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Original Article

Protective effects of prostaglandin E_1 on human umbilical vein endothelial cell injury induced by hydrogen peroxide

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Aim: To investigate the protective effects of prostaglandin E_1 (PGE_1) against H_2O_2-induced oxidative damage on human umbilical vein endothelial cells (HUVECs).
Methods: HUVECs were pretreated with PGE_1 (0.25, 0.50, and 1.00 μmol/L) for 24 h and exposed to H_2O_2 (200 μmol/L) for 12 h, and cell viability was measured by the MTT assay. LDH, NO, SOD, GSH-Px, MDA, ROS, and apoptotic percentage were determined. eNOS expression was measured by Western blotting and real-time PCR.
Results: PGE_1 (0.25−1.00 μmol/L) was able to markedly restore the viability of HUVECs under oxidative stress, and scavenged intracellular reactive oxygen species induced by H_2O_2. PGE_1 also suppressed the production of lipid peroxides, such as MDA, restored the activities of endogenous antioxidants including SOD and GSH-Px, and inhibited cell apoptosis. In addition, PGE_1 significantly increased NO content, eNOS protein, and mRNA expression.
Conclusion: PGE_1 effectively protected endothelial cells against oxidative stress induced by H_2O_2, an activity that might depend on the up-regulation of NO expression.

Keywords: prostaglandin E_1; human umbilical vein endothelial cells; hydrogen peroxide; apoptosis; nitric oxide

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Introduction

The endothelium is located in a strategic anatomical position within the blood vessel wall and acts as a barrier between the blood and vascular smooth muscle. Therefore, the functional integrity of the endothelium is essential to prevent vascular leakage and the formation of vascular diseases[1]. Generation of reactive oxygen species (ROS) by vascular endothelial cells is involved in several clinical conditions such as atherosclerosis, hypercholesteremia and disseminated intravascular coagulation, among others[2, 3]. The up-regulation of ROS in vascular lesions will exert detrimental effects on the peroxidation of membrane lipids, endothelium-derived enzyme inactivation, apoptotic occurrences, *etc*[4]. In contrast, antioxidants that react preferentially with ROS to inactivate them might have therapeutic applications in treating ROS-induced endothelial injury.

Prostaglandin E_1 (Alprostadil, PGE_1), which is an important member of the prostaglandin family, is a product of arachidonic acid metabolism by cyclooxygenase. PGE_1 has vasodilator effects on the systemic and pulmonary circulation[5] and cardio-protective effects during ischemia and reoxygenation[6]. In addition, beneficial effects on angiogenesis, platelet aggregation, blood viscosity and fibrinolysis have been observed[7–13]. These observations may be partly mediated by nitric oxide (NO) and vascular endothelial growth factor (VEGF)[14–16]. Moreover, increasing attention has now been paid to the cytoprotective action and anti-oxidant activity of PGE_1. PGE_1 can protect human retinal pigment epithelial cells against oxidative injury[17] and prevent the production of ROS in the intestinal mucosa of methotrexate-treated rats[18]. However, no direct evidence has been provided about the protective effects of PGE_1 on oxidative stress-induced vascular endothelium dysfunction, which is an important contributor to the development of cardiovascular diseases.

In the present study, we used human umbilical vein endothelial cells (HUVECs) to examine the protective effects of PGE_1 under oxidative stress, as well as the underlying mechanisms involved in this protection.

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Materials and methods

Chemicals and reagents

PGE_1 was purchased from the National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Hydrogen peroxide (H_2O_2), dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), vitamin C (Vc), Hoechst 33258, and N^G-Nitro-*L*-arginine methylester (*L*-NAME) were obtained from Sigma (St Louis, MO, USA). The reagent kits for the measurement of the levels of LDH, MDA, SOD, NO, and GSH-Px were purchased from Nanjing Institute of Jiancheng Bioengineering (Nanjing, China). The annexin V-FITC apoptosis detection kit was obtained from Jingmei (Shenzhen, China). Rabbit anti-eNOS antibody, mouse anti-β-actin antibody, goat-anti-rabbit-IgG-HRP, goat-anti-mouse-IgG-HRP and Western blotting Luminol reagent were obtained from Santa Cruz (Santa Cruz, CA, USA). 2′,7′-dichlorofluorescein diacetate (DCFH-DA), TRIzol reagent and M-MLV reverse transcriptase were obtained from Invitrogen (Carlsbad, CA, USA), and Green Real-time PCR Master Mix was from Toyobo (Osaka, Japan). All other reagents used were of analytical grade.

Cell culture and treatment

HUVECs were maintained in Endothelial Cell Medium (ECM) supplemented with 5% fetal bovine serum and 1% Endothelial Cell Growth Supplement (ECGS) in Poly-*L*-Lysine (PLL) pretreated flasks at 37 °C in a 5% CO_2 incubator. HUVECs, ECM, fetal bovine serum, ECGS and PLL were all purchased from Sciencell Research Laboratories (Carlsbad, CA, USA).

HUVECs were pretreated with ECM containing PGE_1 (0.25, 0.50, and 1.00 μmol/L) or Vc (1.5 mmol/L). Then, the culture supernatant was removed and the cells were exposed to H_2O_2 (200 μmol/L) diluted in ECM for 12 h at 37 °C until further assay.

Cell viability measurement

The viability of HUVECs was measured by the MTT assay[19]. The absorbance (A) at 570 nm in each well was determined with a microplate autoreader (Bio-Rad, Hercules, CA, USA). The viability of HUVECs in each well was presented as a percentage of the control group.

Measurement of intracellular ROS

Measurement of intracellular ROS was based on ROS-mediated conversion of nonfluorescent 2′,7′-DCFH-DA into DCFH[20]. Following the same procedure as the incubation studies, cells were harvested and incubated with DCFH-DA (10 μmol/L) diluted in ECM at 37 °C for 30 min. Subsequently, cells were washed with PBS three times and analyzed by flow cytometry performed on a FACScalibur cell analyzer using Cell Quest software (Becton Dickinson, Mountain View, CA, USA).

Preparation of cell lysate

The cells were treated as described above, and the culture supernatant was collected for analysis of LDH and NO release. The cells were scraped from the plates into ice-cold RIPA lysis buffer and the protein concentration was determined by the bicinchoninic acid (BCA) method, using BSA as a reference standard. Cell lysate was stored at -80 °C until used for the detection of MDA, SOD, and GSH-Px.

Measurement of LDH and NO release, as well as intracellular SOD, GSH-Px and MDA

LDH, an indicator of cell injury, was detected after exposure to H_2O_2 with an assay kit according to the manufacturer's protocol. Enzyme activity was expressed as units per liter and the absorbance was read at 440 nm.

The concentration of nitrites (NO_2^-) and nitrates (NO_3^-), stable end products of nitric oxide (NO), was determined by the Griess reaction. NO production was determined by measuring the optical density at 550 nm and was expressed in units per liter.

The concentration of MDA and the activities of SOD and GSH-Px were determined using commercially available kits. All procedures complied with the manufacturer's instructions. The results of the MDA, SOD, and GSH-Px assays were expressed as units per milligram of protein. The assay for SOD activity was based on its ability to inhibit the oxidation of hydroxylamine by $O_2^{\cdot-}$ produced from the xanthine–xanthineoxiase system. One unit of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%. The assay for GSH-Px activity was assayed by quantifying the rate of oxidation of reduced glutathione to oxidized glutathione by H_2O_2, as catalyzed by GSH-Px. One unit of GSH-Px was defined as the amount that reduced the level of GSH at 412 nm by 1 μmol/L in 1 min/mg protein. MDA was measured at a wavelength of 532 nm by reacting with thiobarbituric acid (TBA) to form a stable chromophoric product. Values for the MDA level were expressed in units per milligram of protein.

Flow cytometric evaluation of apoptosis

Cells grown in 6-well plates were harvested and washed. Samples were double-stained by Annexin V-FITC and propidium iodide (PI) at room temperature for 30 min in the dark and quantitatively analyzed by flow cytometry performed on a FACScalibur cell analyzer using CellQuest software (Becton Dickinson, Mountain View, CA, USA).

Nuclear morphology

Cell death was determined by Hoechst 33258 and PI double fluorescent staining. Cells were cultured on cover slides and treated as described previously. Samples were stained with PI (10 μg/mL) and Hoechst 33258 (10 μg/mL) and then fixed with 4% paraformaldehyde. For each cover slide, 1000−1500 cells were examined under a fluorescence microscope (Olympus Corp, Tokyo, Japan) and photographed with a digital camera (Olympus DP70, Japan).

Western blot analysis

Samples (40 μg protein) from the cell lysate were applied to 10% SDS-PAGE, followed by transfer to nitrocellulose mem-

branes. Blots were then blocked with 5% skim milk in TBST for 1 h and incubated with rabbit anti-eNOS and mouse anti-β-actin antibodies at dilutions recommended by the supplier. Then, the membrane was washed, and primary antibodies were detected with goat-anti-rabbit-IgG-HRP or goat-anti-mouse-IgG-HRP. Protein expression levels were determined by analyzing the signals captured on the nitrocellulose membranes using Western blotting Luminol reagent and Konica X-ray film (Konica, Tokyo, Japan).

Quantitative real-time PCR assays

Total RNA was isolated using TRIzol reagent according to the manufacturer's instructions. The cDNA was synthesized from 2 μg RNA using M-MLV reverse transcriptase. Real-time quantitative PCR analysis was carried out in a MyiQ single color real time PCR detection instrument (Bio-Rad, Hercules, CA, USA). Specific sense and antisense primers used were as follows: eNOS sense: 5′-CCTTCTGTGTGGGAGAGGAT-3′, and antisense: 5′-TTGTAGCCTGGAACATCTTCC-3′; β-actin sense: 5′-CACTGTGTTGGCGTACAGGT-3′, and anti-sense 5′-TCATCACCATTGGCAATGAG-3′. The reaction was conducted with an initial denaturing step at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 55 °C for 10 s and 72 °C for 15 s. The reaction was terminated by a cooling step at 4 °C. Each experiment was performed in duplicate and all data were analyzed using Bio-Rad iQ5 Software.

Statistical analysis

All values are expressed as the mean±SD. The data were analyzed by a one-way ANOVA and a two-tailed Student's *t*-test. $P<0.05$ was considered statistically significant.

Results

Effects of PGE_1 on the viability of H_2O_2-induced HUVECs

The effects of PGE_1 on the viability of H_2O_2-induced HUVECs were evaluated by MTT analysis. The survival rate of HUVECs was 65.92%±3.48% after exposure to 200 μmol/L of H_2O_2 for 12 h (Figure 1A). However, pretreatment with PGE_1 (0.25, 0.50, and 1.00 μmol/L) dose-dependently increased the viability of H_2O_2-induced HUVECs. This effect of PGE_1 was significantly attenuated by the NO-inhibitor *L*-NAME. When incubated with 0.0625−2.00 μmol/L PGE_1 alone for 24 h, cell viability did not show marked changes compared with the control group, but significant cytotoxicity was observed with the 4.00 μmol/L PGE_1 treatment for 24 h (data not shown). Therefore, a range of 0.25−1.00 μmol/L PGE_1 concentrations was used throughout subsequent experiments. Furthermore, addition of PGE_1 (0.25, 0.50, and 1.00 μmol/L) simultaneously with or immediately after H_2O_2 treatment also produced protective outcomes (Figure 2).

To further investigate the protective effects of PGE_1, the LDH assay, another indicator of cell toxicity, was performed. LDH release was minimal in the vehicle treated control group (25.40±2.80 U/L) and a dramatic increase (106.82±12.71 U/L) was observed after 12 h exposure to 200 μmol/L H_2O_2. However, pre-treatment with PGE_1 (0.25, 0.50, and 1.00 μmol/L) attenuated the H_2O_2-induced increase in LDH release to 83.49±7.05, 56.32±5.84, and 41.11±4.73 U/L, respectively (Figure 1B).

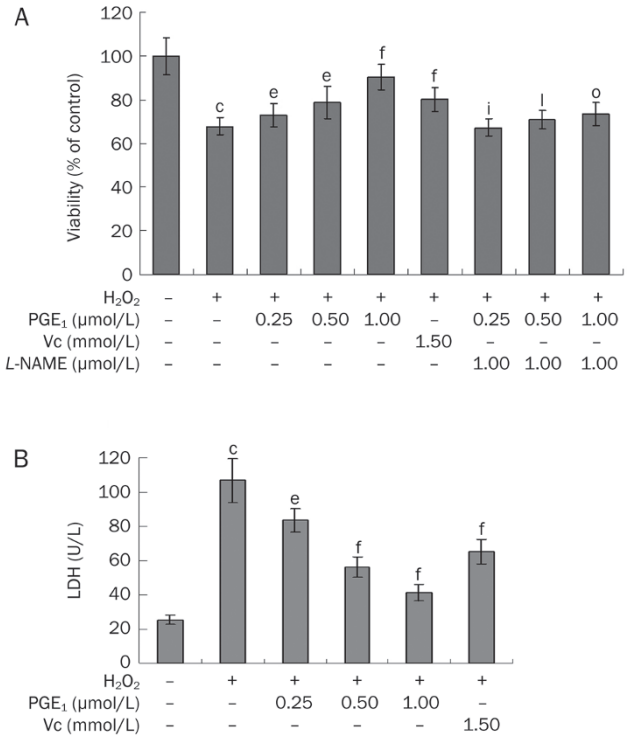

Figure 1. Effects of PGE_1 on cell viability of H_2O_2-induced HUVECs as evaluated by MTT analysis (A) and LDH release (B). Values are expressed as mean±SD. $n=6$. $^cP<0.01$ *vs* control group; $^eP<0.05$, $^fP<0.01$ *vs* the H_2O_2-alone group; $^iP<0.01$ *vs* the H_2O_2+0.25 μmol/L PGE_1 group; $^oP<0.01$ *vs* the H_2O_2+0.50 μmol/L PGE_1 group; $^oP<0.01$ *vs* the H_2O_2+1.00 μmol/L PGE_1 group.

Intracellular radical scavenging ability of PGE_1

The intracellular ROS concentration was determined by measuring the intensity of DCFH fluorescence, as the intensity of fluorescence reflects enhanced oxidative stress.

The DCFH fluorescence intensity in the H_2O_2-treated control group increased to 244.82%±7.24%, whereas pre-incubation with PGE_1 (0.25, 0.50, and 1.00 μmol/L) significantly reduced the increase in fluorescence induced by H_2O_2 in a concentration-dependent manner (Figure 3).

Effects of PGE_1 on SOD and GSH-Px activities and MDA content

The effects of PGE_1 on lipid peroxidation and endogenous antioxidant preservation were also measured. Treatment with H_2O_2 (200 μmol/L, 12 h) decreased the activities of SOD and GSH-Px to 59.6%±6.42% and 54.3%±4.49%, respectively, compared to no treatment. However, pre-incubation with PGE_1 (0.25, 0.50, and 1.00 μmol/L) or Vc (1.5 mmol/L) significantly attenuated the changes in SOD and GSH-Px activity (Table 1).

Additionally, treatment with H_2O_2 (200 μmol/L, 12 h) increased intracellular MDA levels by 79.1%±4.32%, while pre-incubation with PGE_1 (0.25, 0.50, and 1.00 μmol/L) or Vc

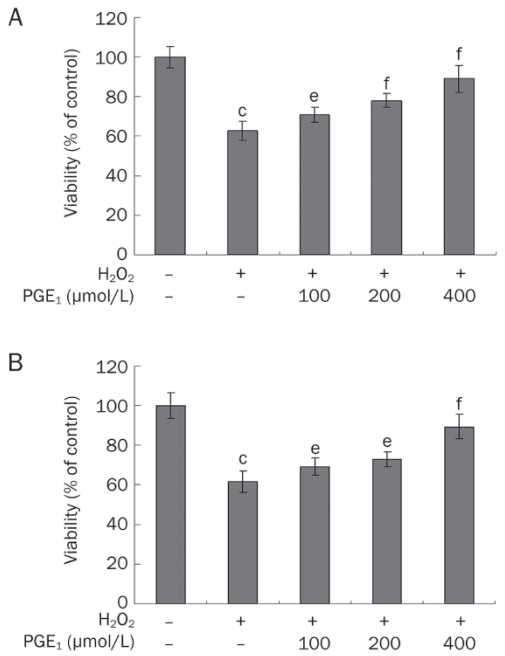

Figure 2. Effects of PGE_1 on cell viability. (A) HUVECs were co-incubated with H_2O_2 and PGE_1 for 24 h, and then cell viability was evaluated by MTT analysis. (B) HUVECs were pre-incubated with H_2O_2 for 1 h and then exposed to PGE_1 for 24 h. The viability of cells was evaluated by MTT analysis. Values are expressed as mean±SD. n=6. ^cP<0.01 *vs* control group; ^eP<0.05, ^fP<0.01 *vs* the H_2O_2-alone group.

(1.5 mmol/L) markedly attenuated the increase to 151.2%, 139.2%, 120.7%, and 151.1% of the control group, respectively (Table 1).

Table 1. Effects of PGE_1 on SOD and GSH-Px activities as well as MDA content in H_2O_2-induced HUVECs. Values are mean±SD. n=6. ^cP<0.01 *vs* control group; ^eP<0.05, ^fP<0.01 *vs* the H_2O_2-alone group.

Parameters	SOD (U/mg protein)	GSH-Px (U/mg protein)	MDA (U/mg protein)
Control	21.39±0.46	124.01±12.20	1.27±0.08
H_2O_2	12.76±1.37^c	67.28±5.56^c	2.27±0.05^c
H_2O_2+0.25 μmol/L PGE_1	14.54±0.52^e	76.69±3.80^f	1.98±0.07^f
H_2O_2+0.50 μmol/L PGE_1	17.21±0.89^f	101.10±4.33^f	1.76±0.11^f
H_2O_2+1.00 μmol/L PGE_1	20.13±1.17^f	113.47±4.25^f	1.53±0.13^f
H_2O_2+1.5 mmol/L Vc	17.46±0.78^f	82.59±2.54^f	1.91±0.13^f

Effects of PGE_1 on H_2O_2-induced apoptosis

The effects of PGE_1 against H_2O_2-induced apoptosis were measured using a FACScalibur cell analyzer and fluorescence microscope. Using Annexin V-FITC and PI staining, the apoptotic percentage of untreated cells was determined to be 8.05%±1.91% (Figure 4A) in the present experiment. After treatment with H_2O_2 for 12 h, the percentage of apoptotic cells increased to 37.0%±1.94% (Figure 4B). Upon pretreatment with PGE_1 (0.25, 0.50, and 1.00 μmol/L), the apoptotic rate induced by H_2O_2 decreased to 27.15%±1.22%, 22.20%±2.02%, and 16.60%±0.93%, respectively (Figure 4C, 4D, 4E).

In the control group, little morphological sign of nuclear damage or chromatin condensation was observed (Figure 5A). Once injured by H_2O_2, cells exhibited condensed nuclei, apoptotic bodies and detachment indicative of cell death (Figure 5B). However, pre-incubation with PGE_1 (0.25, 0.50, and 1.00 μmol/L) before H_2O_2-injury significantly attenuated cell damage (Figure 5C, 5D, 5E).

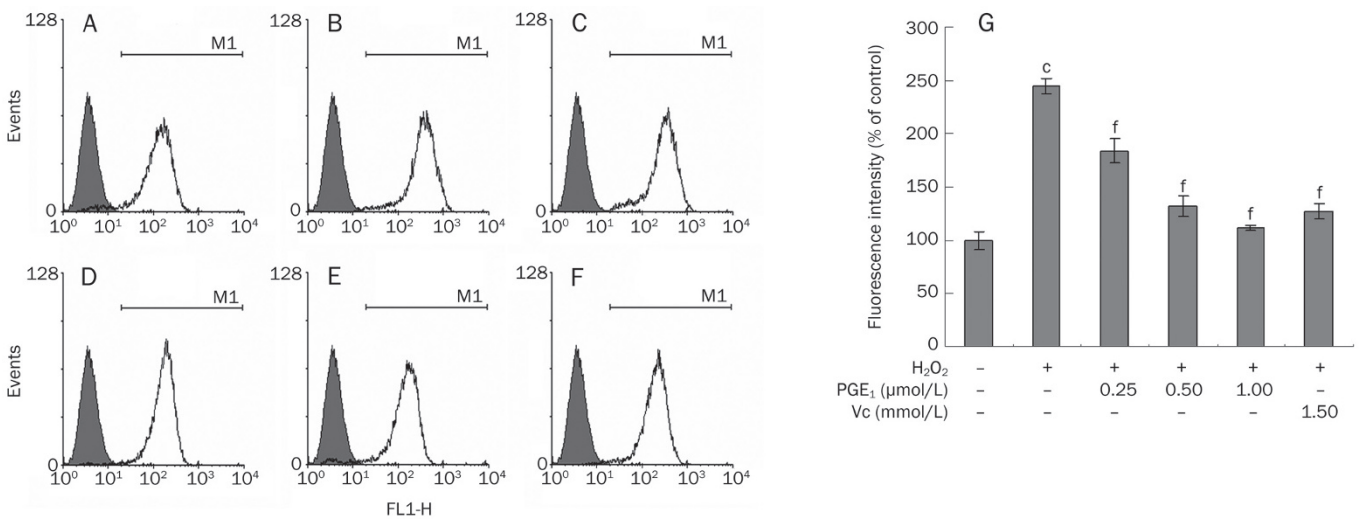

Figure 3. Effects of PGE_1 on the production of intracellular ROS was determined by measuring the intensity of DCFH fluorescence. (A) Control group; (B) H_2O_2-alone group; (C) H_2O_2+0.25 μmol/L PGE_1 group; (D) H_2O_2+0.50 μmol/L PGE_1 group; (E) H_2O_2+1.00 μmol/L PGE_1 group; (F) H_2O_2+1.5 mmol/L Vc group; (G) Effects of PGE_1 on the production of intracellular ROS. The values are expressed as percentages of the fluorescence intensity. Mean±SD. n=4. ^cP<0.01 *vs* control group; ^fP<0.01 *vs* the H_2O_2-alone group.

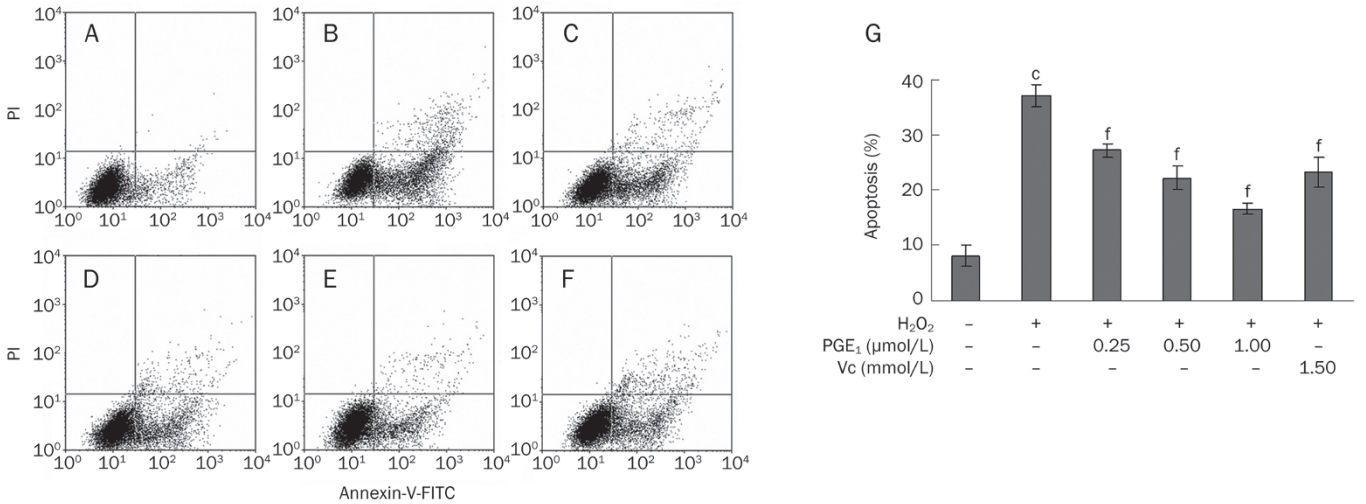

Figure 4. Effects of PGE_1 against H_2O_2-induced apoptosis in cultured HUVECs by flow cytometric analysis. (A) Control group; (B) H_2O_2-alone group; (C) H_2O_2+0.25 μmol/L PGE_1 group; (D) H_2O_2+0.50 μmol/L PGE_1 group; (E) H_2O_2+1.00 μmol/L PGE_1 group; (F) H_2O_2+1.5 mmol/L Vc group; (G) Protective effects of PGE_1 against H_2O_2-induced apoptosis in H_2O_2-induced HUVECs. Values are expressed as mean±SD. n=4. ^cP<0.01 *vs* control group; ^fP<0.01 *vs* the H_2O_2-alone group.

Detection of NO release in culture supernatant and expression of eNOS protein and mRNA in HUVECs

To investigate the mechanisms that mediate the antioxidative effect of PGE_1, the NO release and eNOS expression were measured. Pre-incubation with PGE_1 (0.25, 0.50, and 1.00 μmol/L) was observed to significantly increase NO content in a dose-dependent manner (Figure 6A). However, co-incubation with 1 μmol/L *L*-NAME and PGE_1 for 24 h before H_2O_2 treatment significantly reduced NO release, as well as cell viability, compared to the PGE_1 treatment group (Figures 1A and 6A).

In parallel, the eNOS protein (Figure 6B) and mRNA (Figure 6D) levels in HUVECs were also assayed. Pre-incubation with PGE_1 (0.25, 0.50, and 1.00 μmol/L) also triggered eNOS protein and mRNA expression markedly in a dose-dependent manner. These results were consistent with the observed change in NO content over the course of the experiments.

Discussion

The results presented here indicated that PGE_1 protected endothelial cells from H_2O_2-induced injury. It decreased the amount of ROS, LDH, and MDA, decreased the percentage of apoptotic cells and increased SOD and GSH-Px activity. Furthermore, PGE_1 increased NO release and upregulated eNOS protein and mRNA expression.

Oxidative stress plays a critical role in endothelial dysfunction[21] and is a critical pathogenic factor in the development of cardiovascular diseases, such as atherosclerosis, hypercholesteremia and disseminated intravascular coagulation[22]. Here, we demonstrated that H_2O_2, a precursor of other ROS, can markedly increase cell permeability, damage cellular antioxidant defenses and induce endothelial cell apoptosis at 200 μmol/L.

Agents that inhibit production of ROS or enhance cellular antioxidant defenses can protect cells from the damaging effects of oxygen radicals[23]. Our study first proved that PGE_1 not only strikingly scavenged intracellular ROS induced by H_2O_2 (Figure 3), but also effectively increased the viability of H_2O_2-induced endothelial cells (Figure 1). Miho *et al* suggest that PGE_1 protects human retinal pigment epithelial cells from oxidative injury[17] and prevents the production of ROS in the intestinal mucosa of methotrexate-treated rats[18]. These results indicate that the protective effect is associated with PGE_1 scavenging intracellular ROS.

Lipid peroxidation is one of the primary events in free radical-mediated cell injury. MDA is a by-product of lipid peroxidation induced by excessive ROS and is widely used as a biomarker of oxidative stress[24]. Antioxidant enzymes, such as SOD and GSH-Px, however, are thought to be effective for the augmentation of antioxidant defenses in endothelial cells[25]. In our study, when endothelial cells were incubated with PGE_1, these H_2O_2-induced cellular events were almost completely blocked (Table 1). These results jointly suggest that the scavenging of ROS might be related to the increased activities of antioxidant enzymes.

Hydrogen peroxide not only decreased the viability of cells, but also induced cell apoptosis[21]. Previous studies have shown that PGE_1 protects human liver sinusoidal endothelial cells and endothelial progenitor cells from apoptosis[26, 27]. In the present study, we demonstrate that 200 μmol/L H_2O_2 induces massive cell apoptosis, while PGE_1 (0.25−1.00 μmol/L) prevents the H_2O_2-induced apoptosis in a concentration-dependent manner (Figures 4 and 5). Thus, we presumed that PGE_1 prevented cell apoptosis due to decreased intracellular ROS and increased antioxidant enzymatic activities.

Nitric oxide (NO) is a soluble gas continuously synthesized by the endothelium and has a wide range of biological properties that maintain vascular homeostasis, including modulation of vascular dilator tone, regulation of local cell growth, and

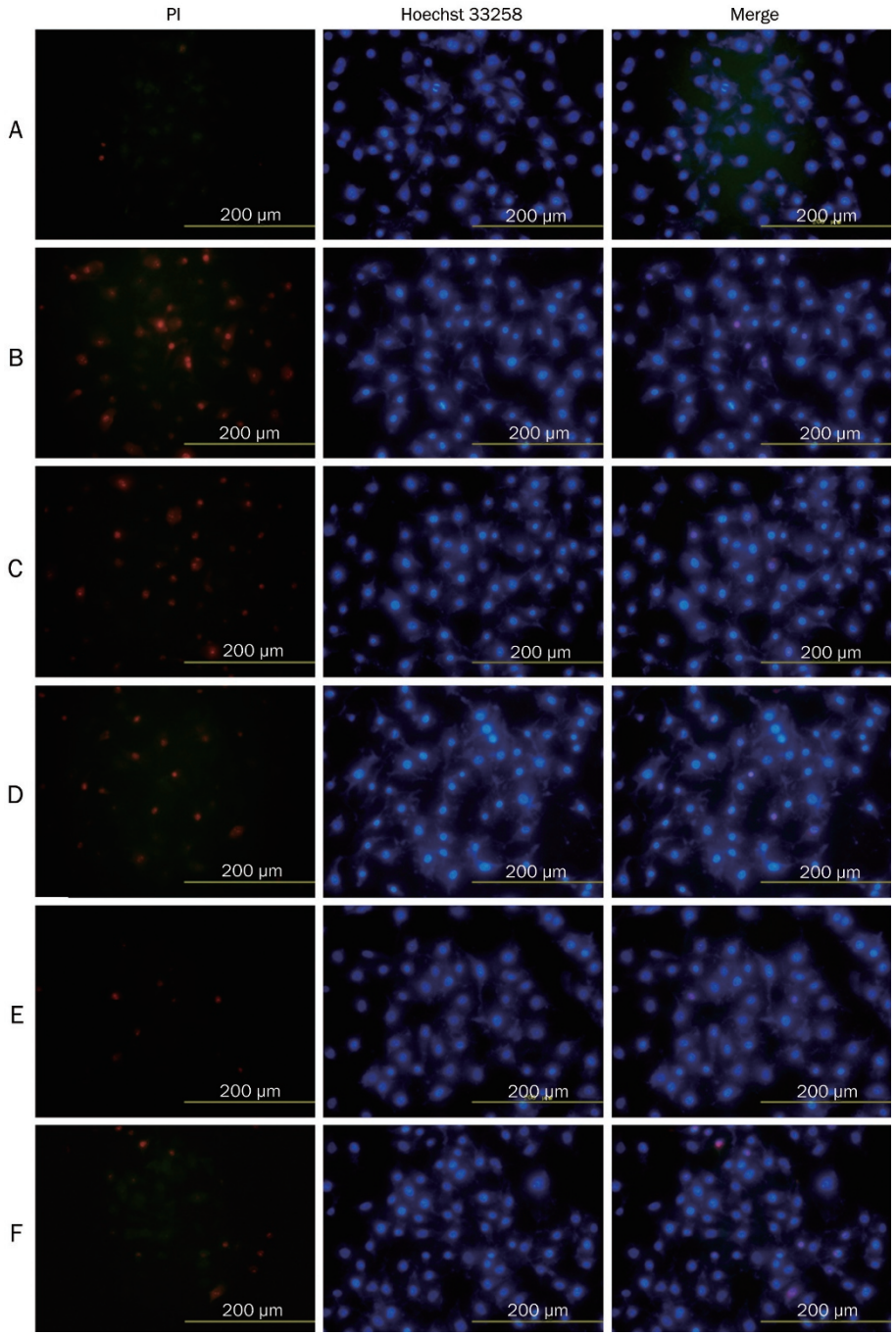

Figure 5. Protective effects of PGE_1 on H_2O_2-induced HUVEC death as visualized by Hoechst 33258/PI staining. (A) Control group; (B) H_2O_2-alone group; (C) H_2O_2+0.25 μmol/L PGE_1 group; (D) H_2O_2+0.50 μmol/L PGE_1 group; (E) H_2O_2+1.00 μmol/L PGE_1 group; (F) H_2O_2+1.5 mmol/L Vc group.

protection of the vessel from injury as a consequence of platelets and cells circulating in blood[28]. Endothelial dysfunction, defined as impaired endothelium-dependent vasorelaxation, is usually attributed to a deficiency of nitric oxide[29]. Our studies show that pretreatment with PGE_1 significantly increases NO and eNOS expression compared to H_2O_2-alone (Figure 6). The increasing NO content is paralleled by a reduction of cell injury induced by H_2O_2 (Figures 1A and 6A). NO terminates chain reactions during lipid peroxidation, as observed in model lipid systems of low-density lipoprotein oxidation and in cells[14, 30]. These results suggest that the NO pathway plays an important role in the protective activity of prostaglandin E_1 against H_2O_2-induced oxidative injury, but the mechanisms are still unclear.

PGE_1 activates adenylate cyclase and increases intracellular cAMP via binding to prostanoid receptor subtypes EP2, EP3, EP4, and IP[31]. The cAMP-responsive elements (CRE) within the human eNOS promoter play an important role in the inducible expression of the human eNOS gene[32, 33]. Progressive 5′-deletion and site-specific mutation analyses defined the beraprost-responsive sequences as CRE, located at -733 and -603 within the human eNOS promoter[32, 33], and the hypoxia-

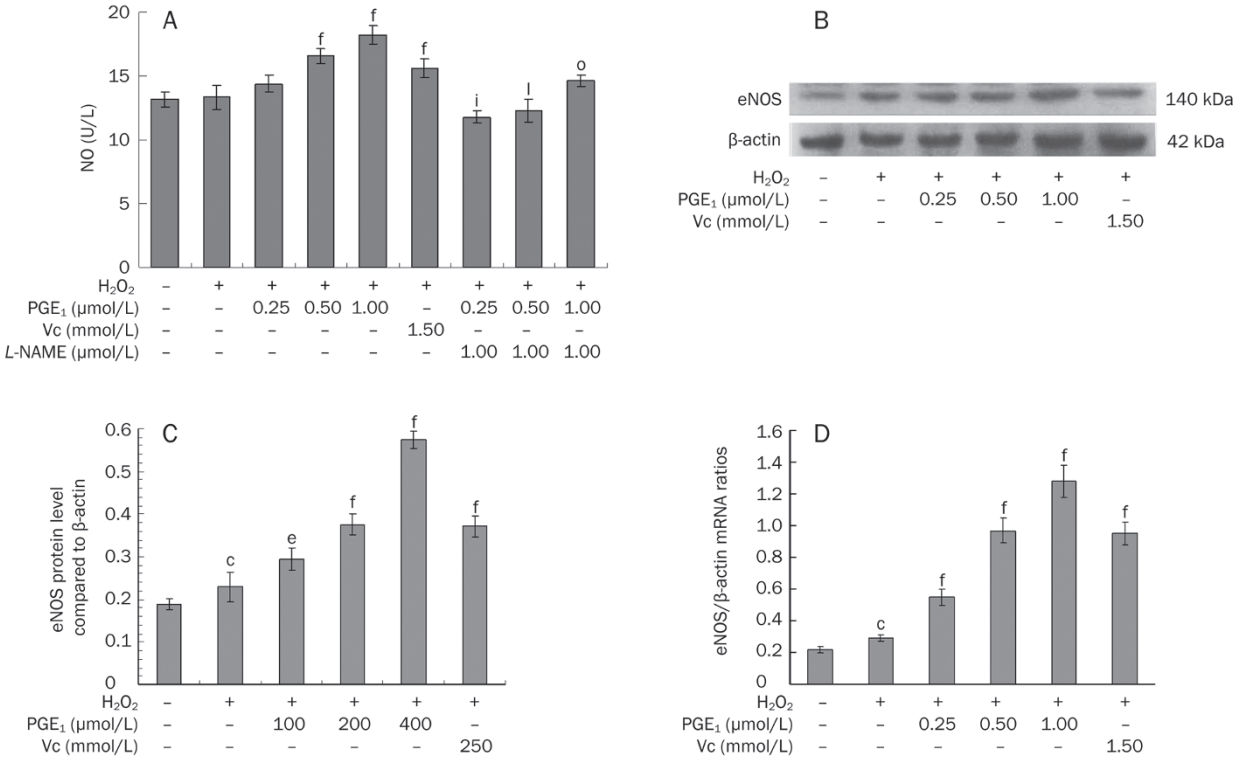

Figure 6. Effects of PGE_1 on NO release (A), eNOS protein (B, C) and mRNA expression (D). Values are expressed as mean±SD. n=6. (A) ^fP<0.01 *vs* the H_2O_2-alone group; ^lP<0.01 *vs* the H_2O_2+0.25 μmol/L PGE_1 group; ^lP<0.01 *vs* the H_2O_2+0.50 μmol/L PGE_1 group; ^oP<0.01 *vs* the H_2O_2+1.00 μmol/L PGE_1 group. (C, D) ^cP<0.01 *vs* the empty control group; ^eP<0.05, ^fP<0.01 *vs* the H_2O_2-alone group.

responsive sequences located at -924 to -921[34], while the PGE_1-responsive sequences are still unclear. PGE_1 increases eNOS expression through PKA pathway activation and cAMP-responsive element binding protein (CREB) phosphorylation. Phosphorylated CREB predominantly binds to CRE, which leads to increased promoter activity and upregulation of the eNOS protein and mRNA expression[32–34]. Previous studies have also reported that PKA signaling increased phosphorylation of Ser-1177 and dephosphorylation of Thr-495 to activate eNOS, which led to the upregulation of NO[35]. Activation of perinuclear EP3 receptors induced eNOS expression, which depends on nuclear envelope K (Ca) channels, protein kinases, and NF-kappaB[36]. Studies also showed that MAPK is a central pathway to transmit the actions of PGE_1 on eNOS[14]. The mechanism by which PGE_1 upregulates NO expression requires further research before it is well understood.

As our study was limited to the cell level, further studies are needed to confirm the cardiovascular protective effect of PGE_1 in organs or humans. As stated above, we concluded that pretreatment of HUVECs with PGE_1 significantly protected those cells from H_2O_2-induced cell death, which was mediated, at least in part, by a mechanism linked to the up-regulation of NO expression. It is noteworthy that the finding of the present study may shed light on the pharmacological basis for the clinical application of PGE_1 for the treatment of atherosclerosis and acute coronary syndrome, which are relevant to endothelial cell death.

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Author contribution

Wen-tong FANG designed and performed the research, analyzed and interpreted data, and drafted the paper; Hong-jian LI and Liao-sheng ZHOU designed the research, analyzed data, critically evaluated the manuscript for its important intellectual content, and corrected the manuscript.

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