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RESEARCH ARTICLE

Relationship between protective effects of rosiglitazone on endothelium and endogenous nitric oxide synthase inhibitor in streptozotocin-induced diabetic rats and cultured endothelial cells

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Abstract

Background Previous investigations have indicated that the level of asymmetric dimethylarginine (ADMA) is increased in diabetic patients and animals, and rosiglitazone has a protective effect on the endothelium. In the present study, we tested the relationship between protective effects of rosiglitazone and ADMA in streptozotocin (STZ)-induced diabetic rats and cultured endothelial cells.

Methods Blood samples were collected from carotid artery. Vasodilator responses to acetylcholine (ACh) in the isolated aortic rings were measured, and serum concentrations of glucose, lipid, nitrite/nitrate, ADMA and tumour necrosis factor-α (TNF-α) were determined. Cultured endothelial cells were treated with ADMA, and the concentrations of intercellular adhesion molecule (ICAM-1), TNF-α, and the activity of nuclear factor-κB (NF-κB) were determined.

Results Vasodilator responses to ACh were decreased markedly and the serum concentrations of TNF-α, nitrite/nitrate and ADMA were increased significantly in diabetic rats. Rosiglitazone (3, 10 or 30 mg/kg) produced a significant reduction of the inhibition of vasodilator responses to ACh, but had no effect on the serum concentrations of glucose, lipid, nitrite/nitrate and ADMA in diabetic rats. ADMA (30 μM) significantly increased the activity of NF-κB and elevated the levels of ICAM-1 and TNF-α, and pre-treatment with rosiglitazone (10 or 30 μM) markedly inhibited the increased activity of NF-κB and reduced the elevated levels of TNF-α and ICAM-1 induced by ADMA in cultured endothelial cells.

Conclusions Rosiglitazone improves endothelial function in diabetic rats, which is related to the reduction of the inflammatory response induced by ADMA. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords rosiglitazone; diabetes mellitus; asymmetric dimethylarginine (ADMA); tumour necrosis factor-α (TNF-α); intercellular adhesion molecule (ICAM-1); nuclear factor-κB (NF-κB)

Introduction

There is substantial evidence that endothelium-dependent vasodilatation is impaired in both animals and patients with diabetes [1,2]. Recent studies have indicated that the impairment of endothelium-dependent vasodilatation was associated with the increased level of asymmetric dimethylarginine ADMA, an endogenous inhibitor of nitric oxide synthase (NOS) instreptozotocin STZ-induced diabetic rats [3]. ADMA, besides inhibiting NOS, may be a new pro-inflammatory factor and plays an important role in the development of endothelial dysfunction. For example, incubation of ADMA with endothelial cell markedly increased NF-κB activity and monocyte chemoattractant protein-1 (MCP-1) expression, and potentiated the adhesiveness of endothelial cells [4,5]. Clinical studies have also shown that the level of ADMA and the content of C-reactive protein (CRP) and tumour necrosis factor-α (TNF-α) were strongly interrelated [6].

Rosiglitazone, a peroxisome proliferations activated receptor gamma (PPAR gamma) agonist, is an insulin sensitizer widely used in the treatment of type 2 diabetes with insulin resistance. There is evidence that rosiglitazone attenuated reduction of vasodilator responses to acetylcholine (ACh) in type 2 diabetic rats [7] and improved insulin resistance and decreased the serum concentration of ADMA in insulin resistance patients [8]. PPAR gamma can control the expression of genes implicated in the inflammatory response via negative interference with different inflammatory pathways, such as NF-κB, activator protein-1 (AP-1), signal transducer and activator of transcription-1 (STAT-1) and nuclear factor of activated T cells-dependent transcription (NFAT) [9]. PPAR gamma activators inhibited expression of vascular adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) in activated endothelial cells and significantly reduced monocyte/macrophage homing to atherosclerotic plaques [10]. Rosiglitazone attenuated the development of hypertension and reduced endothelial dysfunction and prevented upregulation of pro-inflammatory mediators in angiotensin (Ang) II-infused rats [11]. According to facilitation of endothelial dysfunction by inflammatory reaction in STZ-induced diabetic rats and stimulation of some cytokines generation by ADMA, and anti-inflammatory properties of rosiglitazone, we tested the relationship between protective effects of rosiglitazone on the endothelium and ADMA in STZ-induced diabetic rats.

Materials and methods

Animals and experimental design

Male Sprague–Dawley rats weighing 160–200 g were obtained from Xiang-Ya Medical College. They received humane care in compliance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health (NIH publication 86–23, revised 1986). Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg, STZ) dissolved in 0.1 M citrate buffer (pH 4.5). The control animals were injected with equal volume of vehicle. After 3 days following STZ administration, animals showing fasting blood glucose higher than 250 mg/dl were considered as diabetic rats. For rosiglitazone, the diabetic rats were treated with rosiglitazone (3, 10 or 30 mg/kg, i.g.) once a day for 6 weeks. Rosiglitazone was dissolved in a vehicle containing 0.5% saline carboxymethyl cellulose.

Cell culture and treatment

The human umbilical vein endothelial cells (HUVECs, ATCC, CRL-2480) were obtained from Tumor Research Institute of Peking Medical University (Peking, China). HUVECs were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin and 100g/mL streptomycin. When they reached sub-confluence, the cells were transferred into 24-well culture dishes and the culture medium was replaced by the serum-free medium. Cell numbers were $6.1 \pm 0.3 \times 10^6$ cells/well ($n = 4$). Cells were counted by trypan blue exclusion and showed >95% viability.

Cell injury was induced by treatment with ADMA (3, 10 or 30 μM) for 24, 48 or 72 h. For rosiglitazone, endothelial cells were exposed to rosiglitazone (3, 10, 30 μM) for 1 h, and then exposed to ADMA (30 μM) for 48 h in the presence of rosiglitazone.

Organ chamber experiments

The rats were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.). After blood samples were collected from the artery, the thoracic aorta was rapidly isolated and cut into rings of 3 mm length. The rings were suspended horizontally between two stainless steel wires and mounted in a 5 mL organ chamber filled with warm (37 °C) and oxygenated (95% O_2 and 5% CO_2) Krebs' solution. The Krebs' solution had the following composition (mM): NaCl, 119.0; $NaHCO_3$, 25.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2$, 2.5; and glucose, 11.0. One of the ring ends was connected to a force transducer. The aortic ring was stretched with 2 g resting force and equilibrated for 60 min, and then pre-contracted with KCl (60 mM). After a maximal response to KCl was obtained, the rings were washed repeatedly with Krebs' solution and equilibrated again for 30 min. To measure vasodilator responses, rings were contracted with phenylephrine to 40 ~ 50% of their maximal contraction. After the contraction stabilized, an accumulative concentration-response curve to acetylcholine ($3 \times 10^{-9} - 10^{-6}$ M) was observed.

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Determination of plasma lipid and glucose

Plasma glucose was determined using a commercially available enzyme kit (Baoding Changcheng Clinical Agent Co. Hebei, China). Plasma lipid was determined by 7060 Hitachi biochemical instrument.

Determination of nitrite/nitrate concentration

The levels of nitric oxide (NO) in plasma were determined indirectly as the content of nitrite and nitrate. The levels of nitrite/nitrate were measured as previously described [12]. Briefly, nitrate was converted to nitrite with *Aspergillus* nitrite reductase and the total nitrite was measured with the Griess reagent. The absorbance was determined at 540 nm with a spectrophotometer.

Determination of ADMA concentration

The protein in plasma was removed using 5-sulfosalicylic acid (5-SSA). The level of ADMA was measured by high-performance liquid chromatography (HPLC) as described previously with some modification [13]. HPLC was carried out using a Shimadzu LC-6A liquid chromatography with Shimadzu SCL-6A system controller and Shimadzu SIC-6A autosampler. *O*-phthaldialdehyde adducts of methylated amino acids and internal standard ADMA produced by pre-column mixing were monitored using a model RF 530 fluorescence detector set at $\lambda^{ex} = 338$ and $\lambda^{em} = 425$ nm on a resolve C_{18} column. Samples were eluted from the column using a linear gradient containing mobile phase A composed of 0.05 M (pH 6.8) sodium acetate-methanol-tetrahydrofuran (81 : 18 : 1 v : v : v) and mobile phase B composed of 0.05 mM sodium acetate-methanol-tetrahydrofuran (22 : 77 : 1 v : v : v) at a flow-rate of 1 mL/min.

Determination of TNF-α and ICAM-1concentration

TNF-α levels in plasma and the conditioned medium were assayed by enzyme-linked immunosorbent assay (ELISA). The values were measured at 450 nm by a microplate reader (Biotek). The standard curve for TNF-α measured by this ELISA was linear from 16 to 250 pg/mL.

ICAM-1 levels in the conditioned medium were assayed by ELISA. The values were measured at 450 nm by a microplate reader (Biotek). The standard curve for ICAM-1 measured by this ELISA was linear from 31 to 2000 pg/mL.

Electrophoretic mobility shift assay

Endothelial cells were seeded into 25 cm^2 culture flasks at an optimal density of 10^5 cells/mL. Cells were washed with phosphate buffered saline (PBS) twice and incubated with 400 µl buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM,, EDTA, 0.1 mM ethylene glycol-bis(beta-aminoethyl ether)acetate (EGTA), 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF)) for 15 min on ice. Then 25 µL 10% NP-40 solution was added. After vortexing, cell lysates were centrifuged by 1400 *g* for 2 min at 4 °C and nuclei were resuspended in 50 µL of buffer B (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mMEGTA, 25% glycerine 1 mM DTT, 1 mM PMSF) and vigorously vortexed at 4 °C for 15 min. Nuclear lysates were next centrifuged at 1400 *g* for 5 min and the supernatant containing the nuclear proteins was carefully removed. Protein aliquots were either frozen at −70 °C or immediately used for electrophoretic mobility shift assay (EMSA) as described in Ref. 14.

The EMSA for determining the NF-κB DNA-binding activity was performed by incubating aliquots of nuclear extracts containing 15 µg total protein with γ-32^P-labelled double-stranded NF-κB specific oligonucleotide probe (sense: 3′-TCA ACT CCC CTG AAA GGG TCC G-5′; 5′-AGT TGA GGG GAC TTT CCC AGG C-3′) by T4 polynucleotide kinase. The labelled probe was purified through Sephadex G-25. After 10 min of incubation at room temperature, the mixture was run on a 4% non-denaturing polyacrylamide gel in 0.5 × Tris-Borate-EDTA (TBE) buffer. After electrophoresis, the gels were dried and the DNA-protein complexes were detected by autoradiography. Specificity was determined by the addition of an excess of unlabeled NF-κB oligonucleotide or unlabeled AP-2 oligonucleotide to the nuclear extracts before formation of DNA-protein complexes.

Reagents

STZ, ADMA, dimethyl sulfoxide (DMSO), phenylephrine and ACh were obtained from Sigma. Rosiglitazone was obtained from Glaxo Smith Kline company (Peking, China). DMEM was obtained from Gibco. Glucose kit was obtained from Baoding Changcheng Clinical Agent Co. (Hebei, China). Nitric oxide (NO) assay kits were obtained from Ju-Li Biological Medical Engineering Institute (Nanjing, China). TNF-α and ICAM-1 ELISA kits were obtained from Jingmei Biotech Co., Ltd. Gel shift assay system for determination of NF-κB activity was obtained from Promega.

Statistic analysis

Results are expressed as mean ± SEM. Data were analysed by ANOVA followed by the unpaired Student's *t*-test for multiple comparisons. The significance level was chosen as $p < 0.05$.

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Results

Effect of rosiglitazone on plasma levels of glucose and lipid in diabetic rats

The changes in plasma levels of glucose and lipid are shown in Table 1. Blood glucose level was increased in STZ-induced diabetic rats. Rosiglitazone treatment did not have any effect on plasma glucose level in diabetic rats. Plasma triglyceride level of diabetic rats showed a slight increase ($p > 0.05$). Rosiglitazone produced a slight decrease in the plasma level of triglyceride in diabetic rats ($p > 0.05$).

Effect of rosiglitazone on vasodilator responses to acetylcholine in diabetic rats

In the presence of phenylephrine, acetylcholine (3×10^{-9}–10^{-6} M) caused a concentration-dependent relaxation in the isolated rat aorta. Vasodilator responses to acetylcholine were significantly decreased in STZ-induced diabetic rats (Figure 1). Rosiglitazone (3, 10 or 30 mg/kg) significantly attenuated the impairment of vasodilator responses to ACh in diabetic rats.

Effect of rosiglitazone on plasma levels of nitrite/nitrate in diabetic rats

The plasma concentration of nitrite/nitrate was significantly increased in STZ-induced diabetic rats. Treatment with rosiglitazone (3, 10 or 30 mg/kg) had no effect on the increased concentration of nitrite/nitrate in STZ-induced diabetic rats (Figure 2(A)).

Effect of rosiglitazone on plasma levels of ADMA in diabetic rats

Plasma concentration of ADMA was significantly increased in STZ-induced diabetic rats. Rosiglitazone (3, 10 or

Table 1. Effect of rosiglitazone on plasma glucose and lipid

groups	*n*	Plasma glucose mM	HDL mM	TG mM	TC mM	LDL mM
CON	6	6.89 ± 0.80	0.54 ± 0.06	0.59 ± 0.09	1.28 ± 0.14	0.53 ± 0.05
DM	6	19.33 ± 0.81**	0.61 ± 0.03	0.87 ± 0.19	1.13 ± 0.13	0.49 ± 0.03
+RSG (L)	5	18.31 ± 0.84	0.56 ± 0.06	0.76 ± 0.23	1.20 ± 0.12	0.63 ± 0.03
+RSG (M)	5	19.43 ± 1.01	0.49 ± 0.04	0.51 ± 0.15	1.09 ± 0.05	0.71 ± 0.02
+RSG (H)	5	18.95 ± 0.91	0.70 ± 0.05	0.54 ± 0.06	1.12 ± 0.15	0.69 ± 0.03

CON, normal rats; DM, diabetic rats; +RSG (L), +RSG (M) and +RSG (H): rosiglitazone at the dose of 3, 10 or 30 mg/kg, respectively. Values are mean ± SEM ($n = 5$–6).
**$p < 0.01$ compared to CON.

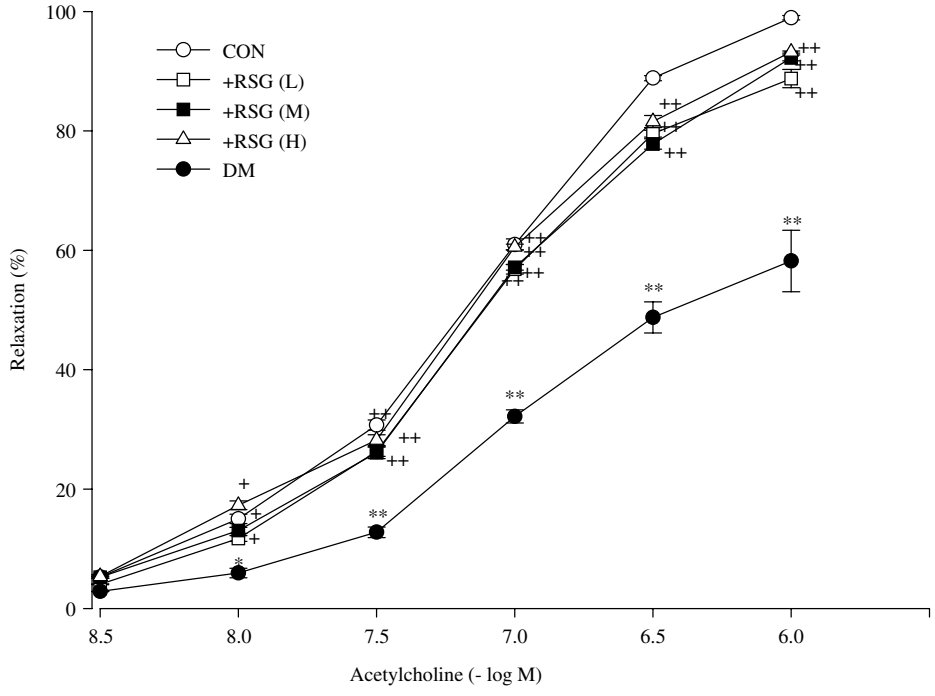

Figure 1. Effect of rosiglitazone on vasodilator responses to acetylcholine in diabetic rats. CON: normal rats; DM: diabetic rats; +RSG (L), +RSG (M) and +RSG (H): rosiglitazone at the dose of 3, 10 or 30 mg/kg respectively. Values are mean ± SEM ($n = 5$–6). *$p < 0.05$, **$p < 0.01$ compared with CON; $^{+}p < 0.05$, $^{++}p < 0.01$ compared with DM

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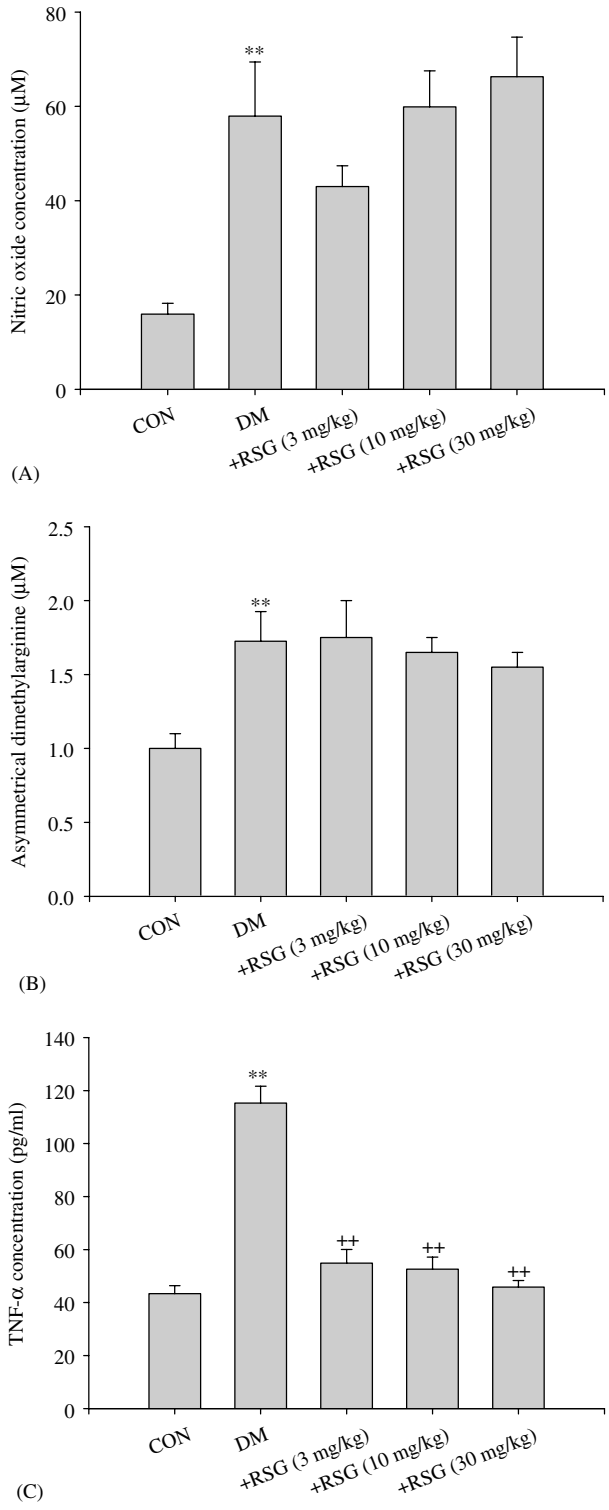

Figure 2. (A) Effect of rosiglitazone on the plasma level of nitrite/nitrate in diabetic rats. Values are mean ± SEM ($n = 5–6$). $^{**}p < 0.01$ compared with CON. (B) Effect of rosiglitazone on the plasma level of ADMA in diabetic rats. Values are mean ± SEM ($n = 5–6$). $^{**}p < 0.01$ compared with CON. (C) Effect of rosiglitazone on the plasma level of TNF-α in diabetic rats. Values are mean ± SEM ($n = 5–6$). $^{**}p < 0.01$ compared with CON; $^{++}p < 0.01$ compared with DM

30 mg/kg) treatment had no effect on the increased concentration of ADMA in STZ-induced diabetic rats (Figure 2(B)).

Effect of rosiglitazone on plasma levels of TNF-α in diabetic rats

Plasma concentrations of TNF-α were significantly increased in STZ-induced diabetic rats. Rosiglitazone (3, 10 or 30 mg/kg) significantly inhibited the elevated concentration of TNF-α in STZ-induced diabetic rats (Figure 2(C)).

Effects of ADMA and rosiglitazone on the content of ICAM-1 in the conditioned medium of endothelial cell

Incubation of endothelial cells with ADMA (10, 30 μM) for 48 h caused a significant increase in the level of ICAM-1 (Figure 3(A) and (B)). Treatment with ADMA at the dose of 30 μM for 48 h caused a significant increase in the concentration of ICAM-1. Rosiglitazone (10 or 30 μM) significantly inhibited the elevated concentration of TNF-α by ADMA (30 μM). However, rosiglitazone itself had no effect on concentration of ICAM-1 (Figure 4(A)).

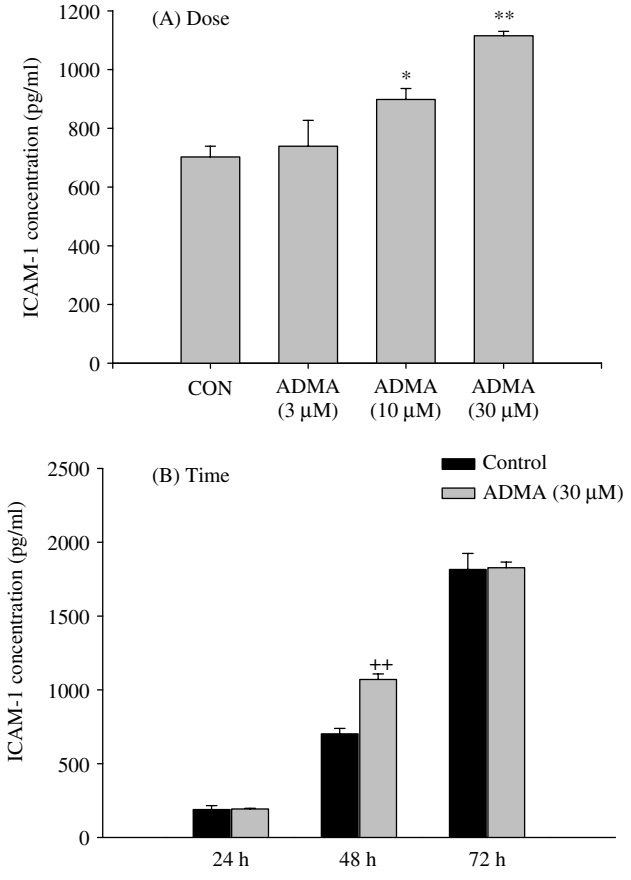

Figure 3. Effects of ADMA on the content of ICAM-1 in the conditioned medium of endothelial cells. Dose-effect reaction of ADMA (A) endothelial cells were treated with ADMA at the dose of 3, 10 or 30 μM for 48 h. Values are mean ± SEM ($n = 4$). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with CON; time-effect reaction of ADMA (B) endothelial cells were treated with ADMA at the dose of 30 μM for 24, 48 or 72 h. Values are mean ± SEM ($n = 4$). $^{++}p < 0.01$ compared with CON at corresponding time

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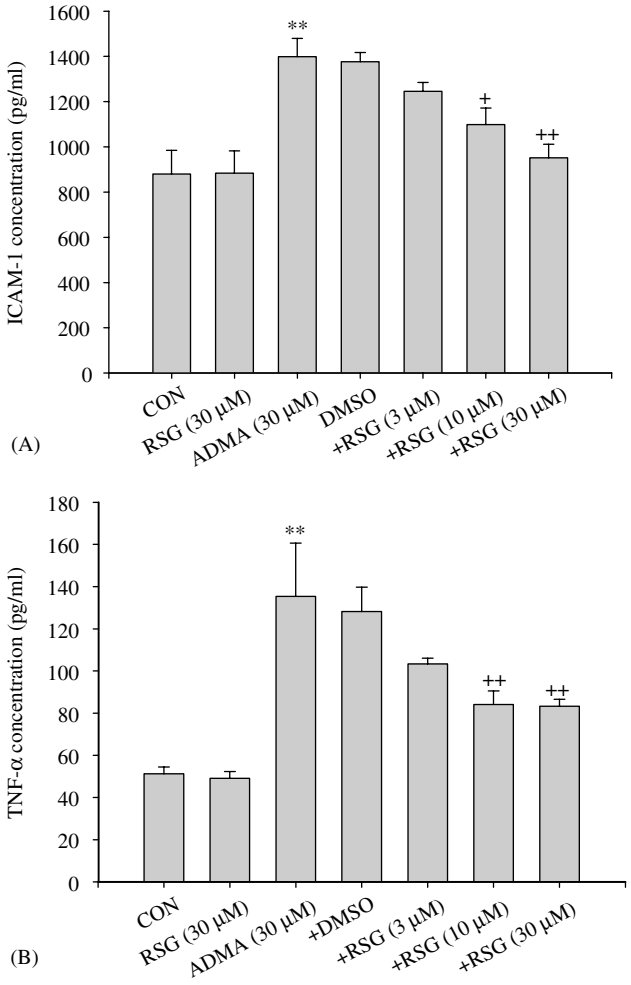

Figure 4. (A) Effects of rosiglitazone on the content of ICAM-1 in the conditioned medium of endothelial cells. Endothelial cells were treated with ADMA at the dose of 30 μM for 48 h. Values are mean ± SEM ($n = 4$). $^{**}p < 0.01$ compared with CON; $^{+}p < 0.01$, $^{++}p < 0.01$ compared to ADMA. (B) Effects of rosiglitazone on the content of TNF-α in the conditioned medium of endothelial cells. Endothelial cells were treated with ADMA at the dose of 30 μM for 48 h. Values are mean ± SEM ($n = 4$). $^{**}p < 0.01$ compared with CON; $^{++}p < 0.01$ compared to ADMA

Effects of rosiglitazone on the content of TNF-α in the conditioned medium of endothelial cell

Treatment with ADMA at the dose of 30 μM for 48 h caused a significant increase in concentration of TNF-α in cultured endothelial cells. Rosiglitazone (10 or 30 μM) significantly inhibited the elevated concentration of TNF-α by ADMA (30 μM). However, rosiglitazone itself had no effect on the concentration of TNF-α (Figure 4(B)).

Effect of rosiglitazone on NF-κB activity of endothelial cell

NF-κB was significantly activated in cultured endothelial cells treated with ADMA (30 μM) for 48 h. Rosiglitazone

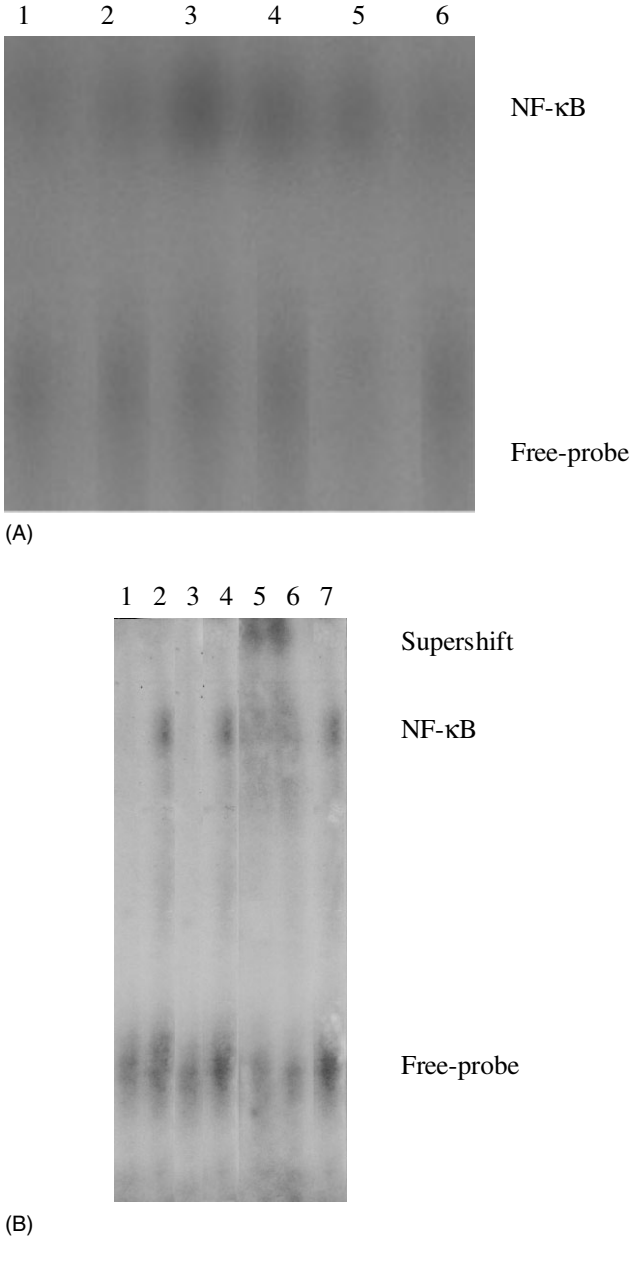

Figure 5. (A) Effect of rosiglitazone on NF-κB activity in endothelial cells. 1: control; 2: rosiglitazone (30 μM); 3: ADMA (30 μM); 4: ADMA+ rosiglitazone(3 μM); 5: ADMA+ rosiglitazone (10 μM) and 6: ADMA+ rosiglitazone (30 μM). (B) Results of supershift and competitive EMSA assay of ADMA-induced NF-κB activity in endothelial cells. Endothelial cells were incubated with 30 μM of ADMA for 48 h. l: nuclear extract was incubated with unlabeled NF-κB probe; 2: nuclear extract was incubated with γ-^{32}PATP labelled NF-κB probe; 3: nuclear extract was incubated with 100-fold molar excess of unlabeled NF-κB probe; 4: nuclear extract was incubated with 100-fold molar excess of unlabeled AP_2 probe; 5: nuclear extract was incubated with Ab against the NF-κB subunits P65/RelA; 6: nuclear extract was incubated with Ab against the NF-κB subunits P50 and 7: nuclear extract was incubated with Ab against c-Rel

(3, 10 or 30 μM) significantly inhibited the increased activity of NF-κB by ADMA (Figure 5(A)).

The specificity of the shifted bands in EMSA was verified by a competition assay. All the shifted bands

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were suppressed by incubation with 100-fold excess of unlabeled NF-κB probe and were unchanged by competition with a similar amount of another irrelevant AP2 oligonucleotide (Figure 5(B)). These results support that rosiglitazone treatment resulted in an alteration in ADMA-induced NF-κB activation in HUVECs.

To confirm that the protein in the protein – DNA complexes found in the nuclear extracts from ADMA stimulated endothelial cells was NF-κB, the antibodies against the p65 and p50 subunits of NF-κB were used for supershift analysis (Figure 5(B)). Incubation with the anti-p65 antibody or anti-p50 antibody resulted in an almost complete supershift of the protein – DNA complexes with the HUVECs. This indicates that the NF-κB dimers in the ADMA stimulated HUVECs contained the p65 and p50 subunits.

Discussion

Diabetes mellitus has an increased prevalence of cardiovascular diseases, which have become the primary cause of morbidity and mortality in patients with diabetes mellitus. High glucose, high lipid, insulin resistance and a number of inflammatory cytokines contribute to endothelial dysfunction in diabetes mellitus, and endothelial dysfunction may be implicated in the pathogenesis of diabetic vascular complications.

NO, an important local regulatory factor in cardiovascular tissues, is synthesized from L-arginine by NOS in endothelial cells. NO possesses complex cardiovascular actions including regulation of vascular smooth muscle tone, protecting endothelial cells and inhibiting vascular smooth muscle cell proliferation [15]. NOS includes two constitutive isoforms of cNOS, endothelial NOS and neuronal NOS, and an inducible isoform (inhibiting nitric oxide synthase (iNOS)) [16]. Under pathological condition, the expression of iNOS is increased greatly and a large amount of NO produced. Excessive NO forms NO_2, N_2O_3 and N_2O_4, which are harmful to the human body. L-arginine analogues such as ADMA, which is present in blood of both humans and animals, can inhibit NOS *in vivo* and *in vitro* [17,18]. There is substantial evidence that the elevated level of ADMA was associated with impairment of endothelium-dependent vasodilatation in animals and individuals with diabetes [3,19,20]. These results suggest that ADMA may be a risk factor for endothelial dysfunction in diabetes mellitus.

Rosiglitazone, widely used in the treatment of type 2 diabetes with insulin resistance, can improve insulin resistance at the level of insulin receptor [21], and glucose and lipid metabolism by activating PPAR gamma. Rosiglitazone therapy for insulin resistance ameliorated endothelial dysfunction independent of glucose control in type 2 diabetes [7]. In the present study, treatment with rosiglitazone also markedly improved endothelial function, but had no effect on blood glucose concentration in STZ-induced diabetic rats. It is known that rosiglitazone reduces plasma glucose level by increasing insulin sensitivity. To rule out that the beneficial effects of rosiglitazone on the vascular endothelium result from improving glucose metabolism, we used the model of STZ-induced diabetic rats, which exists in insulin-deficient rather than insulin-resistant. The results of the present study also revealed that treatment with rosiglitazone did not change the plasma level of lipid parameters, suggesting that beneficial effects of rosiglitazone on the vascular endothelium were not because of improving lipid metabolism in STZ-induced diabetic rats.

Chronic insulin treatment not only prevented the elevation of serum ADMA, but also improved the impairment of endothelium-dependent relaxation in STZ-induced diabetic rats [3]. A positive correlation exists between insulin resistance and plasma concentrations of ADMA, and pharmacological intervention with rosiglitazone enhanced insulin sensitivity and reduced ADMA levels in insulin-resistant patients [8]. On the basis of the inhibitory effects of ADMA on NOS activity, we considered the possibility that the beneficial effect of rosiglitazone on the endothelium was because of a reduction of ADMA level was considered. However, the present results revealed that rosiglitazone markedly improved endothelial function without decreasing plasma levels of ADMA in STZ-diabetic rats. As has been previously reported [22], in the present study the plasma level of NO was increased. The increased level of NO was associated with the increased activity of iNOS in early STZ-induced diabetic rats [23]. In the present study, rosiglitazone did not affect the increased level of NO in STZ-induced diabetic rats. These results do not support the hypothesis that rosiglitazone improves endothelial function by the reduction of endogenous ADMA level.

A great deal of information has demonstrated that inflammatory mediators play a role in the development of diabetes mellitus. TNF-α, ICAM-1 and CRP levels are elevated in diabetes mellitus [24–27], and the elevated level of CRP and IL-6 predicts the development of type 2 diabetes mellitus [28]. More recently, it was reported that pre-incubation of ADMA with endothelial cell markedly increased NF-κB activity and MCP-1 expression, and potentiated the adhesiveness of endothelial cells [4,5]. Clinical studies have also shown that the level of ADMA and CRP and TNF-α content were strongly interrelated [6]. These findings suggest that ADMA, beside inhibiting NOS, may be a new inflammatory factor and plays an important role in the development of endothelial dysfunction in diabetic rats. The present results revealed that the serum concentration of TNF-α was significantly increased, concomitantly with increased level of ADMA in STZ-diabetic rats, and incubation of endothelial cells with ADMA caused a significant increase in the level of TNF-α. These results suggest that ADMA may directly damage endothelial cells by inducing inflammatory reaction.

Rosiglitazone is a PPAR gamma agonist. PPAR gamma can control the expression of genes implicated in the inflammatory response via negative interference with different inflammatory pathways, such as NF-κB, AP-1,

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STAT-1 and NFAT [9]. PPAR gamma activators inhibited expression of VCAM-1 and ICAM-1, which reduce monocyte/macrophage homing to atherosclerotic plaques in activated endothelial cells [10]. Rosiglitazone reduced levels of MCP-1, matrix metalloproteinase-9 (MMP-9) and the pro-inflammatory marker CRP in patients with type 2 diabetes [29,30]. The present results revealed that the serum concentration of TNF-α was significantly increased, which was significantly inhibited by rosiglitazone in STZ-diabetic rats. To further test whether the protective effect of rosiglitazone on endothelium is because of inhibition of ADMA-induced inflammatory reaction, the effect of rosiglitazone on NF-κB activity and the levels of ICAM-1 and TNF-α were examined in HUVECs treated with ADMA. The results revealed that pre-treatment with rosiglitazone markedly inhibited the increased activity of NF-κB and the increased levels of TNF-α and ICAM-1 induced by ADMA. These results suggest that the inhibitory effect of rosiglitazone on ADMA-induced inflammatory reaction involved the NF-κB pathway in endothelial cells.

In summary, the present study suggests that rosiglitazone improves endothelial function in streptozotocin-induced diabetic rats, and the protective effects of rosiglitazone on the endothelium are related to reduction of inflammatory reaction induced by ADMA.

Acknowledgements

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