



## Pioglitazone abrogates cyclosporine-evoked hypertension via rectifying abnormalities in vascular endothelial function

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### ABSTRACT

In addition to insulin sensitization, the thiazolidenedione drug pioglitazone exhibits favorable circulatory effects. Here, we hypothesized that pioglitazone protects against the hypertension and related vascular derangements caused by the immunosuppressant drug cyclosporine (CSA). Compared with vehicle (olive oil)-treated rats, chronic treatment with CSA (20 mg/kg/day s.c., for 14 days) increased blood pressure (BP), reduced the aortic protein expression of phosphorylated eNOS (p-eNOS), and impaired responsiveness of isolated aortas to endothelium-dependent vasorelaxations induced by carbachol. The effects of CSA on BP, aortic p-eNOS, and carbachol relaxations were abolished upon concurrent administration of pioglitazone (2.5 mg/kg/day). Serum levels of adiponectin, an adipose tissue-derived adipokine, were not altered by CSA but showed significant elevations in rats treated with pioglitazone or pioglitazone plus CSA. The possibility that alterations in the antioxidant and/or lipid profile contributed to the CSA-pioglitazone BP interaction was investigated. Pioglitazone abrogated the oxidative (aortic superoxide dismutase), lipid peroxidation (aortic malondialdehyde), and dyslipidemic (serum LDL levels and LDL/HDL ratio) effects of CSA. Histologically, CSA caused focal disruption in the endothelial lining of the aorta and this effect disappeared in rats co-treated with pioglitazone. Collectively, pioglitazone abrogates the hypertensive effect of CSA via ameliorating detrimental changes in vascular endothelial NOS/NO pathway and oxidative and lipid profiles caused by CSA.

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### 1. Introduction

Hypertension is one of the most troublesome effects often associated with the use of the immunosuppressant drug cyclosporine. Approximately 80% of patients treated with CSA after kidney transplantation develop hypertension [1]. Also, CSA causes hypertension in 70% of liver transplant patients and almost 100% of cardiac transplant recipients [2,3]. This clinical problem has been replicated in experimental animals in reported studies including ours [4,5]. Possible contributing factors include increased sympathetic [4], endothelin [6] and angiotensin activities [5], vascular endothelium dysfunction [7], interference with the testosterone-mediated vascular control [8,9], and deterioration of the cellular antioxidant profile [10,11].

Pioglitazone, like other thiazolidenedione drugs (TZD), reduces blood glucose level through improving insulin resistance. TZD produce their effect via binding to peroxisome proliferator-activated gamma receptors (PPAR $\gamma$ ), a nuclear receptor that

modulates among several other functions the transcription of genes essential for glucose transport, lipid homeostasis and metabolism, and arterial inflammation and atherogenesis [12]. In addition to their insulin sensitizing action, TZD have beneficial circulatory effects such as: (i) facilitation of endothelium-dependent vasodilation [13], (ii) inhibition of voltage-dependent calcium channels [14], (iii) inhibition of cell proliferation and migration, which precedes the development of vascular lesions [15], (iv) improving the antioxidant profile [13], and (v) increasing the expression and plasma levels of adiponectin [16]. The latter is a hormone derived from adipose tissue with protective effects against hypertension [17], endothelium dysfunction [18], and oxidative stress [19].

Despite their favorable circulatory effects, it is not known whether TZDs can abrogate the hypertensive and vasculotoxic effects of CSA. This interesting possibility was investigated in the current study at the integrative (blood pressure) and in vitro (vascular reactivity) levels in rats that received chronic treatment of CSA, pioglitazone or their combination. The effect of either drug or their combination on endogenous mediators and ameliorators of endothelial dysfunction was investigated to gain insight into the molecular mechanisms that underlie their functional interaction.

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## 2. Materials and methods

Male Wistar rats (200–240 g, Faculty of Pharmacy, University of Alexandria, Egypt or Charles River, Raleigh, NC, USA) were used in the present study. Experiments were performed in strict accordance with institutional guidelines.

### 2.1. Intravascular cannulation

The method described in our previous studies [4,8] for intravascular cannulation and BP measurement in rats was adopted. Briefly, rats were anesthetized with thiopental (50 mg/kg i.p.). Catheters were placed into the abdominal aorta and vena cava via the left femoral vessels for measurement of arterial pressure and intravenous injections, respectively.

### 2.2. Rat isolated aortic ring preparations

Isolation of the rat aorta and recording of isometric contraction were performed as described in our previous studies [20,21]. Rats were euthanized with an overdose of thiopental sodium (100 mg/kg) and thoracic aortas were removed, trimmed free of connective tissues and cut into ring segments 3 mm in length. Aortic rings were mounted in 10 ml organ baths containing physiological solution at 37 °C and aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The physiological solution was composed of the following (in mM): NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, and glucose 11.1. Aortic rings were mounted in organ baths by means of two stainless steel wire hooks inserted through the lumen of the ring. One of the hooks was anchored to a stationary pin at the bottom of the organ bath and the other was connected to an isometric force-displacement transducer (Grass FT-03C) which was connected to a Grass polygraph (Model 7D) for recording isometric contractions of the aorta. An optimum resting tension of 1.5 g was placed on the tissue and an equilibration period of 1 h was allowed before the start of the experiment, with the bath fluid being replaced every 15 min. To study aortic responsiveness to vasorelaxants, aortas were precontracted with the  $\alpha_1$ -adrenoceptor agonist phenylephrine. To acclimatize the preparation, phenylephrine (1  $\mu$ M) was added to the organ bath on two separate occasions during the 1 h equilibration [20].

### 2.3. Western blotting

For the determination of total and phosphorylated aortic eNOS protein levels, the rat aorta was homogenized on ice in a homogenization buffer [50 mM Tris (pH 7.5), 0.1 mM EGTA, 0.1 mM EDTA, 2  $\mu$ M leupeptin, 1 mM phenylmethylsulfonyl fluoride, 0.1% (vol/vol) Nonidet P-40, 0.1% SDS, and 0.1% deoxycholate]. After centrifugation (12,000 g for 10 min), protein in the supernatant was quantified (Bio-Rad protein assay system; Bio-Rad, Hercules, CA). Protein extracts (50  $\mu$ g per lane) were run on a 4–12% SDS-polyacrylamide gel electrophoresis gel (Invitrogen, Carlsbad, CA) and electroblotted to nitrocellulose membranes. Blots were blocked for 120 min at room temperature in tris-buffered saline/tween20 (TBS/T) buffer (100 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween 20) containing 5% non-fat milk. They were then incubated overnight at 4 °C with mouse antibody to total eNOS (1:2,500; BD Biosciences, San Jose, CA) or rabbit antibody to phospho-eNOS(Ser1177) (1:500, Cell Signaling Technology<sup>®</sup>, Inc. Danvers, MA) in TBS/T buffer containing 5% BSA. After 3 washes with TBS/T buffer, the blots were incubated for 60 min at room temperature with the appropriate horseradish peroxidase-linked species-specific anti-IgG (1:2,000, GE Healthcare BIO-Sciences Corp, Piscataway, NJ). After 3 washes with the TBS/T buffer, the blots were detected by enhanced chemiluminescence

system and exposed to an X-ray film. Equivalent sample loading was confirmed by stripping membranes with blot restore membrane rejuvenation solution (SignaGen Laboratories, Gaithersburg, MD) and reprobing with anti-actin antibody (Sigma). Proteins bands were quantified by measuring the integrated density (mean density  $\times$  area) using the NIH Image software (version 1.62); data was normalized in relation to actin, and expressed as a percent of control (olive oil) as in our previous studies [22,23].

### 2.4. Protocols and experimental groups

#### 2.4.1. Effect of pioglitazone on CSA-evoked hypertension

Four groups of conscious rats ( $n = 5$ –6 each) pre-instrumented for BP measurement were employed in this study to determine the effect of CSA on BP in absence or presence of pioglitazone. Rats were allocated to receive one of the following chronic treatments: (i) olive oil, (ii) CSA, (iii) pioglitazone, or (iv) CSA plus pioglitazone. Cyclosporine (20 mg/kg, dissolved in olive oil) or equal volume of the vehicle was injected s.c. in single daily doses for 14 consecutive days. This dose of cyclosporine has been shown to elevate BP and impair vasorelaxant activity [4,9,24]. Pioglitazone was mixed with food and served to rats in a dose of 2.5 mg/kg/day for 14 days. Rats were instrumented for BP measurement 2 days before starting drug treatments. BP was measured right before starting drug/vehicle treatment (day 0) and 3, 5, 8, 10, 12, and 14 days after the commencement of drug/vehicle administration. For BP measurement on any given day, the arterial catheter was connected to a Gould–Statham pressure transducer (Oxnard, CA, U.S.A.) and BP was displayed on a PowerLab system (ADInstruments, Inc., Colorado, USA). A period of at least 30 min was allowed at the beginning of each experiment for BP and heart rate stabilization. The blood and vasculature collected from these animals was used for the biochemical (serum adiponectin) and Western blot studies.

Two additional groups of rats ( $n = 6$ –7 each) were employed to investigate the effect of captopril, an angiotensin converting enzyme inhibitor, on the hypertensive effect of CSA. Rats were treated with captopril alone (10 mg/kg/day i.p.) or combined with CSA (20 mg/kg/day) for 14 consecutive days.

#### 2.4.2. CSA-pioglitazone interaction on aortic vasorelaxations

Another four groups of rats ( $n = 7$ –8) were used to evaluate the effects of pioglitazone on the CSA-induced alterations in aortic responsiveness to endothelium-dependent and independent relaxations and aortic eNOS protein expression. As described in the preceding experiment, rats received olive oil, CSA, pioglitazone, or CSA plus pioglitazone. On the day of the experiment, rats were sacrificed, thoracic aortic rings were prepared as described earlier. Two cumulative concentration-response curves to stepwise cumulative addition of carbachol ( $3 \times 10^{-8}$  to  $3 \times 10^{-4}$  M) or SNP ( $1 \times 10^{-10}$  to  $1 \times 10^{-5}$  M) were established in aortic rings precontracted with phenylephrine (1  $\mu$ M) as described in our previous studies [20,25]. Each new addition was made after the response to the previous addition had attained a steady state. A wash period of 60 min was allowed after the first curve to help the muscle relax to baseline tension. The aorta was then re-contracted with phenylephrine (1  $\mu$ M) to permit construction of the SNP concentration-response curve.

#### 2.4.3. CSA-pioglitazone redox, adiponectin and lipid interactions

Four groups of rats ( $n = 6$ –7 each) were utilized and received similar treatments as described under section 2.4.2. After 14 days of chronic treatment, overnight-fasted rats were sacrificed and aortas were quickly removed, washed in ice-cold saline, blotted dry, weighed and homogenized in ice-cold saline (Kinematica GmbH PT 45180, Lucerne, Switzerland). Tissues homogenates were

divided into portions and stored at  $-20^{\circ}\text{C}$  till used for the determination of TBARS [26] and SOD [27]. Blood samples were collected and spun at 1200 g for 10 min and serum was aspirated, divided into aliquots and stored at  $-20^{\circ}\text{C}$  till analyzed. Serum samples were also prepared and used for the determination of triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and glucose using the Randox<sup>®</sup> assay kit (Randox Laboratories Ltd., United Kingdom). The total lipids, low density lipoprotein cholesterol (LDL-C), LDL/HDL ratio were computed. Serum adiponectin was measured by Adiponectin (rat) ELISA (ALPCO Immunoassays, Salem, NH, USA). For histopathological examination, cross sections of the aorta were fixed in 10% formaldehyde, stained by Haematoxylin and Eosin stain, and examined under a light microscope.

## 2.5. Drugs

Carbachol (carbamoylcholine chloride), sodium nitroprusside (Sigma Chemical Co., St. Louis, MO, U.S.A.), thiopental (Thiopental, Biochemie GmbH, Vienna, Austria), povidone-iodine solution (Betadine, Nile Pharmaceutical Co., Cairo, Egypt) and Penicid (Cid Pharmaceutical Co., Cairo, Egypt) were purchased from commercial vendors. Cyclosporine, pioglitazone hydrochloride, and captopril were gifts from Novartis Pharma, AG (Basel, Switzerland), Takeda Pharmaceutical Company Ltd (Osaka, Japan), and PHARCO Pharmaceutical Co. (Alexandria, Egypt), respectively.

## 2.6. Data analysis and statistics

Values are expressed as means  $\pm$  S.E.M. Mean arterial pressure (MAP) was calculated as diastolic pressure + one third pulse pressure (systolic–diastolic pressures). Nonlinear regression was used to fit sigmoidal curves to individual concentration–response curves to determine agonist potency ( $\text{EC}_{50}$ ), the dose of carbachol or SNP giving half the maximum vasodilation, and maximum effect ( $E_{\text{max}}$ ). The analysis of variance (ANOVA) followed by a Newman–Keuls post-hoc analysis was used for multiple comparisons with the level of significance set at  $P < 0.05$ .

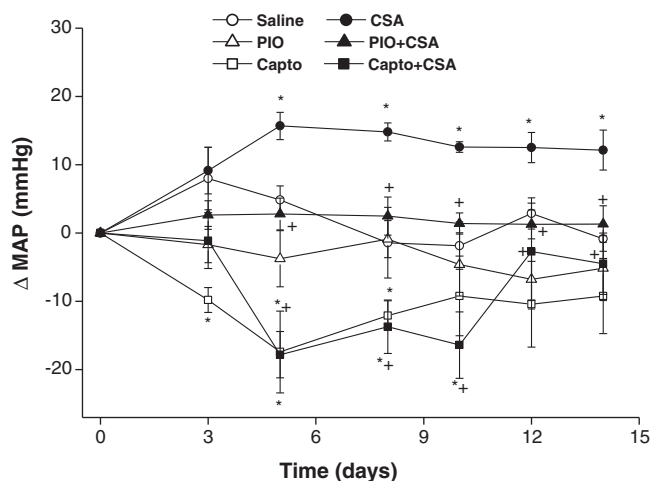
## 3. Results

### 3.1. Effect of pioglitazone on CSA-evoked hypertension

Baseline MAP values in rats subsequently receiving vehicle, pioglitazone, captopril, CSA, pioglitazone plus CSA, or captopril plus CSA were not statistically different ( $107 \pm 6$ ,  $106 \pm 5$ ,  $102 \pm 4$ ,  $103 \pm 4$ ,  $105 \pm 4$ , and  $102 \pm 3$  mm Hg, respectively). Chronic treatment of rats with CSA (20 mg/kg/day for 14 days) resulted in significant increases in MAP compared with vehicle-treated animals (Fig. 1). The hypertensive effect of CSA appeared 5 days after starting CSA regimen and continued through the remaining period of the study (Fig. 1). Concurrent treatment with pioglitazone (2.5 mg/kg/day) or captopril (10 mg/kg/day) for 14 days virtually abolished CSA-induced hypertension (Fig. 1). While treatment with pioglitazone alone had no effect on MAP, captopril caused reductions in MAP that were statistically significant compared with control values during the first 10 days of the study (Fig. 1).

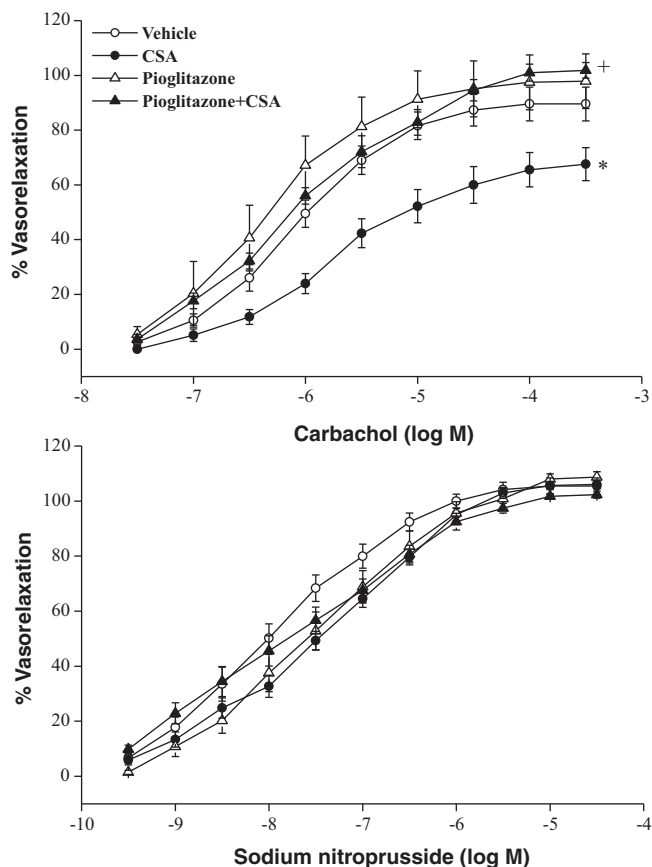
### 3.2. Effect of CSA–pioglitazone combination on aortic vasorelaxation and p-eNOS content

Changes evoked by chronic treatment with CSA, pioglitazone, or their combination on vasorelaxant responses of the aorta to carbachol or SNP are shown in Figs. 2 and 3. The cumulative addition of carbachol ( $3 \times 10^{-8}$  to  $3 \times 10^{-4}$  M) or SNP ( $1 \times 10^{-10}$  to  $1 \times 10^{-5}$  M) resulted in concentration-related relaxations of

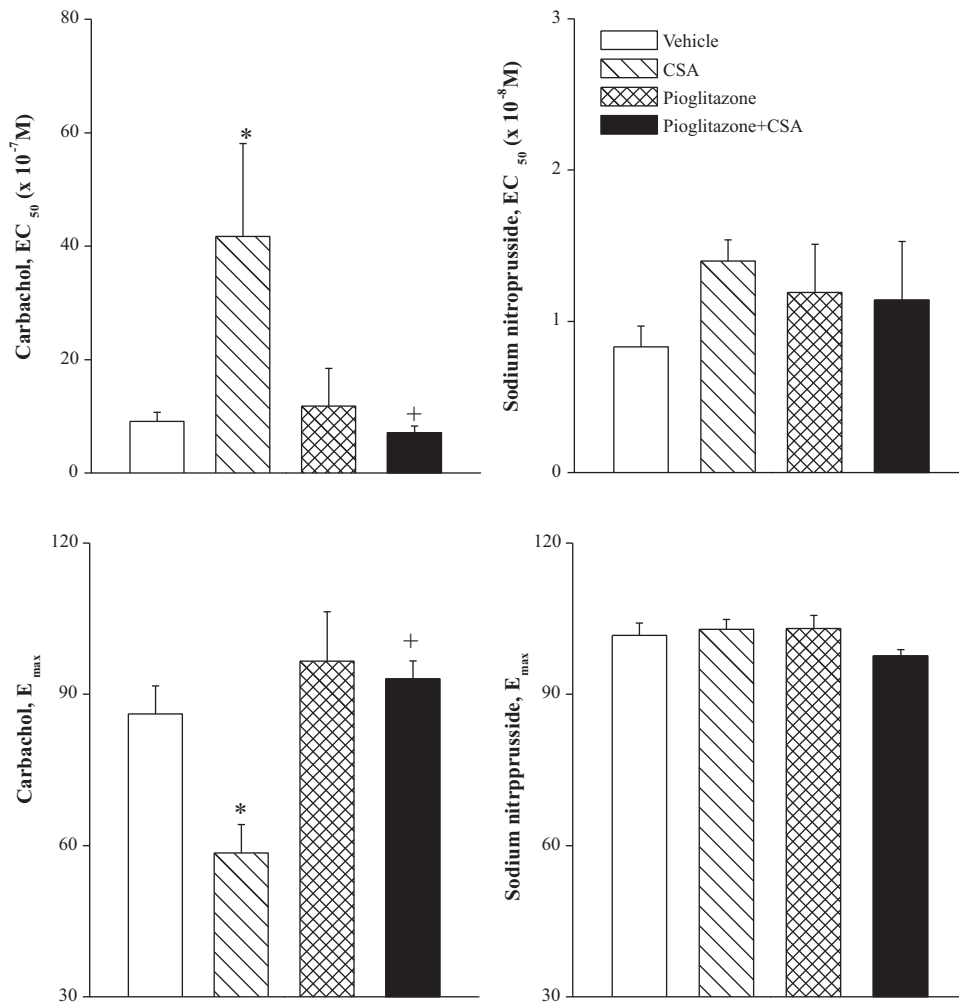


**Fig. 1.** Changes in mean arterial pressure (MAP) of conscious rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (PIO, 2.5 mg/kg/day), captopril (Capto, 10 mg/kg/day), or their combination for 14 days. Values are means  $\pm$  S.E.M. of 5–7 observations. \* $P < 0.05$  compared with vehicle (olive oil) values, + $P < 0.05$  compared with CSA values.

phenylephrine ( $1 \mu\text{M}$ ) precontracted aortic rings (Fig. 2). Compared with corresponding vehicle (olive oil) values, chronic treatment with CSA (20 mg/kg/day for 14 days) caused significant reductions in the vasorelaxant responses to carbachol (Fig. 2). The  $\text{EC}_{50}$  ( $41 \times 10^{-7} \pm 16 \times 10^{-7}$  vs.  $9 \times 10^{-7} \pm 2 \times 10^{-7}$  M) and  $E_{\text{max}}$  ( $58 \pm 6$  vs.  $86 \pm 5\%$ ) of carbachol in aortic preparations of CSA-treated



**Fig. 2.** Vasorelaxant effects of carbachol or sodium nitroprusside in phenylephrine ( $1 \mu\text{M}$ )–precontracted aortic rings obtained from rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (2.5 mg/kg/day) or their combination for 14 days. Values are means  $\pm$  S.E.M. of 7–8 observations. \* $P < 0.05$  compared with vehicle (olive oil) values, + $P < 0.05$  compared with CSA values.



**Fig. 3.** The vasorelaxant potency ( $EC_{50}$ ) and maximum effect ( $E_{max}$ ) of carbachol or sodium nitroprusside in phenylephrine ( $1 \mu M$ )-precontracted aortic rings obtained from rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (2.5 mg/kg/day) or their combination for 14 days. Values are means  $\pm$  S.E.M. of 7–8 observations. \* $P < 0.05$  compared with vehicle (olive oil) values, † $P < 0.05$  compared with CSA values.

rats were significantly higher and lower, respectively, than those of control values. The CSA-evoked attenuation of carbachol responses disappeared in preparations treated simultaneously with pioglitazone (2.5 mg/kg/day, Fig. 2). Also, the concurrent exposure to CSA and pioglitazone restored the  $EC_{50}$  and  $E_{max}$  values of carbachol to near-control values (Fig. 3). In preparations treated with pioglitazone alone, the relaxant responses (Fig. 2) and  $EC_{50}$  and  $E_{max}$  values (Fig. 3) of carbachol were not statistically different from control values. Unlike carbachol, the relaxant responses elicited by SNP were not affected by CSA, pioglitazone, or their combination (Figs. 2 and 3).

Western blotting showed that compared with vehicle values, 2-week CSA treatment significantly decreased phosphorylated eNOS expression in aortic tissues whereas total eNOS remained unchanged (Figs. 4 and 5). The decrease in p-eNOS expression caused by CSA was abrogated in rats treated concurrently with pioglitazone (Figs. 4 and 5).

### 3.3. CSA-pioglitazone interaction on adiponectin, SOD and lipid profiles

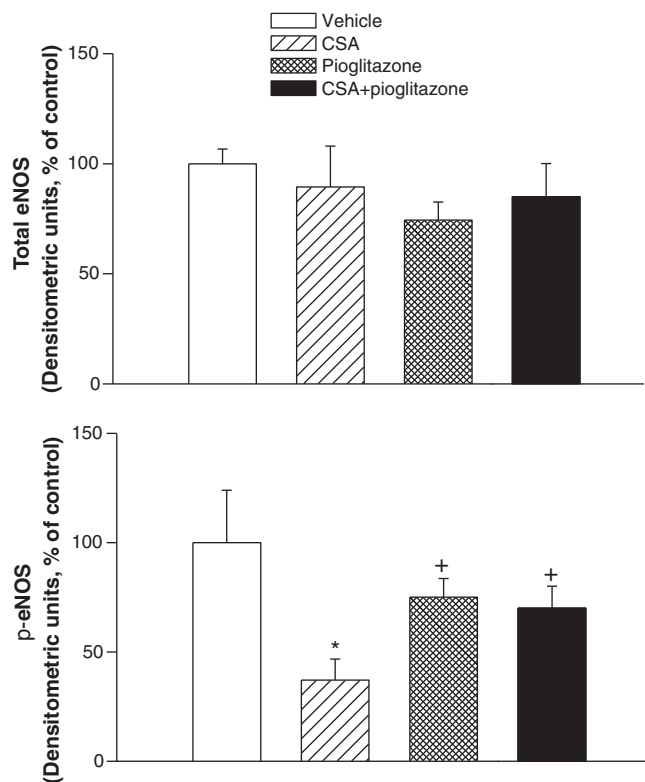
Figs. 6 and 7 illustrate changes caused by different drug treatments in serum adiponectin, aortic SOD and lipid profiles. Compared with vehicle-treated rats, serum adiponectin was not altered in rats treated with CSA but showed significant increases in rats treated with pioglitazone or pioglitazone plus CSA (Fig. 6A).

CSA caused significant reduction in aortic SOD activity (Fig. 6B) and increase in MDA (Fig. 6C). Also, serum LDL (Fig. 7E) and LDL/HDL ratio (Fig. 7G) were increased by CSA treatment. On the other hand, serum total lipids, total cholesterol, triglycerides, HDL, VLDL, and glucose were not affected by CSA (Fig. 7). The deleterious effects of CSA on the antioxidant and lipid profiles disappeared upon concurrent administration of pioglitazone (Figs. 6 and 7). Histological examination of the aorta revealed that chronic exposure to CSA was associated with focal disruption in the endothelial lining of the aorta (Fig. 8). This effect of CSA was virtually abolished in rats treated simultaneously with pioglitazone (Fig. 8). No apparent histological changes were demonstrated in smooth muscle and connective tissue layers in the aortas of different rat preparations (Fig. 8).

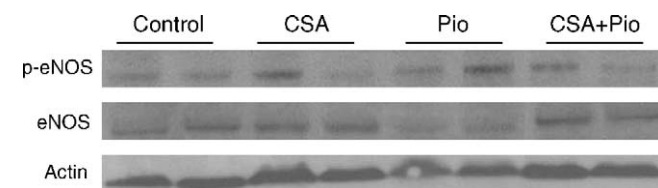
## 4. Discussion

The current study is the first to report on the potential protective effect of the insulin sensitizing drug pioglitazone against the hypertensive and deleterious vascular effects of chronic CSA. Concurrent pioglitazone administration virtually abolished the CSA-induced hypertensive response. The following findings explain, at least partly, pioglitazone counteraction of CSA-induced hypertension: (i) pioglitazone ameliorated the functional (impairment of endothelium-dependent relaxations), protein





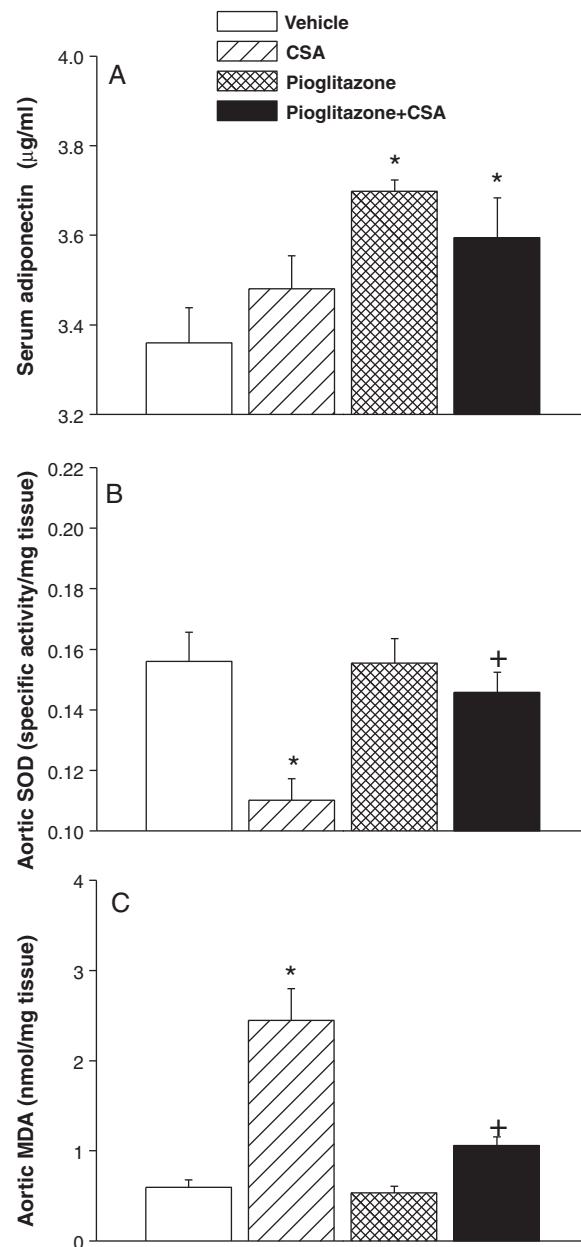
**Fig. 4.** The protein expression of total or phosphorylated eNOS (p-eNOS) in aortic tissues of Wistar rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (2.5 mg/kg/day) or their combination for 14 days. Values are means  $\pm$  S.E.M of 6–7 observations. \* $P$  < 0.05 compared with vehicle (olive oil) values, † $P$  < 0.05 compared with CSA values.



**Fig. 5.** Illustrative gels depicting total or phosphorylated eNOS (p-eNOS) expression in homogenates of aortic tissues of Wistar rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (2.5 mg/kg/day) or their combination for 14 days.

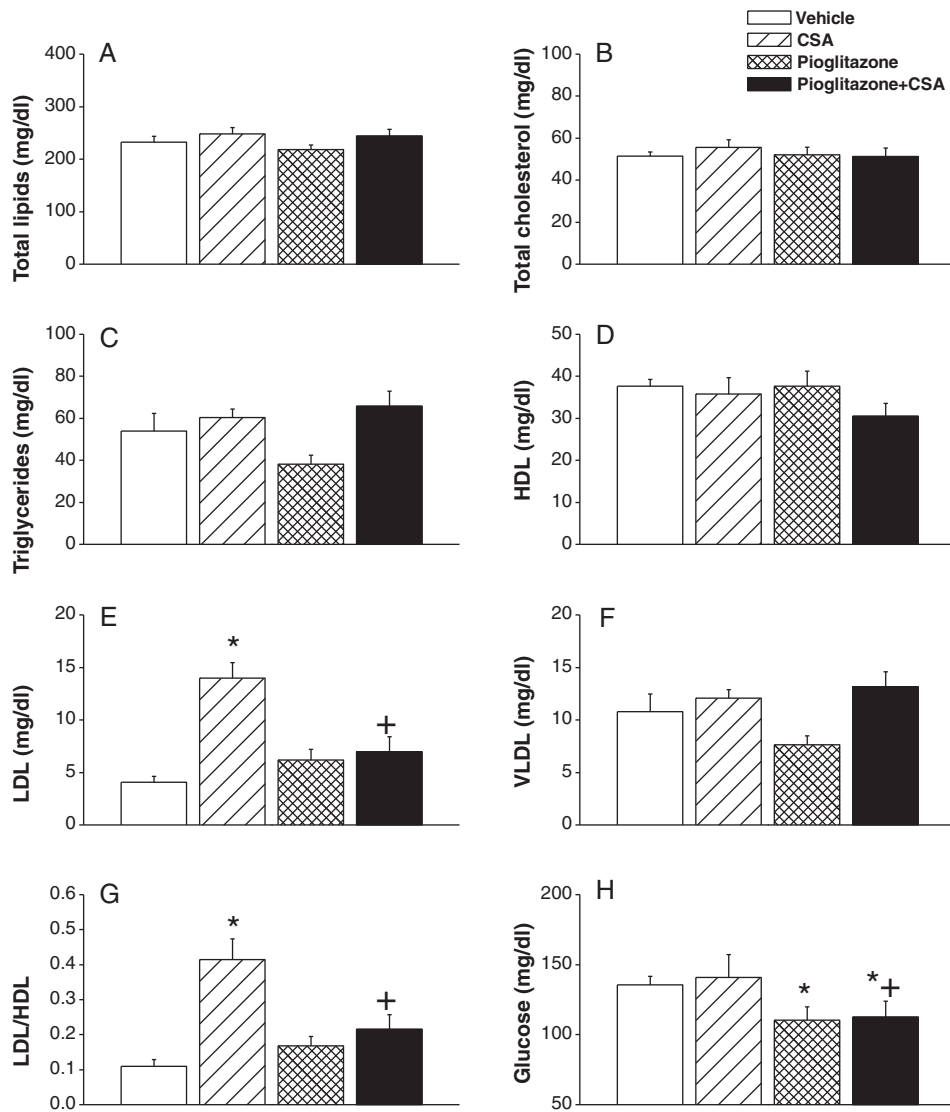
expression (reduced p-eNOS), and structural (disruption of vascular endothelial lining) abnormalities caused by CSA in the rat aorta, (ii) the CSA-induced lipid peroxidation, oxidative stress, and dyslipidemia were also abrogated by concurrent treatment with pioglitazone, and (iii) pioglitazone increased circulating adiponectin levels. These findings established convincing evidence that pioglitazone offsets the hypertensive effect of CSA via ameliorating significant vascular derangements caused by CSA.

We reasoned that concurrent exposure to pioglitazone would mitigate or at least minimize the hypertensive action of CSA and potentially related vasculotoxic manifestations. This assumption was ascertained in the current study because the hypertensive effect of CSA disappeared when rats were treated simultaneously with pioglitazone. We focused on vascular NOS/NO signaling because of its importance in CSA-evoked endothelial dysfunction and hypertension [28] and in the favorable vascular effects of pioglitazone [13]. Several lines of evidence are presented here that highlight a pivotal role for endothelial NOS/NO signaling in the pioglitazone amelioration of CSA-evoked endothelial dysfunction and hypertension. Histopathological and functional studies



**Fig. 6.** Plasma adiponectin (panel A) and aortic superoxide dismutase (SOD, panel B) and malondialdehyde (MDA, panel C) levels in rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (2.5 mg/kg/day) or their combination for 14 days. Values are means  $\pm$  S.E.M. of 6–7 observations. \* $P$  < 0.05 compared with vehicle (olive oil) values, † $P$  < 0.05 compared with CSA values.

showed that pioglitazone circumvented the CSA-induced disruption in the aortic endothelial lining and impairment in endothelium-dependent vasorelaxant responses elicited by carbachol. This favorable effect of pioglitazone on endothelium-dependent vasorelaxations appears to be directly related to its ability to counterbalance the decline in p-eNOS expression caused by CSA in vascular smooth muscle. Notably, our finding that eNOS protein was expressed in the rat aorta is consistent with previous reports, which detected appreciable amounts of eNOS in aortic tissue homogenates [29]. Because treatment with CSA, pioglitazone, or their combination failed to alter aortic responsiveness to sodium nitroprusside, it is unlikely that alterations in aortic smooth muscle reactivity to NO or downstream effectors, e.g. GC/cGMP pathway, contributed to the vascular interaction between CSA and pioglitazone.

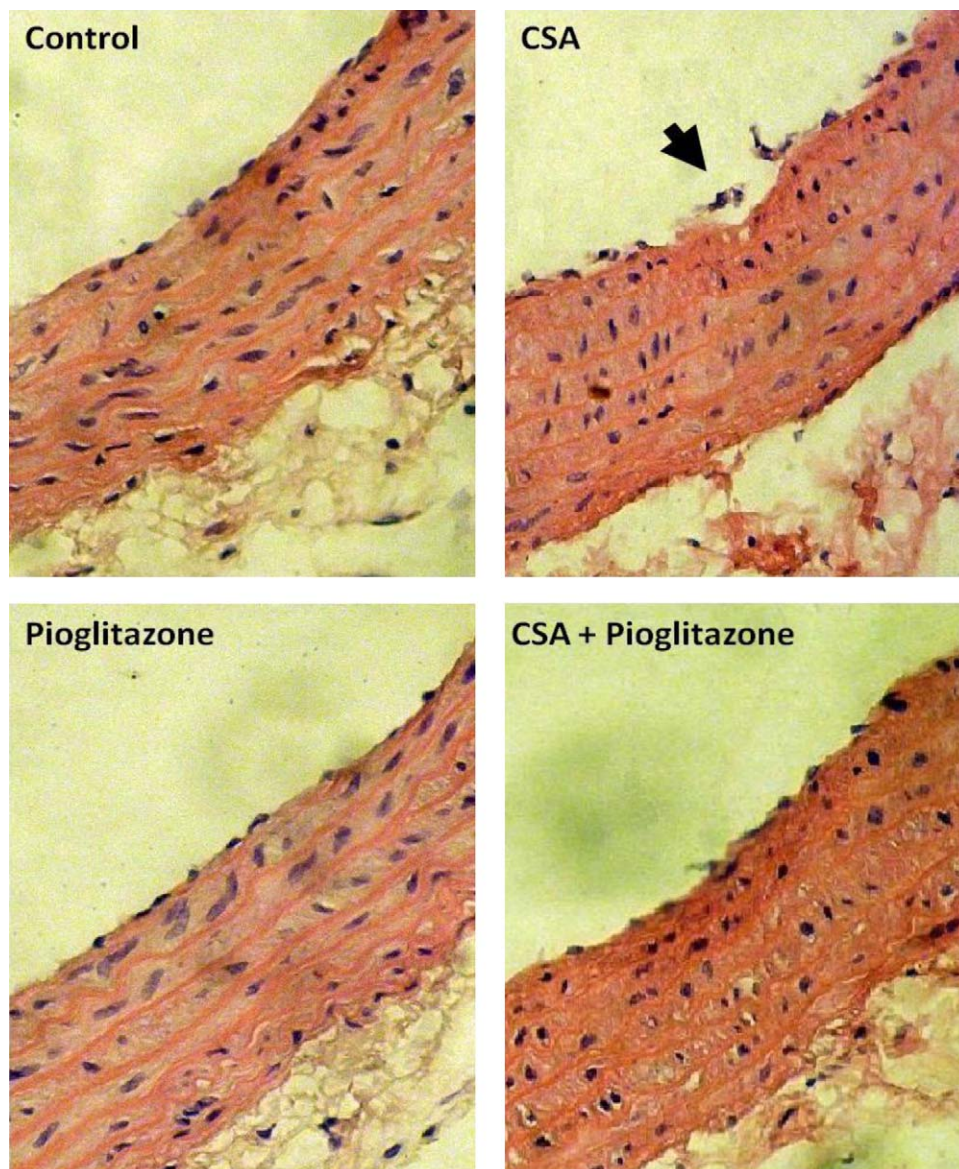


**Fig. 7.** Serum glucose and lipid levels in rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (2.5 mg/kg/day) or their combination for 14 days. Values are means  $\pm$  S.E.M. of 6–7 observations. \* $P < 0.05$  compared with vehicle (olive oil) values, + $P < 0.05$  compared with CSA values.

The role of oxidative stress in the hypertensive and vasculotoxic actions of CSA and the abrogation of these effects by pioglitazone was evaluated by measuring the activity of SOD in aortic tissues. SOD constitutes a biologically important group of metalloprotein enzymes that protect cells against the cytotoxic effect of the superoxide anion [30]. The lipid peroxidation product MDA, which reflects oxidative destruction of polyunsaturated fatty acids of biological membranes [31], was also assessed. Our observations that CSA caused significant decrease and increase in aortic SOD and MDA contents, respectively, are in line with the established role of oxidative stress caused by reactive oxygen species and lipid peroxides in the pathophysiology of CSA-induced hypertension, vascular injury, and endothelium dysfunction [10,11,19]. The subsequent increases in vascular reactive oxygen species, e.g.  $O_2^{\cdot-}$  and  $H_2O_2$ , interfere with endothelial cell integrity and attenuate endothelium-dependant relaxations and elevates BP [19,32]. By the same token, the capability of concurrently administered pioglitazone to abrogate the CSA-induced alterations in SOD and MDA points to a key role for improved redox and lipid peroxidation states in the protective effect of pioglitazone against hypertension and vascular toxicity evoked by CSA. These findings are in harmony with reported favorable effects of pioglitazone in other pathological models of oxidative stress. For example, pioglitazone nor-

malizes the activity of CAT, GPx and SOD in spontaneously hypertensive hyperlipidemic rats [33]. Similar protective effects for pioglitazone are demonstrated in cell cultures stimulated by high concentrations of glucose, where pioglitazone reduces MDA and restores SOD activity [34].

One more evidence that implicates oxidative stress in the vascular interaction between pioglitazone and CSA emerged from the assessment of the lipid profile. Our findings showed that the increases caused by CSA in serum LDL and LDL/HDL ratio were abrogated in rats treated concurrently with pioglitazone. Notably, clinical studies highlighted that long-term administration of CSA increases total cholesterol, triglycerides, LDL and/or VLDL, which correlate with increased risk of atherosclerosis [35,36]. The dyslipidemic effect of CSA has been attributed to the facilitation of cholesterol synthesis via increasing 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity [36] and inhibition of cholesterol-26-hydroxylase [37]. Indeed, the CSA-induced dyslipidemia and elevated LDL together with the increased levels of reactive oxygen radicals promote the oxidation of lipoproteins and formation of proatherogenic ox-LDL [38]. The latter is believed to inactivate NO and induce endothelial dysfunction [11]. Our finding that pioglitazone favorably affects the dyslipidemic effect of CSA is consistent with reports that activation of PPAR $\gamma$  induces



**Fig. 8.** Hematoxylin and Eosin stained photomicrographs ( $\times 100$ ) of aortas obtained from rats treated with cyclosporine (CSA, 20 mg/kg/day), pioglitazone (2.5 mg/kg/day) or their combination for 14 days. In contrast to the intact endothelial cell layer seen in vehicle (olive oil) preparations, the aorta of CSA-treated rats shows focal disruption of the endothelial lining (arrow). This effect of CSA disappears in preparations treated concurrently with pioglitazone.

monocyte/macrophages differentiation and uptake of ox-LDL [39] and inhibits the production of inflammatory enzymes such as iNOS and matrix metalloproteinase-9 [40], and pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6 [41].

The possibility that alterations in circulating adiponectin contributed to the CSA-pioglitazone hemodynamic interaction was investigated. We are not aware of any study that investigated the effect of CSA on adiponectin production. Although serum adiponectin was found to be decreased in renal transplant recipients on CSA therapy [10], it is not clear whether the reduction in adiponectin level is a direct effect of CSA or it occurs secondary to the after-transplantation improvement in kidney and vascular functions. In the present study, serum adiponectin remained unaltered in CSA-treated rats, thereby arguing against a possible role for adiponectin in the hypertensive and vasculotoxicity induced by CSA. Nonetheless, because serum adiponectin was increased by pioglitazone, the probability that adiponectin was responsible, at least partly, for the favorable effect of pioglitazone on the hypertensive and vascular effects of CSA cannot be ruled out. Indeed, the beneficial circulatory effects of pioglitazone have been

attributed, among other things, to increases in the expression and plasma level of adiponectin [16]. As mentioned earlier, adiponectin acts to counterbalance rises in BP [17] and endothelium dysfunction [18]. More studies are clearly needed to identify more precisely the role of adiponectin, and possibly other adipokines, in the CSA-pioglitazone hemodynamic interaction.

The current study establishes that pioglitazone could be an effective therapeutic regimen for combating the hypertensive action of CSA. In this regard, pioglitazone appears to be as effective as the standard antihypertensive drug captopril. The latter prevented CSA-evoked hypertension in this experimental study as well as in reported clinical studies [42]. The questions remain, however, whether (i) the additional endothelial/antioxidant benefits of pioglitazone makes it a better drug than others currently used in clinical practice, and (ii) other glitazones, e.g. rosiglitazone and troglitazone, can similarly abrogate the hemodynamic consequences of CSA. These important issues will be addressed in future studies from our laboratory.

In summary, our functional, biochemical, molecular, and histological findings suggest a protective effect for pioglitazone



against the hypertensive and vasculotoxic effects of CSA. Apparently, the amelioration of the CSA-evoked lipid peroxidation, oxidative stress, and dyslipidemia contributes to the inhibitory effect of pioglitazone on the CSA-evoked hypertension. The clinical importance of this research is warranted particularly in view of recent information that the activation of PPAR $\gamma$  boosts the immunosuppressant effect of CSA via the inhibition of T cell proliferation and IL-2 release [43]. Interestingly, the combined use of PPAR $\gamma$  ligands and low-dose CSA might represent a rationale therapeutic approach for the prevention of CSA nephrotoxicity while maintaining adequate immunosuppression [43,44].

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