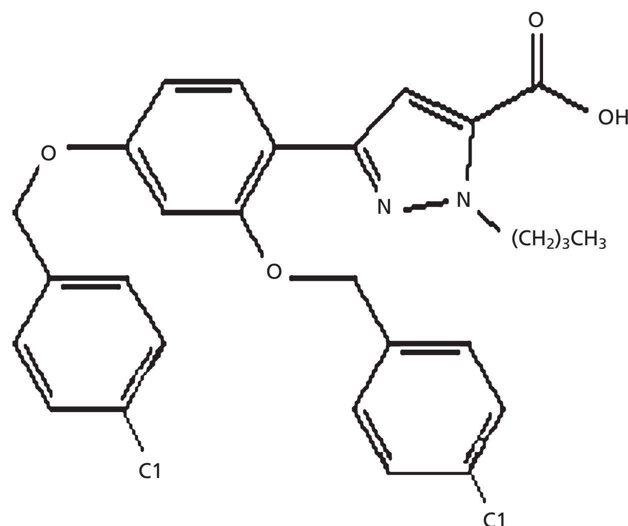

Figure 1 Chemical structure of CPU0213.

erenol injection (Lot no. 6 E20005) was purchased from Shanghai Hefeng Pharmaceutical Co. Ltd (Shanghai, China). Injectable sodium nitroprusside (Lot No. 060401) was purchased from the Beijing Shuanghe Modern Medical Technology Co. Ltd (Beijing, China). Acetylcholine (ACh, Lot No. 20030620) was purchased from Shanghai Sanaisi Reagent Co. Ltd (Shanghai, China). Noradrenaline (Lot No. 122 F-0340) was purchased from Sigma Co. Phenylephrine (Lot No. 103 H-0073) was purchased from Sigma Co. L-N (omega)-nitro-arginine (L-NNA, Lot No. 32H-0038) was purchased from Sigma Co. Kits for measuring triglyceride (TG), cholesterol, malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) (Lot No. 20070625) were purchased from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China). Trizol, AMV reverse transcriptase, dNTP, oligo d(T)18, Taq enzyme and RNase inhibitor were purchased from Promega Corporation (Madison, WI, USA).

Animals

Sixty male Sprague–Dawley (SD) rats, 180–200 g, were obtained from the Experimental Animal Center of Zhejiang province (qualified No. SCXK (SU) 2006-0018). All experiments were carried out in agreement with the Guidelines for the Care and Use of Laboratory Animal approved by the Bureau of Science-Technology of Jiangsu Province, China.

Streptozotocin-injected rats

SD rats, fasted overnight, were injected with single dose of STZ (65 mg/kg, i.p.), following previous practice.[11] On the 7th, 14th, 21st and 28th day after the STZ injection, fasting plasma glucose levels were measured and the glucose levels higher than 16.7 mmol/l on the 7th day were recognized as diabetes. At the beginning of the 5th week, rats injected with STZ were randomly divided for drug interventions for 4 weeks: sildenafil (12 mg/kg/day, p.o.) and CPU0213, a dual endothelin receptor antagonist (30 mg/kg/day, s.c.), respectively. Based on previous reports, the dose of sildenafil[21] and CPU0213[9,11,12] was chosen. The normal and diabetic untreated groups were given the same volume of distilled

water. At the beginning of the 9th week, the vascular activity and biomarkers were measured.

Vascular activity

Thoracic aorta was harvested and cut into 3-mm arterial rings, which were placed in Krebs–Hensleit (KH) solution gassed with 95% O_2 and 5% CO_2 and the vascular tension was recorded according to previous practice.[12] An equilibration time of 2 h was employed during which KH solution was changed every 20 min while the rings were under 1 g tension and were pretreated twice with noradrenaline (1 μmol/l). Phenylephrine (10^{-9} to 10^{-5} mol/l) was then added to generate cumulative dose–response curves. In separate experiments the maximum contraction of arterial rings to phenylephrine was recorded after being incubated with L-NNA at 10^{-4} mol/l for 30 min. The AUC (area under the curve) of the two curves was calculated and compared. The difference between the two curves was taken to measure NO bioavailability, indicating the basal release of NO from the endothelium, which was compared among groups. When the constriction of the vascular rings had reached a stable plateau to phenylephrine (1 μmol/l), acetylcholine (Ach, 10^{-9} to 10^{-5} mol/l) was added to measure and compare the cumulative relaxation. Similarly, in separate vascular rings, sodium nitroprusside (10^{-11} to 10^{-6} mol/l) was finally added to induce maximum relaxation in a dose-dependent manner.

Biochemical measurements in serum

Bioactive substances glucose, TG, cholesterol, MDA and GSH-px were measured in serum, according to the instructions in the assay kits, respectively.

Reverse transcription-polymerase chain reaction (RT-PCR)

A portion of 100 mg aorta was dissected and homogenized in 1 ml of Trizol and the PCR was conducted accordingly.[11] Briefly, into the homogenate, 200 μl of chloroform was added, then mixed and centrifuged at 4°C for 15 min (10 000*g*). The supernatant was collected after several steps for extraction of the dried RNA and the concentration was determined with a UV spectrophotometer. Finally, RNA of adjusted concentration was transcribed into cDNA, which was preserved at −20°C. Semi-quantitative mRNA expression of ET_A, iNOS, MMP-9, NADPH p67phox and 18S was carried out by using sense primers and antisense primers: sense: 5′-ATCGCTGACAATGCTGAGAG-3′, and antisense: 5′-CCACGATGAAAATGGTACAG-3′ for ET_A; sense: 5′-ATCCCGAAACGCTACACTT-3′ and antisense: 5′-TCTGGCGAAGAACAAATCC-3′, for iNOS; sense: 5′-CGTGGCCTAGTGACCTATG-3′ and antisense: 5′-GGATAGCTCGGTGGTGTCCT-3′ for MMP-9; and sense: 5′-GAAAGCATGAAGGATGCCTGG-3′ and antisense: 5′-ATAGCACCAAGATCACATCTCC-3′ for NADPH oxidase p67phox; sense: 5′-GCTGCTGGCACCAGACTT-3′ and antisense: 5′-CGGCTACCACATCCAAGG-3′ for 18S. Products were resolved on 2% agarose gel followed by ethidium bromide staining and a semi-quantitative assay for the images was conducted as previously described.[11]

Western blot

Western blot analysis was performed according to previous practice.[11] Briefly, thoracic aortic rings were homogenized in 400 μl of lysis buffer and the homogenate was centrifuged (10 000*g*) at 4°C for 20 min. Supernatant was loaded into another tube, preserved at −20°C for subsequent use. In 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, these prepared samples were transferred onto cellulose nitrate film, and incubation with the first antibodies (from Wuhan Boster Biological Technology, Wuhan, China) was performed for a further hour. After three washes, the blots were incubated with horseradish peroxidase-conjugated goat secondary antibody immunoglobulin G (1 : 1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for an additional 1 h. 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

All data are expressed as mean ± SD. The between-group comparison was performed with single-factor analysis of variance test. $P < 0.05$ was considered as a statistically significant difference.

Results

Serum biochemical measurements

In the STZ-injected rats blood glucose rose significantly from 7.5 ± 1.6 mmol/l to 29.0 ± 3.0 mmol/l ($P < 0.01$), and the reduction in body weight was significant (295 ± 14 g vs 197 ± 13 g) ($P < 0.01$). These changes did not respond to interventions with either sildenafil or CPU0213. Meanwhile, hypercholesterolaemia and hypertriglyceridaemia were evident and sustained (Table 1), whether or not the interventions were constituted (Table 1). An increase in serum

Table 1 Biochemical changes in serum were found in streptozotocin-injected rats

	Total cholesterol (mmol/l)	Triglycerides (mmol/l)	MDA (nmol/l)	GSH-px (U)
Control	1.56 ± 0.13	0.68 ± 0.11	6.5 ± 1.8	1998 ± 156
STZ injected	4.84 ± 0.50**	1.51 ± 0.19**	15.8 ± 2.9**	1552 ± 153**
STZ + CPU0213	3.81 ± 0.84**	1.33 ± 0.22**	9.9 ± 2.3*,****	1658 ± 123****
STZ + sildenafil	4.24 ± 0.75**	1.41 ± 0.28**	11.4 ± 2.2*,****	1800 ± 275***

GSH-px, glutathione peroxidase; MDA, malondialdehyde. Data are mean ± SD; $n = 6$. *$P < 0.05$, **$P < 0.01$, compared with control; ***$P < 0.05$, ****$P < 0.01$, compared with STZ injected.

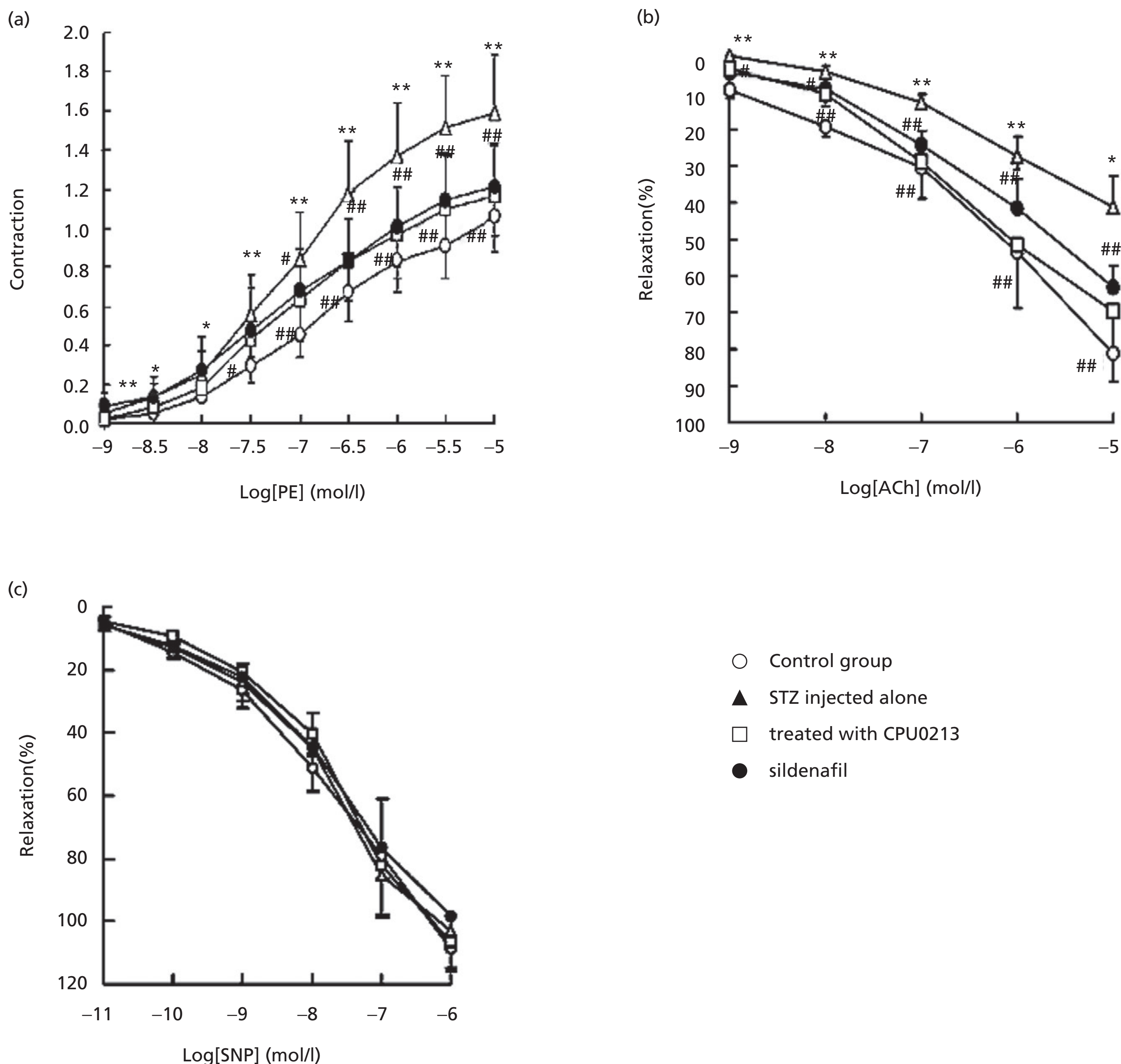

Figure 2 Effect of CPU0213 and sildenafil on the cumulative responses of rat isolated thoracic aortic rings: (a) constricted by phenylephrine (PE), (b) dilatation by acetylcholine (ACh) and (c) relaxation by sodium nitroprusside (SNP). Data are the mean ± SD, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with STZ alone.

MDA production was associated with a decrease in activity of GSH-px, indicating a status of oxidative stress which was attenuated by either CPU0213 or sildenafil in STZ-injected rats (Table 1).

Vascular activity

The cumulative vasoconstrictive curves showed an increase in vascular tension to phenylephrine of the isolated thoracic aortic rings from the STZ-injected rats relative to control (Figure 2a). The maximum contractile tension of normal thoracic aortic rings was 1.06 ± 0.18 g, while the tension was elevated to 1.59 ± 0.29 g ($P < 0.01$) in diabetic tissue. The increased vascular tone was reduced by CPU0213 (1.21 ± 0.19 g, $P < 0.05$) and sildenafil (1.24 ± 0.23 g, $P < 0.05$), respectively. The reduced vasodilator activity to Ach was significant in the STZ-treated rats, and was improved significantly by CPU0213 and sildenafil (Figure 2b). The relaxation to nitroprusside was significant, although no change was found among groups (Figure 2c).

Nitric oxide bioavailability

A decreased NO bioavailability occurred in the thoracic aortic rings as assessed by comparing the AUC (area under the curve) of the cumulative constriction curves in the presence and absence of L-NNA. NO bioavailability in STZ-injected rats was reduced to 37.3% of the control group ($P < 0.\ 01$),

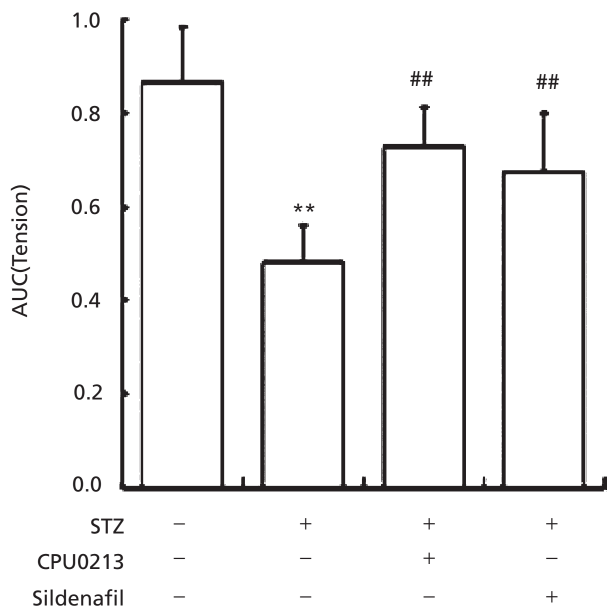

Figure 3 Nitric oxide bioavailability of rat isolated thoracic aorta was measured by functional assessment and was impaired in thoracic aorta of rats injected with STZ alone. It was improved by either CPU0213 or sildenafil. Data are the mean ± SD, $n = 6$, $*P < 0.05$, compared with control; $^{\#}P < 0.05$, compared with STZ alone.

indicating that the basal release of NO in the diabetic vascular endothelium was significantly compromised. After treatment with either CPU0213 or sildenafil, NO bioavailability was increased significantly (Figure 3).

ET_A, NADPH oxidase, iNOS and MMP-9

ET_A was apparently activated in the STZ-injected group, as shown by upregulated mRNA of ET_A relative to control ($P < 0.01$). In addition, mRNA abundance of inflammatory factors iNOS, NADPH oxidase p67phox and MMP-9 increased significantly ($P < 0.01$), in the vascular wall, and was suppressed significantly by either CPU0213 or sildenafil (Figure 4). These changes were confirmed by an increase in the abundance of proteins of these biomarkers in the aortic vasculature. CPU0213 and sildenafil acted effectively to suppress these changes (Figure 5).

Discussion

The vascular abnormality found in diabetic patients characterized as reduced flow-dependent vasodilatation is consistent with the previous findings in STZ-injected rats.[4] An increase in MDA and a decrease in GSH-px indicate a state of inflammatory reactions that may contribute to dysfunction of vascular endothelium in STZ-injected rats. Therapies interrupting the abnormal redox pathways in vascular tissue are helpful in preventing and attenuating cardiovascular complications in the diabetic population.[22] Regarding the overt hyperglycaemia in the STZ-injected rats, an increase in advanced glycation end products stimulates an increase in ROS, inflammatory factors and cytokines and those in turn are responsible for further increasing the genesis of ROS and inflammatory reactions. Activated iNOS plays an important role in mediating tissue damage through forming OONO-• by a combination of ROS with NO and a suppressive effect on the upregulated iNOS leads to an amelioration of abnormal vascular activity.[23] The compromised responses to Ach were improved by CPU0213, an endothelin receptor antagonist, partly through normalizing iNOS expression in the vasculature. Sildenafil attenuates the activated iNOS as does CPU0213.

Vascular abnormality appears to be consequent to endothelial dysfunction through decreased NO bioavailability in association with activated ET_A. The basal release of NO from the vascular endothelium was reduced, as assessed functionally by reduced NO availability, which is related to either depressed activity of endothelial NOS or activated inflammatory factors. Vascular endothelial function is sensitive to oxidative stress and in this regard, an activated ET_A system plays an important role in stimulating production of ROS. Thus, endothelin receptor antagonists are effective in recovering NO bioavailability and vascular endothelium function by suppressing ROS genesis in diabetes.[4,12]

NADPH oxidase serves as the main source of ROS in diabetic complications and a blockade of ET receptors by CPU0213 leading to suppressed NADPH oxidase mitigates changes in diabetic nephropathy[11] and testopathy,[21] and stress-induced cardiomyopathy and vasculopathy.[24] There are two portions of NADPH oxidase, the catalytic (gp91, NOX4, NOX1) and modulatory subunits (p22phox, p47phox and p67phox). NADPH oxidase p67phox is located in the cytosol and on the appearance of inducers such as ET-1, is activated to move to the membrane to complete the activation process by combining with other subunits. The ageing-related impaired cerebrovascular dilatation is due to an increase in p67phox proteins,[25] which is responsible for the oxidative stress involved in the diabetic vascular wall. Concerning the two receptors of ET-1 in activating NADPH oxidase, both ET_A and ET_B are active in stimulating NADPH oxidase p67phox, while the role of ET_A is more dominant than ET_B in cardiac fibroblasts.[13] In this study we confirmed that a dual endothelin receptor antagonist, CPU0213, suppresses upregulated NADPH oxidase p67phox to relieve the oxidant stress and impaired vascular activity. By antioxidative activity an aldose reductase inhibitor (new pyrido[1,2-a]-pyrimidin-4-one derivative, PPO) relieves ischaemic injury in diabetic and non-diabetic hearts and it could share a suppression on NADPH oxidase in the myocardium but further verification is needed.[26]

A significant overexpression of MMP-9 is found in progressive lesions in the atherosclerotic carotid wall caused by hypercholesterolaemia, especially in the shoulder and cap sub-regions of atheroma resulting in an unstable plaque and an upregulated MMP-9 represents a status of inflammatory reaction relating to activated NADPH oxidase in the vascular wall.[27] Abnormal degradation of the extracellular matrix in vascular wall is present in atherosclerosis and diabetes mellitus. Abnormal MMP-9 expression as a causal factor adversely affects the normal interaction between cells and the extracellular matrixes, and promotes vascular smooth muscle cells (VSMCs) to release soluble Fas ligand and tumour necrosis

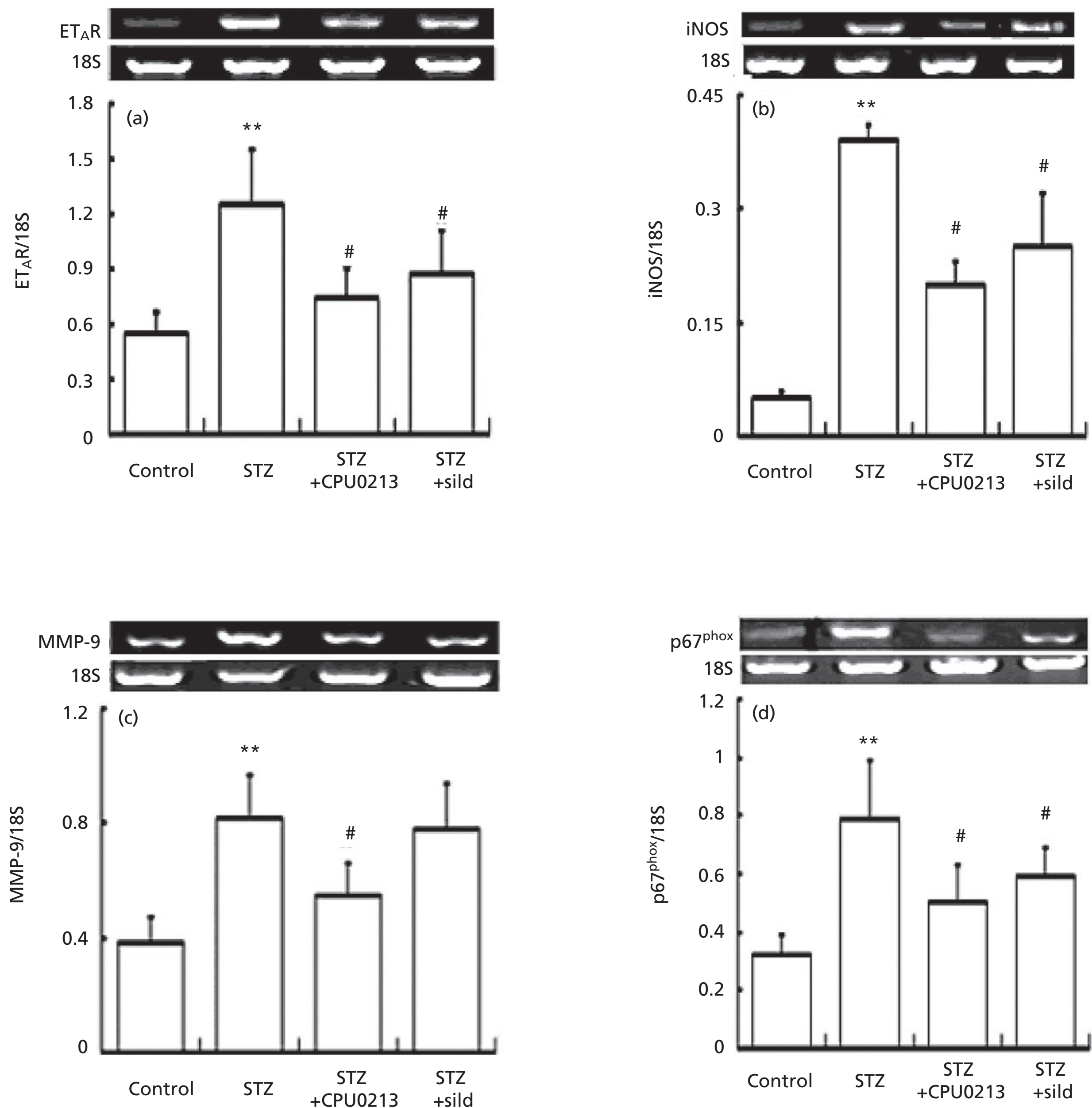

Figure 4 mRNA expression of (a) ET_A (ET_AR), (b) induced nitric oxide (iNOS), (c) MMP-9 and (d) NADPH oxidase $p67^{phox}$ were abnormally upregulated in the rat isolated thoracic aorta. These changes were significantly reversed by CPU0213 (STZ + 0213) and sildenafil (STZ + sild). Data are the mean ± SD, $n = 6$. $^{**}P < 0.01$ compared with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with STZ alone.

factor (TNF), both of which induce further damage to VSMCs by apoptosis and remodelling of the vascular structure.[27] MMP-2 and MMP-9 are upregulated in cardiac fibroblasts during isoproterenol stimulation and both ET_A and ET_B blockers and the dual ET blocker CPU0213 were capable of mitigating these changes.[13]

Additionally, an increased expression of MMP-9 leads to an impairment of Cx43 (connexin 43) in mediating intercellular gap junction communications in diabetic vasculature.[28] Certainly, dysfunctional activity in diabetic vascular beds is mediated by downregulation of Cx43 and Cx40, while upregulation of MMP-9 is present.[29] Upregulated MMP-9 may interfere with small molecules moving through the gap communications via intercellular connexins contributing to vascular remodelling and dysfunction in diabetes.

Suppression of NADPH oxidase or an antioxidant activity may ameliorate vascular dysfunction. Some active components sourced from plant origin having anti-inflammatory and antioxidant activity are worth testing for their potential in attenuating the inflammatory reactions in diabetic vasculature.[30,31]

By inhibiting PDE-5, sildenafil improves NO bioavailability and vascular dilatation markedly. Sildenafil suppresses oxidant stress in diabetic mouse heart[32] and relieves erectile

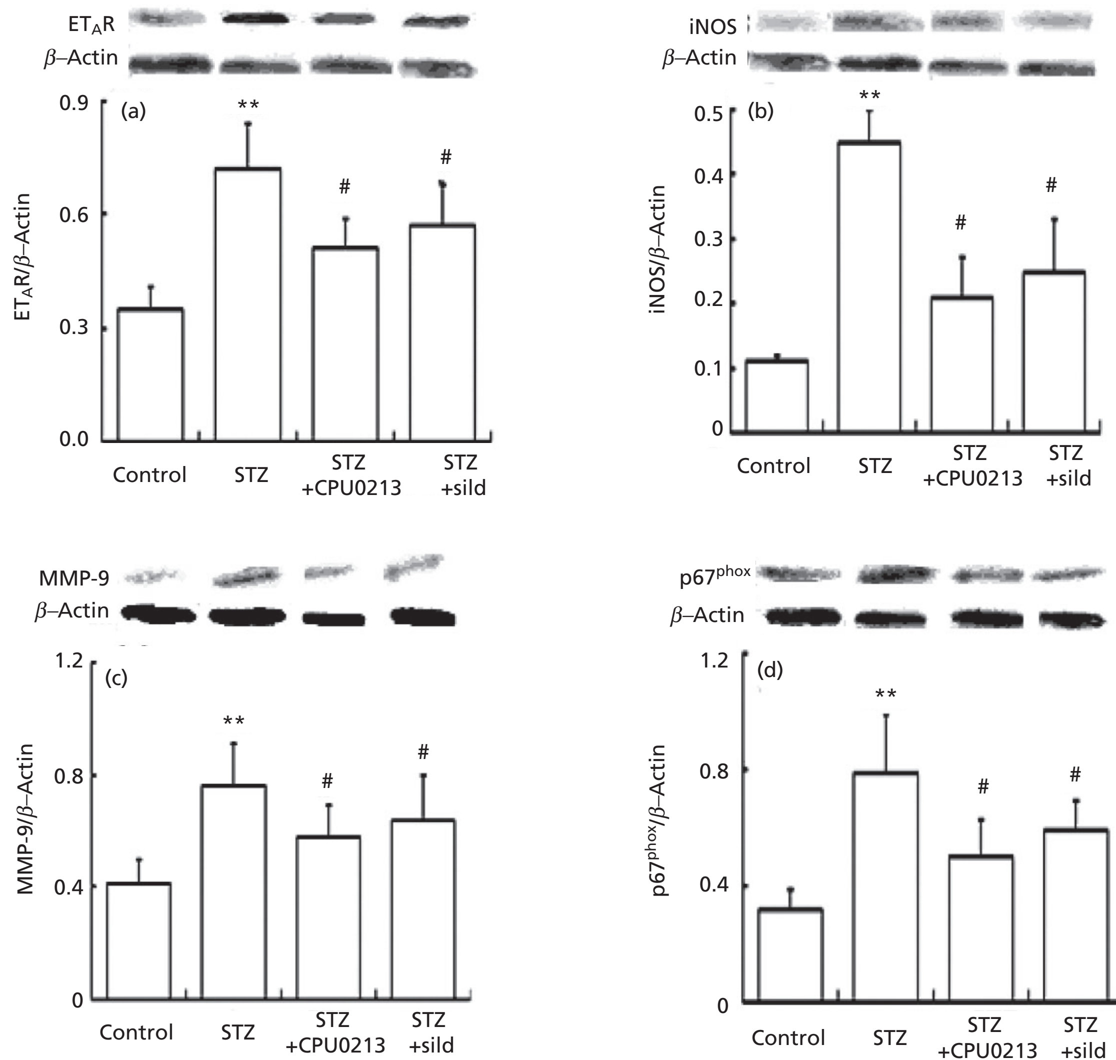

Figure 5 Upregulated protein abundance of (a) ET$_A$ (ET$_A$R), (b) induced nitric oxide (iNOS), (c) MMP-9 and (d) NADPH oxidase p67^{phox} were found in rat isolated thoracic aorta from rats injected with STZ alone. Both CPU0213 (STZ + 0213) and sildenafil (STZ + sild) were effective in normalizing these changes. Data are mean ± SD, $n = 6$. $^{**}P < 0.01$ compared with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with STZ alone.

dysfunction in diabetes, as compared with placebo.[33,34] However, lower responsiveness of erectile dysfunction to sildenafil has been found in diabetic patients[35] and can be reinstituted by a supplement of testosterone preparation.[20]

Data in this study demonstrate that sildenafil administration improves vascular activity in a manner resembling that of the endothelin receptor antagonist CPU0213, by normalizing the abnormal biomarkers in the vascular wall. These findings are consistent with those of chronic medication with sildenafil.[36] The antioxidant and anti-inflammatory activity of sildenafil therapy can be further improved by combination with an antioxidant agent in treating diabetic erectile dysfunction[37] and this observation may support the findings that the abnormal biomarkers in the cavernosal vascular structure show no response to sildenafil alone (unpublished data). The responses of diabetic aortic rings to sildenafil are positive, although it is complicated and the difference between effectiveness of diabetic cavernosal vascular and aortic vascular structure to sildenafil may be tissue-dependent, possibly mainly due to local inflammatory reactions. A combination of sildenafil with bosentan improves the therapeutic outcome in treating pulmonary hypertension compared with sildenafil or bosentan alone.[38] This implies that an enhanced anti-inflammatory activity by combining endothelin receptor antagonists could be helpful in improving sildenafil activity in dealing with inflammatory biomarkers in vascular beds. It is worth investigating suppression of inflammatory cytokines involved in diabetic vascular abnormalities of the cavernosal structure.

Conclusion

An upregulated ET$_A$, iNOS, NADPH oxidase and MMP-9 contribute to diabetic vascular abnormality in rat isolated aortic rings and the endothelin receptor antagonist CPU0213 exerts beneficial effects by restoring the abnormal biomarkers.

Sildenafil affords a relief to the diabetic vascular dysfunction comparable with that using an endothelin receptor antagonist CPU0213 through attenuating the inflammatory biomarkers in the aortic vasculature.

Declarations

Conflict of interest

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

Funding

The study was supported by the National Natural Science Foundation of China, No: 30873112, 30670760 and 81070145.

Acknowledgements

We are grateful to Prof. David Triggle at the State University of New York for his long-time assistance discussing the project and assisting with the English construction of the manuscript.

References

1. Versari D *et al*. Endothelial dysfunction as a target for prevention of cardiovascular disease. *Diabetes Care* 2009; 32(Suppl 2): S314–S321.
2. Kirsch M *et al*. Sildenafil in hypoxic pulmonary hypertension potentiates a compensatory up-regulation of NO-cGMP signaling. *FASEB J* 2008; 22: 30–40.
3. Mather KJ *et al*. Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. *Diabetes* 2004; 53: 2060–2066.
4. Su W *et al*. Upregulated endothelin system in diabetic vascular dysfunction and early retinopathy is reversed by CPU0213 and total triterpene acids from Fructus Corni. *Clin Exp Pharmacol Physiol* 2007; 34: 1228–1233.
5. Type 2 diabetes: target HbA1c of about 7%. *Prescrire Int* 2009; 18: 177.
6. Gore MO, McGuire DK. Cardiovascular disease and type 2 diabetes mellitus: regulating glucose and regulating drugs. *Curr Cardiol Rep* 2009; 11: 258–263.
7. Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008; 358: 2545–59.
8. Vladimirova-Kitova LG *et al*. Evaluation of the relationship between flow-mediated vasodilation and some atherogenic risk markers in severe hypercholesterolemia. *Folia Med (Plovdiv)* 2008; 50: 22–28.
9. Luo L *et al*. Hypercholesterolaemia induces early renal lesions characterized by upregulation of MMP-9 and iNOS and ET(A)R: alleviated by a dual endothelin receptor antagonist CPU0213 and simvastatin. *J Pharm Pharmacol* 2009; 61: 775–780.
10. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; 414: 813–820.
11. Xu M *et al*. Normalizing NADPH oxidase contributes to attenuating diabetic nephropathy by the dual endothelin receptor antagonist CPU0213 in rats. *Am J Nephrol* 2009; 29: 252–256.
12. Xu J *et al*. The endothelin receptor antagonist CPU0213 is more effective than aminoguanidine to attenuate isoproterenol-induced vascular abnormality by suppressing overexpression of NADPH oxidase ETA, ETB, and MMP9 in the vasculature. *J Cardiovasc Pharmacol* 2008; 52: 42–48.
13. Peng HJ *et al*. The separate roles of endothelin receptors participate in remodeling of matrix metalloproteinase and connexin 43 of cardiac fibroblasts in maladaptive response to isoproterenol. *Eur J Pharmacol* 2010; 634: 101–106.
14. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 2004; 24: 816–823.
15. Schulz R *et al*. Recent advances in the understanding of the role of nitric oxide in cardiovascular homeostasis. *Pharmacol Ther* 2005; 108: 225–256.
16. Cui B *et al*. CPU0213, a non-selective ETA/ETB receptor antagonist, improves pulmonary arteriolar remodeling of monocrotaline-induced pulmonary hypertension in rats. *Clin Exp Pharmacol Physiol* 2009; 36: 169–175.
17. Kalani M. The importance of endothelin-1 for microvascular dysfunction in diabetes. *Vasc Health Risk Manag* 2008; 4: 1061–1068.
18. Schäfer A *et al*. Improvement of vascular function by acute and chronic treatment with the PDE-5 inhibitor sildenafil in experimental diabetes mellitus. *Br J Pharmacol* 2008; 153: 886–893.
19. El-Sakka AI. Efficacy of sildenafil citrate in treatment of erectile dysfunction: effect of type 2 diabetes. *Eur Urol* 2004; 46: 503–509.
20. Zhang XH *et al*. Testosterone restores diabetes-induced erectile dysfunction and sildenafil responsiveness in two distinct animal models of chemical diabetes. *J Sex Med* 2006; 3: 253–264.
21. Xu M *et al*. Upregulated NADPH oxidase contributes to diabetic testicular complication and is relieved by strontium fructose 1,6-diphosphate. *Exp Clin Endoc Diab* 2010; 118: 459–465.
22. Pennathur S, Heinecke JW. Mechanisms for oxidative stress in diabetic cardiovascular disease. *Antioxid Redox Signal* 2007; 9: 955–969.
23. Schulz R *et al*. Recent advances in the understanding of the role of nitric oxide in cardiovascular homeostasis. *Pharmacol Ther* 2005; 108: 225–256.
24. Cheng YS *et al*. Isoproterenol disperses distribution of NADPH oxidase, MMP-9, and pPKCepsilon in the heart, which are mitigated by endothelin receptor antagonist CPU0213. *Acta Pharmacol Sin* 2009; 30: 1099–1106.
25. Mayhan WG *et al*. Age-related alterations in reactivity of cerebral arterioles: role of oxidative stress. *Microcirculation* 2008; 15: 225–236.
26. Calderone V *et al*. Anti-ischaemic activity of an antioxidant aldose reductase inhibitor on diabetic and non-diabetic rat hearts. *J Pharm Pharmacol* 2010; 62: 107–113.
27. Stintzing S *et al*. Overexpression of MMP9 and tissue factor in unstable carotid plaques associated with Chlamydia pneumoniae, inflammation, and apoptosis. *Ann Vasc Surg* 2005; 19: 310–319.
28. Brink PR *et al*. Experimental diabetes alters connexin43 derived gap junction permeability in short-term cultures of rat corporeal vascular smooth muscle cells. *J Urol* 2006; 175: 381–386.
29. Zheng YF *et al*. NaHS ameliorates diabetic vascular injury by correcting depressed connexin 43 and 40 in the vasculature in streptozotocin-injected rats. *J Pharm Pharmacol* 2010; 62: 615–621.
30. Chang SH *et al*. Anti-inflammatory effects of Artemisia princeps in antigen-stimulated T cells and regulatory T cells. *J Pharm Pharmacol* 2009; 61: 1043–1050.
31. Sharma SB *et al*. Antidiabetogenic action of Morus rubra L. leaf extract in streptozotocin-induced diabetic rats. *J Pharm Pharmacol* 2010; 62: 247–255.

32. Ebrahimi F *et al*. Sildenafil decreased cardiac cell apoptosis in diabetic mice: reduction of oxidative stress as a possible mechanism. *Can J Physiol Pharmacol* 2009; 87: 556–564.
33. Blonde L. Sildenafil citrate for erectile dysfunction in men with diabetes and cardiovascular risk factors: a retrospective analysis of pooled data from placebo-controlled trials. *Curr Med Res Opin* 2006; 22: 2111–2120.
34. Vardi M, Nini A. Phosphodiesterase inhibitors for erectile dysfunction in patients with diabetes mellitus. *Cochrane Database Syst Rev* 2007; (1): CD002187.
35. Hatzimouratidis K, Hatzichristou DG. Phosphodiesterase type 5 inhibitors: unmet needs. *Curr Pharm Des* 2009; 15: 3476–3485.
36. Aversa A *et al*. Chronic administration of sildenafil improves markers of endothelial function in men with Type 2 diabetes. *Diabet Me* 2008; 25: 37–44.
37. Morano S *et al*. Antioxidant treatment associated with sildenafil reduces monocyte activation and markers of endothelial damage in patients with diabetic erectile dysfunction: a double-blind, placebo-controlled study. *Eur Urol* 2007; 52: 1768–1774.
38. Mouchaers KT *et al*. Endothelin receptor blockade combined with phosphodiesterase-5 inhibition increases right ventricular mitochondrial capacity in pulmonary arterial hypertension. *Am J Physiol Heart Circ Physiol* 2009; 297: H200–H207.