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In Utero Exposure to Fine Particulate Matter Causes Hypertension Due to Impaired Renal Dopamine D_1 Receptor in Offspring

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Abstract

Background/Aims: Adverse environment in utero can modulate adult phenotypes including blood pressure. Fine particulate matter ($PM_{2.5}$) exposure in utero causes hypertension in the offspring, but the exact mechanisms are not clear. Renal dopamine D_1 receptor (D_1R), regulated by G protein-coupled receptor kinase type 4 (GRK4), plays an important role in the regulation of renal sodium transport and blood pressure. In this present study, we determined if renal D_1R dysfunction is involved in $PM_{2.5}$–induced hypertension in the offspring.

Methods: Pregnant Sprague–Dawley rats were given an oropharyngeal drip of $PM_{2.5}$ (1.0 mg/kg) at gestation day 8, 10, and 12. The blood pressure, 24-hour sodium excretion, and urine volume were measured in the offspring. The expression levels of GRK4 and D_1R were determined by immunoblotting. The phosphorylation of D_1R was investigated using immunoprecipitation. Plasma malondialdehyde and superoxide dismutase levels were also measured in the offspring.

Results: As compared with saline-treated dams, offspring of $PM_{2.5}$-treated dams had increased blood pressure, impaired sodium excretion, and reduced D_1R-mediated natriuresis and diuresis, accompanied by decreased renal D_1R expression and GRK4 expression. The impaired renal D_1R function and increased GRK4 expression could be caused by increased reactive oxidative stress

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(ROS) induced by $PM_{2.5}$ exposure. Administration of tempol, a redox-cycling nitroxide, for 4 weeks in the offspring of $PM_{2.5}$-treated dam normalized the decreased renal D_1R expression and increased renal D_1R phosphorylation and GRK4 expression. Furthermore, tempol normalized the increased renal expression of c-Myc, a transcription factor that regulates GRK4 expression.

Conclusions: In utero exposure to $PM_{2.5}$ increases ROS and GRK4 expression, impairs D_1R-mediated sodium excretion, and increases blood pressure in the offspring. These studies suggest that normalization of D_1R function may be a target for the prevention and treatment of the hypertension in offspring of mothers exposed to $PM_{2.5}$ during pregnancy.

Keywords

Dopamine D_1 receptor; Fetal origins of adult disease; Hypertension; Oxidative stress; Fine particulate matter

Introduction

Hypertension is the largest contributor to global disease burden, accounting for 7% of global disability-adjusted life years, and the most common modifiable risk factor for cardiovascular diseases [1, 2]. Although the mechanisms that cause hypertension are not completely understood, a large number of studies have revealed that abnormal fetal programming is an important factor contributing to the development of hypertension [3, 4]. Adverse environmental stimuli, experienced during a critical period of development in utero and early life, induce the programming of adult susceptibility to cardiovascular diseases, including hypertension [5–7]. Among those environmental factors, air pollution is the most important and common and important one. Several pieces of evidences have shown that maternal exposure to fine particulate matter ($PM_{2.5}$) increases the blood pressure of the offspring. A study in 1, 131 mother-infant pairs in Boston found that exposure to $PM_{2.5}$ in late pregnancy was positively associated with newborn systolic blood pressure [8]. Animal studies also confirmed that exposure to $PM_{2.5}$ during in utero and early life development in mice increases susceptibility to heart failure [9, 10].

There are many mechanisms that cause hypertension. However, the kidney, by regulating sodium excretion, is vital in the control of blood pressure. Renal sodium excretion is under the control of natriuretic and anti-natriuretic factors. An important natriuretic factor is dopamine which decreases renal tubular sodium reabsorption by inhibition of sodium transporters, exchangers, and ion channels, including the Na^+-H^+ exchanger and Na^+-K^+-ATPase [11–14]. Dopamine exerts it actions via dopamine receptors, that are classified into two families: D_1-like receptors (D_1 and D_5 receptors), which stimulate adenylyl cyclase activity; and D_2-like receptors (D_2, D_3 and D_4 receptors), which inhibit adenylyl cyclase activity. Stimulation of renal dopamine receptors, especially the D_1 receptor (D_1R), induces natriuresis and diuresis and decreases blood pressure [11–14]. In spontaneously hypertensive rats (SHRs) or hypertensive patients, the renal D_1R is hyper-phosphorylated and dysfunctional, which are ascribed to increased G protein-coupled receptor kinase type 4 (GRK4) expression and activity in hypertensive states [11, 14–18]. In our present study, we sought to determine if a dysfunction of renal D_1R is involved in the hypertension of the offspring of dams exposed to $PM_{2.5}$ and if so, investigate its underlying mechanisms.

Materials and Methods

$PM_{2.5}$ sampling and processing

The sample site was in Daping Hospital, which is approximately 1 km from the Chongqing City center. The nearest main road is 100 m northeast of the hospital. The monitoring site, within a radius of approximately 200 m, is almost completely surrounded by residential areas.

We used a Medium Volume Sampler (MVS, model TH-150, Tianhong Co., Wuhan, China), equipped with a $PM_{2.5}$ filtration system, to collect $PM_{2.5}$ on filters (diameter 150 mm), as reported [19–21]. The flow rate of MVS was adjusted to 30 m^3/h. After sampling, the filters were sheared into small pieces and sonicated with an ultrasonator (KQ-250DE, Shumei, Kunshan, Jiangsu, China) for 3×40 min in ddH_2O. The extracted material was frozen, lyophilized, and weighed to determine the extraction efficiency. The farinose solid was stored at −80°C for future use. The sampling period started on April 8, 2014 and ended on July 28, 2015. A total of 25 filters were used to collect $PM_{2.5}$.

The diameters of the collected particles, measured by a scanning electron microscope (JSM-6610, JEOL, Japan), were equal to or less than 2.5 μm (Fig. 1A and 1B). We also used the energy dispersive spectrometric (EDS) technology to determine roughly the chemical components of the samples (Fig. 1C). The elements in the fine particles consisted mainly of C, Si, Ca, Na and S, with minor amounts of Mg, Fe, Al, K and Zn. So we speculated that the collected particles may have been derived mainly from vehicles' exhaust emission, coal combustion products, such as fly ash, soil and biomass burning products, atmospheric reaction, and cement dust.

Animals and treatment

The experimental protocols were approved by The Third Military Medical University Animal Care and Use Committee. Virgin Sprague–Dawley (SD) rats (250–260 g) were purchased from the Animal Centre of The Third Military Medical University (Chongqing, China). All the rats were given a standard laboratory chow and water was consumed ad libitum. $PM_{2.5}$ was suspended in phosphate buffer (PBS). To minimize aggregation, the particle suspensions were always sonicated for 15 min, 3 times and vortexed before their dilution and prior to intratracheal administration. Control animals received saline treatment.

Pregnant SD rats on gestation day 8, 10, and 12 were continuously anesthetized with isoflurane and placed in a supine position with the neck extended on an angled board. The tongue was pulled straight out of the mouth for the oropharyngeal instillation of $PM_{2.5}$ suspension (1.0 mg/kg in 25 μl) or saline via a sterile syringe [20–23]. The rats were housed individually throughout pregnancy and until delivery. The pups were weaned to regular rat chow at 4 weeks of age. At 12 weeks of age, male offspring of $PM_{2.5}$- and saline-treated dams were randomly assigned into two groups: one group that drank tap water served as control, and the other group drank tap water with 1.0 mmol/L tempol, redox-cycling nitroxide [24], that was changed two times a day for 4 weeks.

Placenta biomarkers detections

At the end of the four-week exposure, the placentae were collected for analysis after cesarean section. Placental levels of interleukin-4 (IL-4), interleukin-6 (IL-6), malondialdehyde (MDA), and the glutathione peroxidase (GSH-Px) were measured by ELISA using commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Blood pressure measurement and urine collection

The blood pressure was measured in conscious rats at 6, 8, 10, 12 and 14 weeks of age. Systolic blood pressure was measured using a computerized noninvasive tail-cuff manometry system in conscious rats (BP-98A; Softron, Tokyo, Japan). The animals were individually restrained in a clear acrylic restrainer at an ambient temperature of 37°C for 15 min. Five blood pressure values were recorded for each rat and the average was taken. To assure the reliability of the measurements, the mice were trained for one week before the experiments in order for the mice to be acclimated to the procedure. Blood pressures were measured between 3:00–5:00 pm.

SD rats were acclimated in metabolic cages for at least 2 days before urine collection. The sodium concentrations in the urine samples were measured by a flame photometer 480 (Ciba Corning Diagnostics, Norwood, MA).

Fenoldopam-induced diuresis and natriuresis

The rats were anesthetized with pentobarbital (50 mg/kg intravenously), placed on a heated board to maintain body temperature at 37°C, and tracheotomized (PE-100). Catheters (PE-50) were placed into the external jugular and femoral veins and left carotid artery for fluid administration and blood pressure monitoring. Systemic blood pressure was monitored electronically using Cardiomax II (Columbus Instruments, Columbus, OH). Laparotomy was performed to expose the left and right ureters, which were then catheterized for urine collection. The right suprarenal artery (which originates from the right renal artery) was located and catheterized (PE-10, heat stretched to 180 μm). After a 60-min stabilization period, a normal saline load equivalent to 5% of body weight was infused intravenously for 30 min. After the acute saline load, a 60-min stabilization period was allowed before starting the urine collections [24, 25].

Five consecutive 40-min urine samples were collected. Vehicle (normal saline) without fenoldopam was given during the first collection period (control). In the next period, the mice received fenoldopam, a D_1-like receptor agonist, at a dose rate of 0.1, 0.5, 1.0 ($\mu g \cdot kg^{-1} \cdot min^{-1}$), a dose that has been shown not to affect blood pressure in previous studies [26, 27]. During the last collection period (recovery), only the vehicle was infused which was considered as the recovery phase of the experiment. The change in all infusates (vehicle and reagents) was commenced 10 min before each period to account for the dead space in the delivery catheter. All infusions (vehicle and reagents) were given at a rate of 40 μl/h; fluid losses throughout the experiment were replaced intravenously with 5% albumin in normal saline at 1% body wt over 30 min. The rats were euthanized with an overdose of pentobarbital (100 mg/kg) at the end of the experiment right after the last sample was collected. Urine samples were stored at −80°C until use.

Immunoblotting

Immediately after euthanasia, renal cortex (upper pole, left kidney) was homogenized in buffer (10 mM Tris HCl, 250 mM sucrose, 2 mM PMSF, protease inhibitor cocktail; pH 7.4), and centrifuged at 24, 000 g for 25 min at 4°C. The upper fluffy layer of the pellet was re-suspended in the homogenization buffer, which was considered as the total protein of renal tissue. Finally, the samples were quickly frozen and stored at −80 °C until use [28, 29].

After boiling the homogenates in sample buffer (35 mmol/L Tris-HCl, pH 6.8, 4% SDS, 9.3% dithiothreitol, 0.01% bromophenol blue, 30% glycerol) at 95°C for 5 min, 60 μg protein were separated by SDS-PAGE (10% polyacrylamide), and then electroblotted onto nitrocellulose membranes (Amersham Life Science, Arlington, TX). The blots were blocked overnight with 5% nonfat dry milk in phosphate buffered saline with Tween 20 (PBST) (0.05% Tween 20 in 10 mmol/L PBS) at 4°C with constant shaking, then incubated with polyclonal rabbit anti-rat GRK4 antibodies (1:400; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. The other antibodies were: anti- D_1R antibody (1:400; Millipore, Billerica, MA), c-Myc polyclonal antibody (1:500; Immunoway Biotechnology Co, Plano, Texas) and rabbit polyclonal GAPDH (1:500; Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were then incubated with infrared-labeled secondary antibodies (donkey anti-rabbit IRDye 800, Li-Cor Biosciences, Lincoln, NE) at room temperature for 1 hr. After washing three times with PBST, the bound complexes were detected using the Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE). The images were analyzed using the Odyssey Application Software to obtain the integrated intensities.

Determination of D_1R phosphorylation by co-immunoprecipitation

Equal amounts of lysates (1.0 mg protein/ml renal cortex total protein extract, as mentioned before) were incubated with D_1R antibody (2 mg, Millipore, Billerica, MA) for 2 h, followed by protein G agarose overnight with rocking at 4°C for 12 hr. The immunoprecipitates were pelleted and washed three times with PBS. Then the pellets were suspended in sample buffer, boiled for 10 min, and subjected to immunoblotting with phosphoserine antibody (1:400, Immunechem, Burnaby, BC, Canada). The bound complexes were detected using the Odyssey Infrared Imaging System (Li-Cor Bioscience). All bands were quantified by densitometry [28, 30].

Biochemical markers of oxidative stress

To assess oxidative stress in the whole body, plasma samples from rats were measured for superoxide dismutase activity using a superoxide dismutase (SOD) Assay Kit (Dojindo Laboratories, Kumamoto, Japan), following the manufacturer's instructions. The level of MDA in the plasma was also quantified using a commercially available kit (Nanjing Jianchen Bioengineering Institute, Nanjing, China).

Statistical Analysis

The data are expressed as mean ± SEM. Comparison within groups was made by repeated measures ANOVA (or paired *t*-test when only 2 groups were compared), and comparison among groups (or *t*-test when only 2 groups were compared) was made by factorial ANOVA with Holm-Sidak test. The differences among groups with two dependent variables (the

administrations of $PM_{2.5}$ and tempol treatment) were determined using the two-way ANOVA, also followed by Holm-Sidak test. A value of $P<0.05$ was considered significant.

Results

Effects of $PM_{2.5}$ exposure on blood pressure and renal function in offspring

First, we authenticated the success of the $PM_{2.5}$ exposure model. We found that the lungs of the $PM_{2.5}$-exposed dams had increased infiltration of inflammatory cells, relative to the control dams (Fig. 2). There were also unabsorbed aggregates of particles that were encapsulated by inflammatory cells (Fig. 2), indicating that $PM_{2.5}$ was successfully infused into the alveoli of the $PM_{2.5}$-exposed dams.

Second, we observed whether placenta is affected by the $PM_{2.5}$ exposure. Similar with other studies [31, 32], our results showed that compared with the control dams, the mass, thickness, longitudinal and transverse diameters and surface area of the placenta were all increased in the $PM_{2.5}$-treated group, relative to the control dams (Table 1). We also checked some indices of inflammation and oxidative stress in the placenta. The results showed that both IL-4 and IL-6 levels (Fig. 3A and 3B) in the $PM_{2.5}$-treated group were higher than the control group. However, there were no differences of MDA and GSH-Px levels between the groups (Fig. 3C and 3D).

We measured, next, the effects of $PM_{2.5}$ exposure on blood pressure and renal function in the offspring. Systolic blood pressures were significantly higher in the offspring of $PM_{2.5}$-treated dams than the offspring of vehicle-treated dams at 10 weeks of age and remained significantly elevated until the end of the study (14 weeks of age) (Fig. 4A). The high blood pressure could be ascribed to sodium and water retention, because 24h urine volume and sodium excretion were both lower in the offspring of the $PM_{2.5}$-treated dams than the offspring of the control dams (Fig. 4B and 4C). The differences in sodium and water excretion between $PM_{2.5}$-treated and vehicle-treated dams were also observed at 10 weeks of age, when the blood pressure differences were different between the two groups. However, there were no differences in the weights of the offspring of vehicle (control)- and $PM_{2.5}$-treated dams (Fig. 4D).

Role of D_1R and GRK4 in $PM_{2.5}$–induced hypertension in offspring

Renal D_1-like receptors play a vital role in the regulation of sodium balance and blood pressure [11–14]. To determine whether or not impaired D_1-like receptor function is involved in the hypertension in the offspring of $PM_{2.5}$-treated dams, we studied the diuretic and natriuretic effects of fenoldopam, a D_1-like receptor agonist, infused directly into the right renal artery, via the right suprarenal artery. The results showed that fenoldopam treatment induced both natriuresis and diuresis in the offspring of control dams, whereas such effects were markedly attenuated in the offspring of $PM_{2.5}$-treated dams (Fig. 5A and 5B).

Impaired renal D_1R function in hypertensive states is due to renal D_1R hyper-phosphorylation, which has been ascribed to the increased GRK4 expression and activity [33–36]. Our present study found that renal D_1R expression was lower in the offspring of

$PM_{2.5}$-treated dams than the offspring of vehicle-treated dams (Fig. 5C). Moreover, renal GRK4 expression was higher in the offspring of $PM_{2.5}$-treated dams than the offspring of vehicle-treated dams (Fig. 5D), indicating that GRK4 may be involved in the dysfunction of renal D_1R in $PM_{2.5}$-induced hypertension.

Role of ROS in the $PM_{2.5}$–induced hypertension in offspring

Both in vivo and in vitro studies have shown that $PM_{2.5}$ exposure leads to increased inflammation, enhanced production of reactive oxidative species (ROS), mitochondrial oxidative DNA damage and incident hypertension [37, 38]. Thus, we wondered whether ROS are also involved in the pathogenesis of hypertension in the offspring of $PM_{2.5}$-treated dams. We found that plasma MDA levels were higher but plasma SOD levels were lower in the offspring of $PM_{2.5}$-treated than vehicle-treated dams (Fig. 6A and 6B), suggesting that ROS may be associated with the increased blood pressure in the offspring of $PM_{2.5}$-treated dams.

To determine further the role of ROS in the hypertension of the offspring of $PM_{2.5}$-treated dams, the 12-week-old offspring of $PM_{2.5}$-treated and control dams were treated with tempol, a redox-cycling nitroxide, an efficient antioxidant [24], for 4 weeks. As shown in Fig. 6A and 6B, tempol treatment decreased the increased plasma MDA levels and increased the decreased plasma SOD levels in the offspring of $PM_{2.5}$-treated dams. Furthermore, not only did tempol increase the $PM_{2.5}$-mediated decrease in urine volume and sodium excretion, but also normalized the $PM_{2.5}$-mediated increase in blood pressure in the offspring. By contrast, tempol had no such effects in the offspring of control dams (Fig. 7A, 7B, and 7C). These results suggest reducing in ROS production can normalize the decreased water and sodium excretion and increased blood pressure of the offspring of $PM_{2.5}$-treated dams.

We also determined whether treatment of tempol could reverse the decreased renal D_1R expression and dysfunction in the offspring of $PM_{2.5}$-treated dams. We found that tempol normalized the increased renal D_1R phosphorylation and decreased D_1R expression in the offspring of $PM_{2.5}$-treated dams (Fig. 8A and 8B). Furthermore, the increased renal GRK4 expression was also normalized by tempol in the offspring of $PM_{2.5}$-treated dams (Fig. 8C). Interestingly, we found that c-Myc, a transcription factor regulating GRK4 [36], which was increased in the offspring of $PM_{2.5}$-treated dams, was also normal by tempol (Fig. 8D), indicating that antioxidants by decreasing c-Myc expression, normalize an increase in GRK4 abundance, leading to normalization of D_1R expression and function in the offspring of $PM_{2.5}$-treated dams.

Discussion

Exposure to ambient particulate matter has been shown to be associated with increased hospital admission, cardiovascular diseases, and cardiopulmonary mortality [39–41]. Hypertension, an established risk factor for cardiovascular disease, has been implicated in the cardiovascular morbidity and mortality related to ambient particulate matter [42]. Epidemiological studies have found that traffic or $PM_{2.5}$ exposure can increase blood pressure in both the short-term and long-term [43, 44], which were also validated in animal

experimental studies [45, 46]. To date, there are no studies that have examined the association between prenatal $PM_{2.5}$ exposure and hypertension in the offspring, although this association has been studied extensively, in other susceptible populations, especially the elderly [43]. Our current study showed that in utero exposure to $PM_{2.5}$ can increase the blood pressure in adult offspring. However, there are no differences in the weights between the adult offspring of vehicle (control)- and $PM_{2.5}$-treated group, suggesting that the offspring of $PM_{2.5}$ exposed mothers are not growth restricted in our present study. Similar with other studies [31, 32], our results also showed that placental morphology is affected by the $PM_{2.5}$ exposures. Oxidative stress markers such as IL-4 and IL-6 levels in the placenta of the $PM_{2.5}$-treated group were also higher than the control group.

The mechanisms by which $PM_{2.5}$ exposures in utero induce cardiovascular disease in the adult offspring are poorly understood. In rodent models, acute and chronic inhalation of $PM_{2.5}$ can elicit systemic vascular inflammation and impair vascular reactivity [47–50]. In addition to arterial dysfunction, $PM_{2.5}$-induced hypertension is associated with decreased urinary sodium excretion [51]. Renal sodium excretion is under hormonal control. For example, dopamine produced in the renal tubules decrease sodium reabsorption by inhibition of sodium exchangers, transporters, ion channels, e.g., apical Na^+-H^+ exchanger in the proximal tubule and thick ascending limb of Henle, and basolateral Na^+-K^+-ATPase in almost all the nephron segments [11–14]. Renal dopamine, independent of renal nerves, plays an important role in maintaining sodium homeostasis and normal blood pressure, especially under conditions of moderate sodium excess. In these situations, the renal dopaminergic system, mainly via the D_1R, is responsible for more than 50% of renal sodium excretion [16–18, 52]. Abnormalities in renal dopamine production and D_1R response to an increased sodium load have been implicated in the diminished natriuretic response and increase in blood pressure in hypertensive patients and rodents [52, 53]. Our current study showed that D_1R-mediated natriuresis and diuresis are impaired in the offspring of $PM_{2.5}$-treated dams. The decreased renal expression and increased phosphorylation of the D_1R in the kidney may be involved in the renal D_1R dysfunction in the hypertension of these offspring.

Increased GRK4 expression and activity are involved in the hyper-phosphorylation, desensitization, and uncoupling of the D_1R from G- and effector proteins. GRK4 activity and expression are increased in hypertensive humans and animal models of hypertension [11, 52, 53]. Our present study found that GRK4 expression is higher in the offspring of $PM_{2.5}$-treated dams, which supports the notion that GRK4 is involved in the impaired function of renal D_1R in the hypertension of these offspring.

Many studies have shown that the impaired intrarenal D_1R signaling in hypertension can cause or be caused by oxidative stress [18, 53]. Hypertensive patients and animals with hypertension have decreased antioxidant capacity and produce excessive amounts of ROS [53, 54]. Antioxidant treatment could mitigate the increased ROS production, in part by increasing antioxidant enzymes, such as SOD and GSH. $PM_{2.5}$ is known to increase serum TNF-α levels, stimulate macrophages to generate ROS, and cause oxidative stress [47–49]. $PM_{2.5}$ compounds, whose composition largely depends on their sources, typically contain organic chemicals, metals, soot, soil, dust, allergens, and acids on their surface. When

inhaled, these particles, alone or through chemical reactions, initiate the production of ROS; a certain amount of ROS are needed for normal cell function but excessive production of these free radicals cause injury in cells, tissues, and organs including lung, heart, and vasculature. To prove the role of ROS in the $PM_{2.5}$-induced hypertension in offspring, we used tempol, a redox-cycling nitroxide, which has been shown to reduce GRK4 expression, restore D_1R expression and function, and normalize blood pressure by decreasing oxidative stress in old and obese rats [24, 33]. Our current study showed higher ROS levels and lower antioxidant capacity in the offspring of $PM_{2.5}$-treated dams. However, the administration of tempol, by restoring the production of ROS to a normal state, ameliorated the $PM_{2.5}$-mediated impairment of natriuresis and diuresis, and normalized the high blood pressure of the offspring from $PM_{2.5}$-treated dams. These beneficial effects of tempol may be related to the normalization of renal D_1R function because tempol treatment also normalized the renal D_1R phosphorylation and expressions of D_1R and GRK4. The transcription factor c-Myc positively regulates GRK4 protein expression via binding to the promoter of GRK4 [34]. We also found that the increased renal c-Myc expression in the offspring of $PM_{2.5}$-treated dams was also normalized by the administration of tempol.

Conclusion

In summary, our study provides direct evidence supporting the hypothesis that $PM_{2.5}$ exposure in utero leads to hypertension in the adult offspring that is caused, by the dysfunction of renal D_1R. Oxidative stress, associated with increased c-Myc expression, enhances GRK4 levels, impairs renal D_1R expression and function, causing a decrease in sodium excretion, and consequently increasing blood pressure of the adult offspring of $PM_{2.5}$-exposed dams. These abnormalities were normalized by the antioxidant, tempol, Therefore, restoration of D_1R function by antioxidants may be a potential therapeutic target for $PM_{2.5}$-mediated hypertension.

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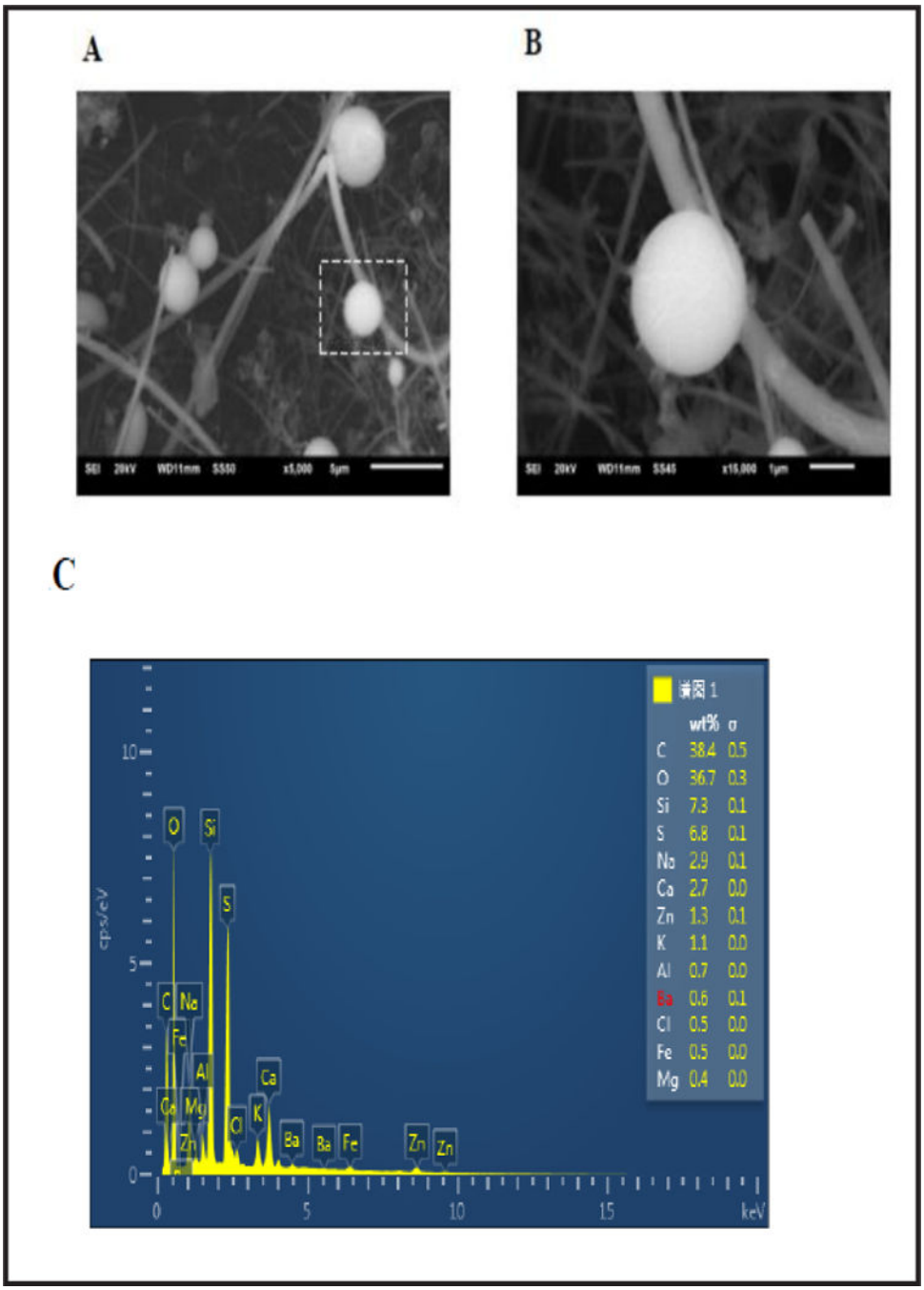

Fig. 1.

Analysis of $PM_{2.5}$ samples. Analysis of $PM_{2.5}$ samples with scanning electron microscope showed that the collected samples were less than 2.5μm in diameter (A). High power image of particles showed the exact size of the particle (B). Main chemical components of collected $PM_{2.5}$ samples were also analyzed by Energy Dispersive Spectroscopy (C).

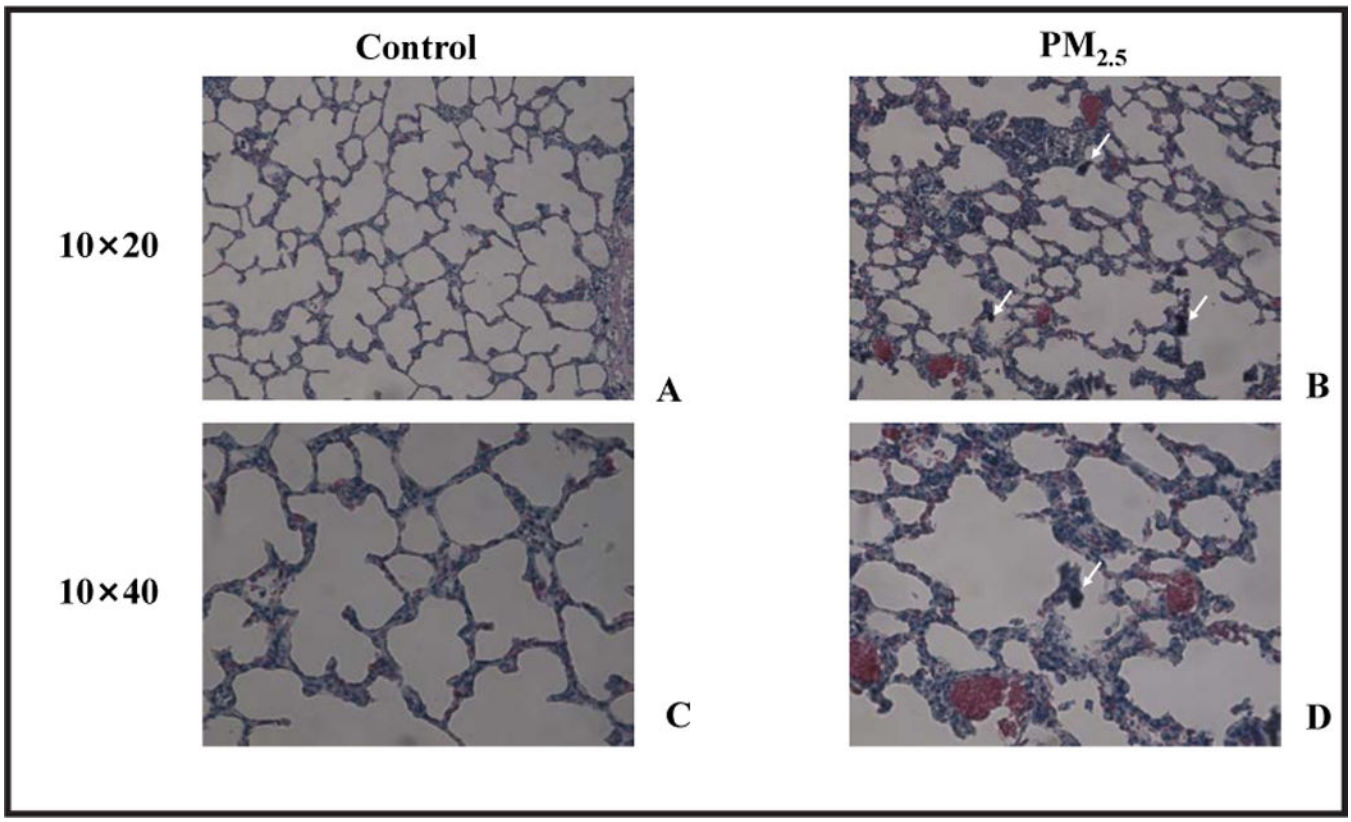

Fig. 2.

Instillation of $PM_{2.5}$ induces pulmonary inflammation and alveolar remodeling. Representative light microscopy sections of lung tissue from control and $PM_{2.5}$-treated dams, showing particulate matter deposition with obvious alveolar inflammatory response (A and B). High power images show unabsorbed particles in the alveoli (C and D).

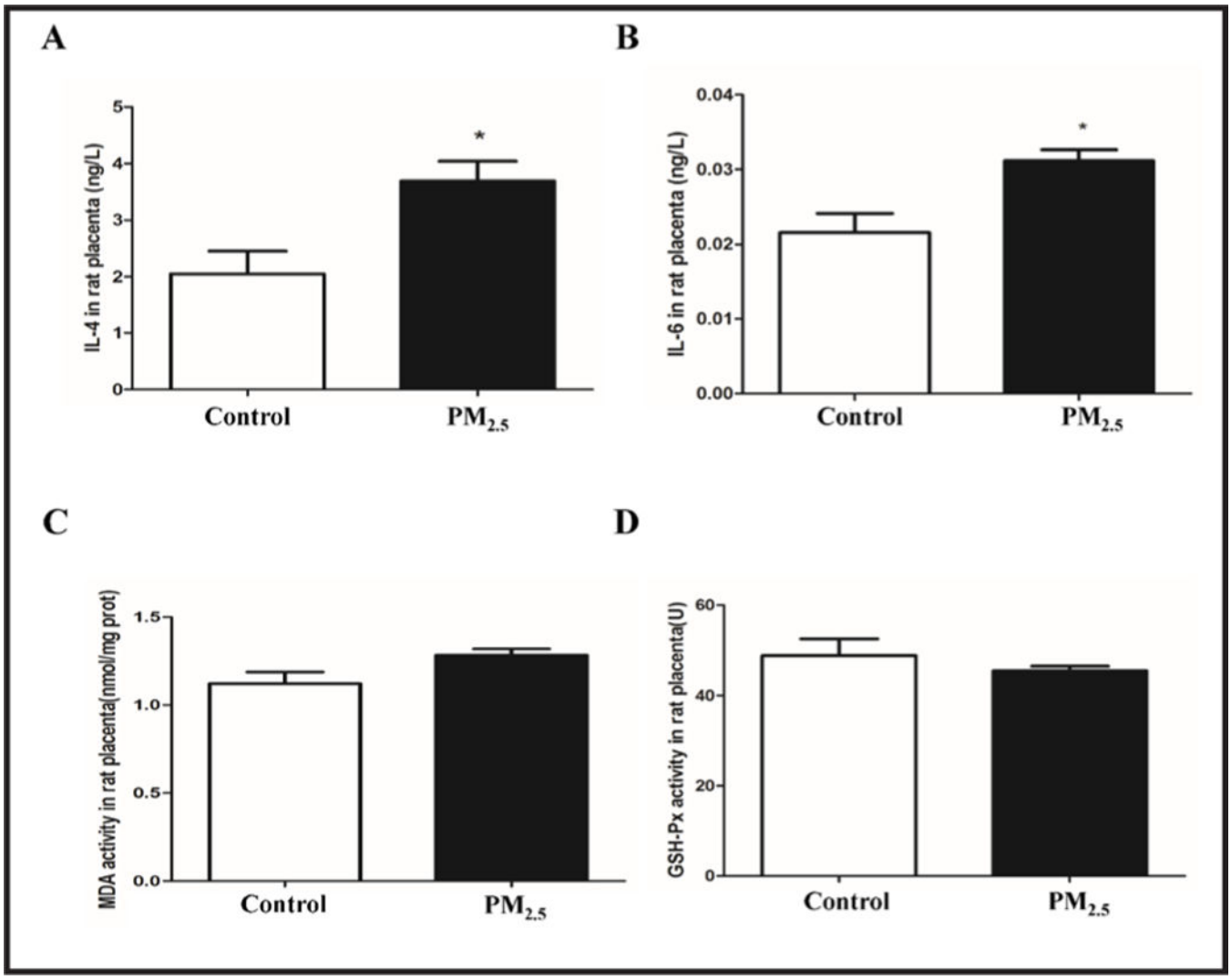

Fig. 3.

Effects of prenatal $PM_{2.5}$ exposure on placental inflammation and oxidative stress biomarkers. Placental inflammation and oxidative stress biomarkers were detected. IL-4 and IL-6 levels (A and B) were higher in the $PM_{2.5}$-treated than the control group (*$P<0.01$). MDA and GSH-Px (C and D) of placental homogenates were not significantly different between the two groups (n=4).

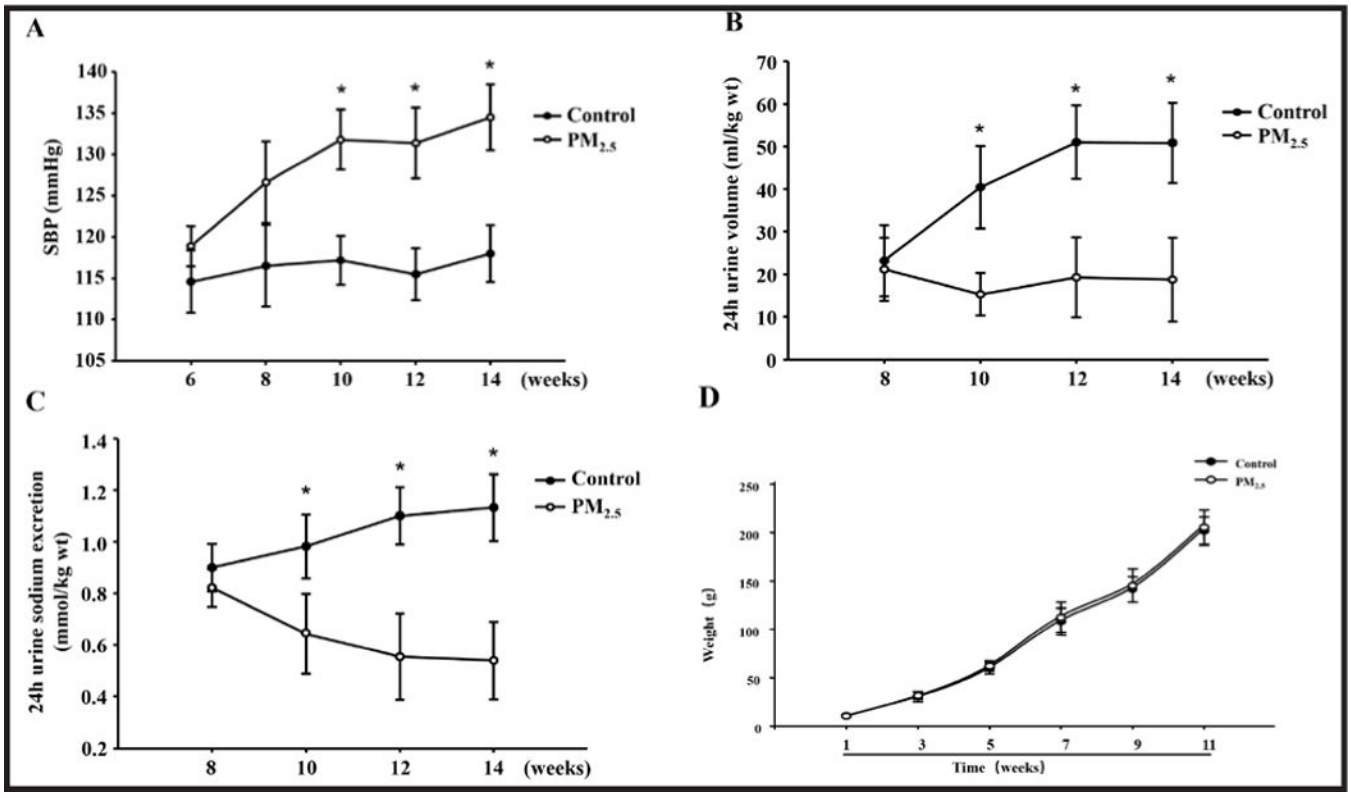

Fig. 4.

Effect of prenatal $PM_{2.5}$ exposure on blood pressure and sodium excretion in adult offspring. Systolic blood pressure (SBP) was measured by the tail-cuff method in 6-, 8-, 10-, 12- and-14-week-old offspring of vehicle- (control) and $PM_{2.5}$-treated dams (A). The 24 h urine volume (B) and sodium excretion (C) were measured in 8-, 10-, 12- and 14-weeks-old offspring of vehicle (control)- and $PM_{2.5}$-treated dams. The weights of the offspring were also observed (D). *$P<0.05$ versus 6 weeks of $PM_{2.5}$-treated dams and offspring of control group (n=12–15).

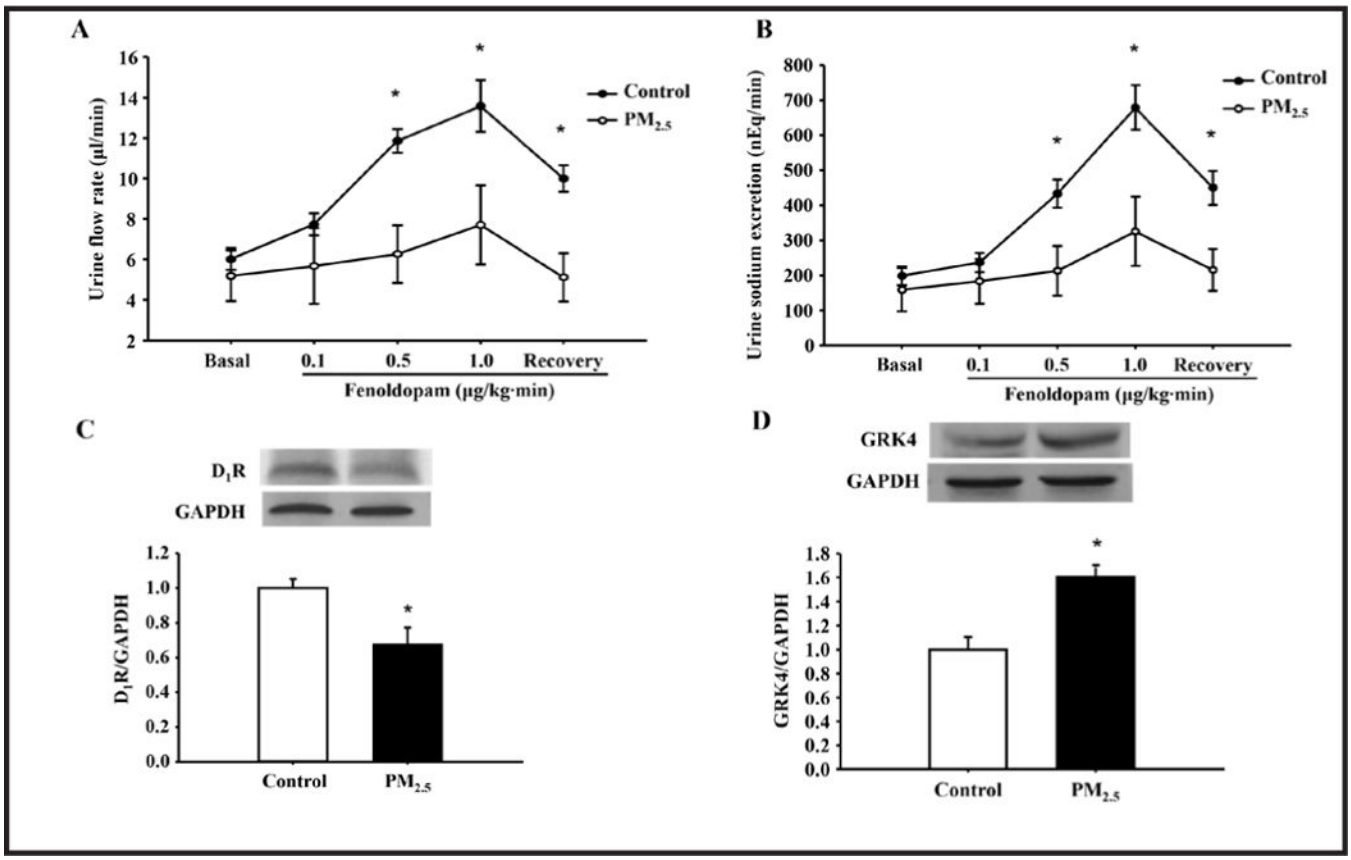

Fig. 5.

Effect of prenatal $PM_{2.5}$ exposure on renal D_1R function in adult Offspring. Urine flow rate (A) and urine sodium excretion (B) were measured in 12-week-old offspring of vehicle (control)- and $PM_{2.5}$-treated dams. Basal: values before fenoldopam administration; Fenoldopam: values during fenoldopam administration; Recovery: values after stopping the fenoldopam infusion. Renal cortical D_1R expression (C) and GRK4 expression (D) were quantified by immunoblotting in renal cortex (total protein) from12-week-old offspring of vehicle (control)- and $PM_{2.5}$-treated dams, *P<0.05 versus offspring of control group (n =8–10).

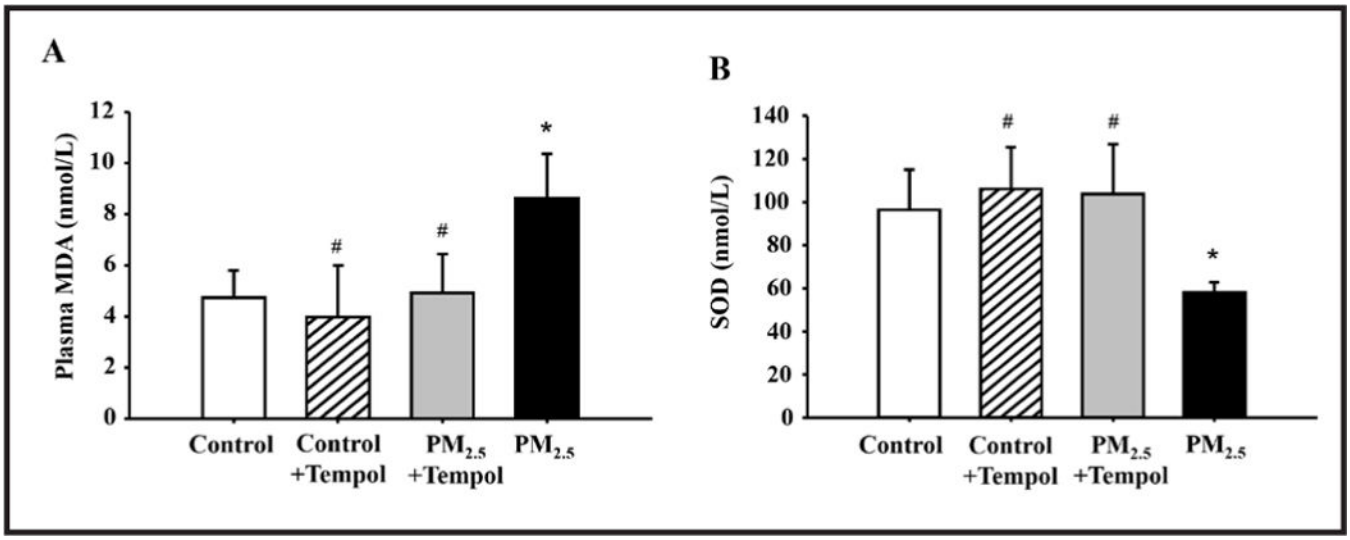

Fig. 6.

Effects of prenatal $PM_{2.5}$ exposure on measures of oxidative stress in adult offspring. Plasma MDA (A) and plasma SOD (B) were quantified in 16-week-old offspring of vehicle (control)- and $PM_{2.5}$-treated dams after treatment with vehicle or tempol (1.0 mmol/L in drinking water) for 4 weeks. *P<0.05 versus offspring of control group, $^{\#}$P<0.05 versus offspring of $PM_{2.5}$-treated group (n=6).

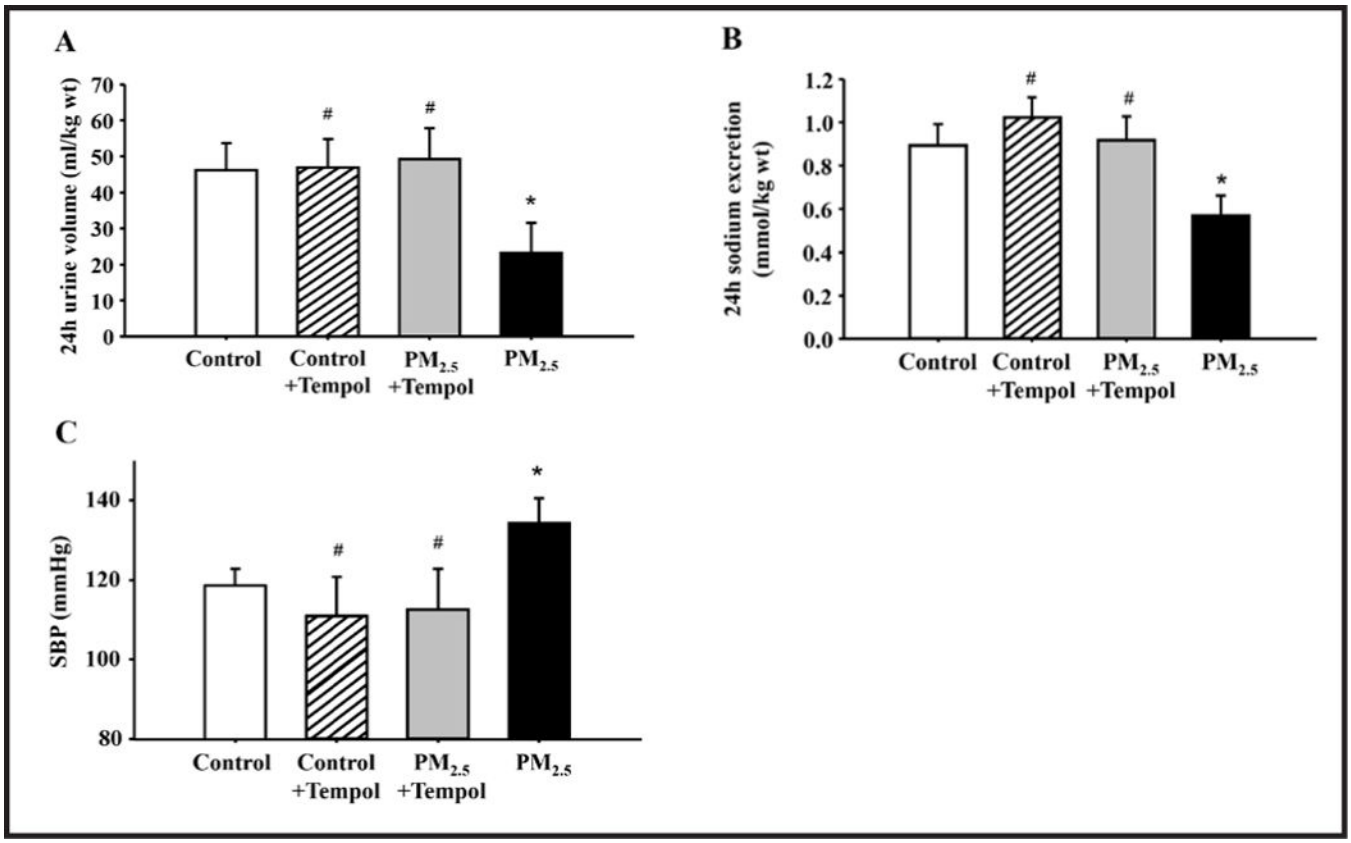

Fig. 7.

Effects of tempol on blood pressure and 24 h urine sodium excretion in adult offspring of $PM_{2.5}$-treated dams. The 24 h urine volume (A), sodium excretion (B) and systolic blood pressure (SBP) (C) were measured in 16-week-old offspring of vehicle (control)- and $PM_{2.5}$-treated dams after treatment with vehicle or tempol (1.0 mmol/L in drinking water) for 4 weeks. *P<0.05 versus offspring of control group, $^{\#}$P<0.05 versus offspring of $PM_{2.5}$-treated group (n =8).

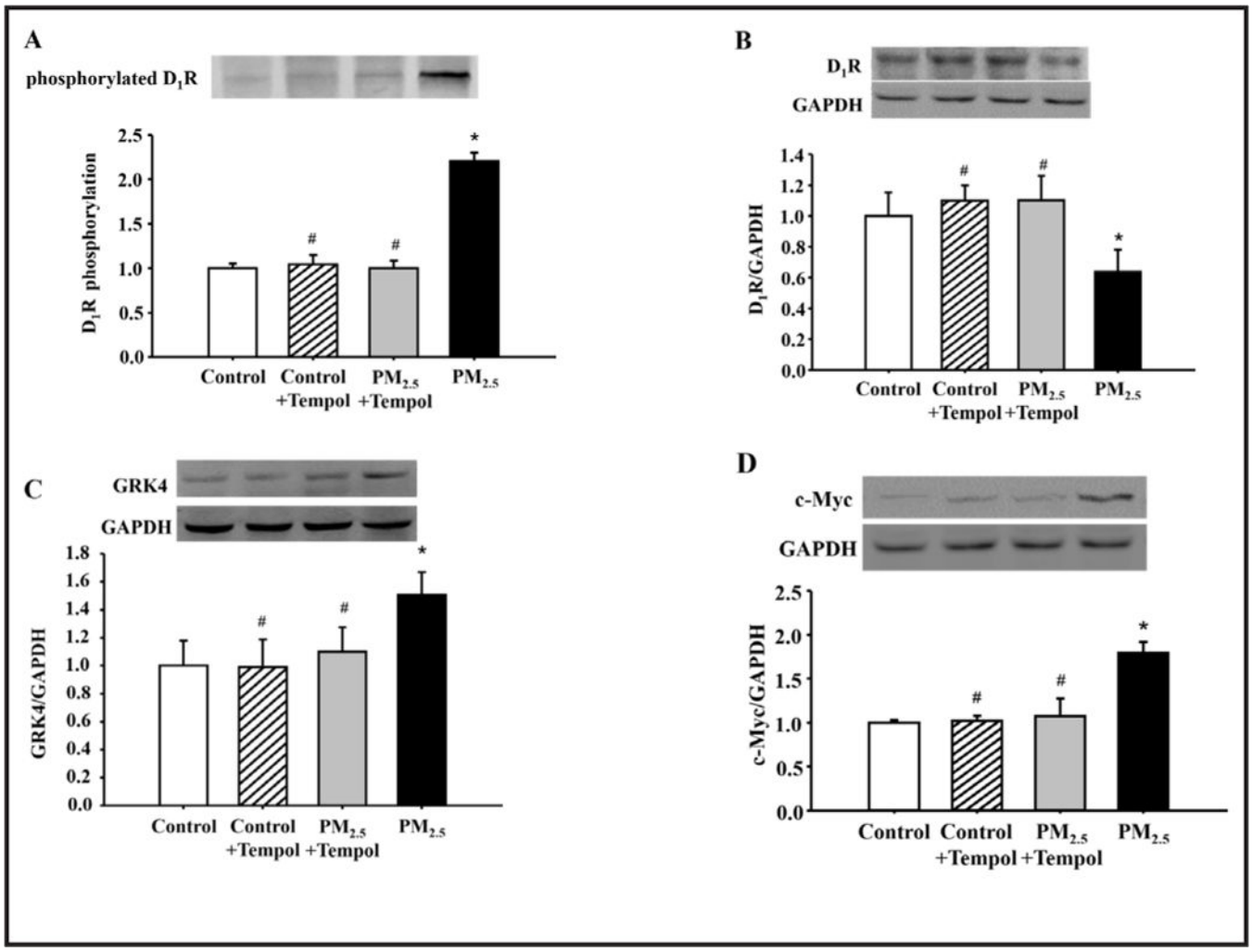

Fig. 8.

Effects of tempol on renal D_1R and GRK4 expressions in adult offspring of $PM_{2.5}$-treated dams. Renal cortical phosphorylation of D_1R (A), total D_1R (B),GRK4 (C), and c-Myc (D) expressions were quantified by immunoblotting in 16-week-old offspring of vehicle (control)- and $PM_{2.5}$-treated dams after treatment with vehicle or tempol (1.0 mmol/L in drinking water) for 4 weeks, *P<0.05 versus offspring of control group, # P<0.05 versus offspring of $PM_{2.5}$-treated group (n =8).

Table 1.

Mass, thickness, longitudinal and transversal diameters, and surface area of the placenta. LD: longitudinal diameter; TD: transverse diameter. Results are shown as mean±SEM.

	Mass (g)	Thickness (mm)	LD (mm)	TD (mm)	Surface area (mm^2)
Control	0.436±0.02	4.00±0.16	13.32±0.3	11.04±0.2	115.9±5.1
$PM_{2.5}$	0.557±0.03*	4.47±0.13*	14.43±0.3*	12.53±0.5*	142.7±8.1*

*P<0.05 versus vehicle-treated (control) group (n=4)