Original Research Article

Nerve growth factor protects against cadmium-induced hypertension in mice via vascular remodeling

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Abstract

Purpose: To investigate the effect of nerve growth factor (NGF) on cadmium ion (Cd^{2+})-induced hypertension in a mouse model, and the mechanism of action involved.

Methods: Hypertension was induced in mice by administration of cadmium chloride ($CdCl_2$) at a dose of 100 mg/L in deionised water. Then, NGF was administered daily for 45 days via the intragastric route. Immunohistochemical technique employing Vectastain ABC kit was used for determination of the levels of matrix metalloproteinases in mice aorta.

Results: Treatment with NGF significantly and dose-dependently alleviated Cd-induced increase in blood pressure in mice ($p < 0.05$). The Cd-induced elevation in mean arterial pressure (MAP) in mice was also reduced on treatment with NGF at doses of 5 and 10 mg/kg. At the two doses of NGF, vascular responsiveness was enhanced in Cd-administered mice. Exposure to NGF dose-dependently reversed Cd-mediated suppression of eNOS expression and elevation in iNOS level. Moreover, NGF at doses of 5 and 10 mg/kg, reversed Cd-mediated enhancement in nitrate/nitrite levels in urine samples, and reversed Cd-induced elevation in vascular smooth muscle cell (VSMC) count and collagen content in mice arterial wall. The NGF treatment also significantly ($p < 0.05$) reduced Cd-induced increases in levels of MMP's.

Conclusion: The present study demonstrates that NGF increased vascularity and arterial stiffness, and protected against Cd-induced hypertension in mice. Moreover, NGF treatment elevated eNOS expression, suppressed iNOS level and inhibited MMP expression in mice exposed to Cd. Thus, NGF has anti-hypertensive potential and may be beneficial in the treatment of hypertension.

Keywords: Hypertension, Nerve growth factor, Cadmium, Oxidative stress

INTRODUCTION

Cadmium ion (Cd^{2+}) is one of the most toxic metals associated with serious health problems in human beings, and its abundance in the environment is on the increase [1]. Cadmium exposure leads to oxidative stress, followed by damage to various organs such as liver, lungs, kidneys and bones, as well as impairment of immune and cardiovascular functions [2]. Studies have revealed that the most critical target of Cd exposure is the vascular system, resulting in

associated disorders such as diabetes and hypertension [3,4]. Multiple epidemiological investigations have found that elevated Cd^{2+} concentrations in blood are associated with high blood pressure [5,6]. Animal experimental investigations have also demonstrated that blood pressure is elevated by Cd exposure [7,8]. Although elevation in blood pressure by Cd exposure has been clearly demonstrated, the mechanism involved is yet to be fully understood.

Studies indicate that Cd exposure initiates oxidative stress which influences vascular cells via multiple ways, resulting in damage and dysfunction of vascular system [9]. Moreover, oxidative stress decreases the levels of the nitric oxide (NO) in the vasculature, leading to inflammation and injury, followed by oxidative changes in lipids, proteins and DNA [10]. Reactive oxygen (ROS) as well as nitrogen species (RNS) regulate vascular function by influencing contraction and dilation, growth and apoptosis, as well as migration of vascular cells [11]. Moreover, ROS and RNS regulate the turnover of extracellular matrix proteins, thereby affecting the stiffness and remodeling of vessels [11]. Thus, enhancement of NO bioavailability, suppression of ROS levels, enhancement of stiffness and remodeling of vascular system have therapeutic effects on hypertension.

Studies have found that anti-oxidant compounds and metal-chelating agents are potential antidotes for disorders associated with metal poisoning. The uncoupling of nitric oxide synthase (eNOS) and activation of MMPs in arterial endothelium are induced by generation of ROS [10]. The present study investigated the effect of nerve growth factor (NGF) on cadmium ion (Cd^{2+})-induced hypertension in a mouse model. The mechanism involved in NGF-mediated alleviation of Cd-induced hypertension was also investigated.

EXPERIMENTAL

Animals

Sixty ICR mice (each weighing about 28 g) were purchased from the Animal Laboratory of Beijing Institute, Chinese Academy of Medical Sciences, China. All mice were maintained at room temperature (22 ± 2 °C) under 12-h dark/12-h dark light cycle, and given free access to water and standard rat chow. The study was conducted according to the guidelines issued by the National Institute of Health, China [12]. Approval for the animal protocols was obtained from the Ethics Committee for Animal Experiments Medical University, China (no. MUBC/17/0013) [12].

Experimental protocols

Following 1-week of acclimatization to laboratory conditions, the mice were assigned randomly to six groups: group I (normal control), group II (given NGF at a dose of 5 mg/kg), group III (given NGF at a dose of 10 mg/kg), group IV (given $CdCl_2$ at a dose of 100 mg/L), group V (given $CdCl_2$ at a dose of 100 mg/L and NGF at a dose of 5 mg/kg), and group VI (given $CdCl_2$ at a dose of 100 mg/Lin addition to NGF at a dose of 10 mg/kg). Mice were given $CdCl_2$ (100 mg/L) in deionised water and NGF for 45 days daily via the intragastric route. Doses of $CdCl_2$ and NGF were decided based on previous reported studies.

Blood pressure, vascular reactivity and heart rate determination

At the end of NGF treatment, all mice were anesthetized by injecting ketamine and xylaxine at doses of 100 mg/kg and 2.5 mg/kg, respectively. Blood pressure measurement and determination of vascular reactivity were carried out in line with protocols in previous reports. The body temperature of the animals was maintained using heating pads. Tracheotomy was done to enable the animals breathe spontaneously. The carotid artery was carefully exposed and then connected with pressure transducer using a polyethylene tubing so as to monitor heart beat and arterial blood pressure using data acquisition system (Biopac System Inc., CA, USA). The administration of vasoactive agents was done through the left side jugular vein cannulated to polyethylene tubing. Initially, baseline measurements were recorded. Then, ACh (10 nmol/kg), a vasodilator that influences the endothelium; SNP (10 nmol/kg SNP), a vasodilator independent of endothelium, and Phe (0.03 mmol/kg) were infused randomly and blood pressure was recorded continuously. After drug infusion, the animals were allowed to stabilize for 5 min so that blood pressure returned to baseline level. Blood pressure changes were determined by comparing with values obtained immediately prior to infusion of test compound.

Measurement of anti-oxidant levels

Mice were sacrificed after completion of treatment by injection of overdose of anaesthesia. Blood samples were taken from aorta of the abdominal region for determination of antioxidants and markers of oxidative stress.

Levels of superoxide anion (O^{2-}) and GSH were measured in blood samples using protocols reported previously [12]. Malondialdehyde (MDA) levels were determined with TBA assay [12], while concentrations of protein carbonyl groups were determined using DNPH method [12]. Urine samples of mice were analysed for NO, nitrate and nitrite levels using previously reported methods.

Protein expression levels of eNOS and iNOS proteins

Western blotting was used to determine the protein expression levels of eNOS and iNOS in mice aortas, as reported earlier [12]. The protein expression levels of eNOS and iNOS were normalized to that of β-actin which served as internal control.

Immunohistochemical assay

Immunohistochemical ready to use ABC kit (Vector Laboratories, Inc., CA, USA) was used for determination of the localization and levels of MMP's in mice aorta [12]. Following dewaxing, mice aortic artery sections were exposed to MMP-2 antibody (ab37150; Abcam) and MMP-9 antibody (ab19016; Millipore). Single batch experiments were conducted using specimens as well as positive control. The stained specimens were examined under a light microscope, and images were recorded at 6400. The content of MMP's in aortic wall was determined by counting the threshold pixels stained by antibodies using Image-Pro Plus Program (Media Cybernetics, MD, USA).

Analysis of vascular wall composition and morphology

The middle part of descending thoracic aorta was fixed in 4 % phosphate-buffered paraformaldehyde after cleaning, and subsequently embedded in paraffin. For determination of smooth muscle cell count (SMCs), thin slices obtained were stained in hematoxylin and eosin (H & E). Collagen and elastin fractions in thoracic aorta were determined by staining the slices with picrosirius red and Miller's elastic stain, respectively [12]. The SMC count was determined by calculating the population of stained nuclei, while collagen and elastin fractions were measured by calculation of the stained threshold pixels from picrosirius red stain and Miller's elastic stain, respectively. The stained sections were examined under the light microscope, while KS400 image system (Carl Zeiss Microscopy) was used to evaluate the aortic walls.

Statistical analysis

The data obtained are expressed as mean ± standard deviation SD) of triplicate measurements. The data were analyzed statistically using one-way analysis of variance (ANOVA) along with Newman–Keuls posthoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

NGF alleviated Cd-induced hypertension

Blood pressures were significantly ($p < 0.05$) raised in mice by Cd administration (Table 1). Moreover, Cd administration led to increased MAP (Table 1). Administration of NGF at doses of 5 and 10 mg/kg to normal mice did not induce any change in blood pressure and MAP. However, NGF significantly and dose-dependently reversed Cd-induced increase in blood pressure in mice ($p < 0.05$). In mice treated with NGF (10 mg/kg), the Cd-induced increase in blood pressure was successfully reduced close to blood pressure of normal mice. In addition, the Cd-induced elevation in MAP in mice was reduced on treatment with NGF at doses of 5 and 10 mg/kg.

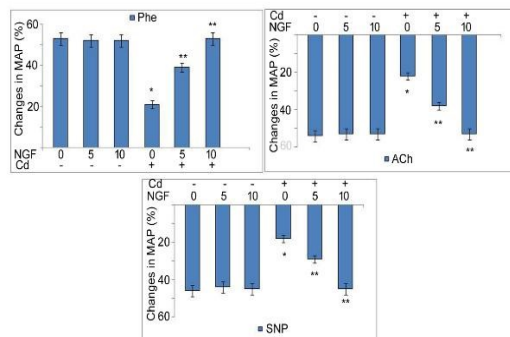## NGF attenuated vascular dysfunction induced by Cd in mice

Mice administered Cd had impaired vasodilating and vasoconstricting profiles, when compared to the control group, as indicated by hypo-responsiveness to Phe, ACh and SNP (Figure 1).

Table 1: Effect of NGF on Cd-induced blood pressure in mice

Variable	Control	NGF (5 mg/kg)	NGF (10 mg/kg)	Cd (Model)	Cd + NGF (5 mg/kg)	Cd + NGF (10 mg/kg)
Systolic pressure (mmHg)	119 ± 3	118 ± 2	119 ± 3	163 ± 4 *	142 ± 5 *#	120 ± 5*#
Diastolic pressure (mmHg)	80 ± 5	79 ± 3	80 ± 0	120 ± 5 *	103 ± 4 *#	81 ± 4#
MAP (mmHg)	97 ± 3	96.8 ± 4	97 ± 4	139 ± 5 *	117 ± 4 *#	97 ± 3#

*$P < 0.05$, vs. normal mice, #$p < 0.05$, vs. Cd group

However, NGF treatment enhanced vascular responsiveness in Cd-administered mice in a dose-based manner. The NGF-induced increase in vascular responsiveness in Cd-administered mice was more effective at a dose of 10 mg/kg than at a dose of 5 mg/kg.

reduced close to their levels in normal mice. The blood GSH level was significantly ($p < 0.05$) suppressed in Cd-administered mice, when compared to the control group. However, NGF treatment reversed the Cd-induced reduction in blood GSH levels in the mice.

Figure 1: Effect of NGF on vascular responsiveness in Cd-administered mice. Baseline measurements were recorded first. Then, Ach, SNP and Phe were infused randomly. Continuous recording of blood pressure was made to determine mean arterial pressure (MAP). *$P < 0.05$, vs. control group; **$p < 0.02$, vs. Cd model group

Figure 2: Effect of NGF on eNOS and iNOS in mice aortas. Mice administered Cd were treated with NGF for 45 days, and eNOS and iNOS levels were assayed using western blotting. *$P < 0.05$, vs. control group; **$p < 0.02$ vs. Cd group

NGF attenuated Cd-induced changes in eNOS and iNOS

Administration of Cd to mice caused marked reduction in eNOS expression and elevation in iNOS level in the aortas (Figure 2). However, NGF treatment dose-dependently reversed the Cd-mediated suppression of eNOS expression and elevation of iNOS level. In the group treated with NGF at a dose of 10 mg/kg, eNOS expression was reduced, and iNOS level was elevated close to those of normal mice.
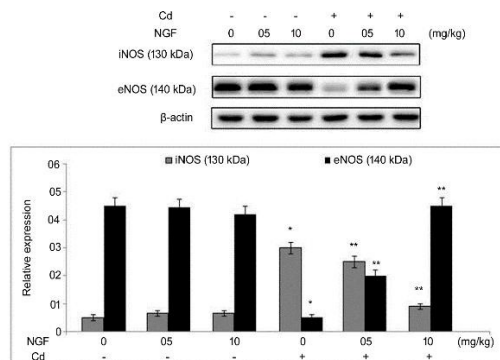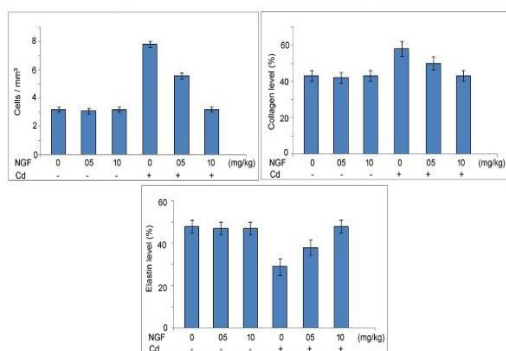
NGF reduced Cd-induced increases in NO

In Cd-exposed mice, nitrate/nitrite levels were significantly increased in urine samples, relative to control mice ($p < 0.05$; Table 2). However, NGF treatment significantly and dose-dependently reduced the Cd mediated enhancement in nitrate/nitrite levels in urine samples. In the group treated with NGF at a dose of 10 mg/kg, the Cd-induced increases in nitrate/nitrite levels in urine samples were

NGF increased vascular remodeling in Cd-administered mice

In Cd-administered mice, arterial wall hypertrophy was caused by significant increases in VSMC count and collagen content ($p < 0.05$; Figure 3). Moreover, Cd administration led to a significant reduction in elastin content of aortic artery wall, relative to normal mice group ($p < 0.05$). However, NGF treatment led to marked decreases in Cd-induced elevation in VSMC count and collagen content in mice arterial wall. Moreover, the Cd-mediated reduction in elastin content in aortic artery wall of mice was significantly reversed by NGF treatment ($p < 0.05$).

NGF suppressed level of MMP's in Cd-exposed mice

The levels of MMP's in Cd-administered mice aortas were enhanced significantly ($p < 0.05$), relative to the corresponding levels in mice in control group (Figure 4).

Table 2: Effect of NGF on nitrate/nitrite levels in Cd-administered mouse urine

Variable	Control	NGF (5 mg/kg)	NGF (10 mg/kg)	Cd (Model)	Cd + NGF (5 mg/kg)	Cd + NGF (10 mg/kg)
Urinary nitrate /nitrite (nmol/mg creatinine)	896± 23	898 ± 34	900 ± 43	2015 ± 58*	1465 ± 41*#	903± 54*#
Blood GSH (mM)	798±32	794 ± 29	799 ± 30	268 ± 30*	499 ± 26*#	800 ± 48#

*$P < 0.05$, vs. normal mice; #$p < 0.05$, vs. Cd group

Figure 3: Effect of NGF on SMC count and content of collagen and elastin in Cd-exposed mice. Mice administered Cd were treated with NGF at doses of 5 and 10 mg/kg for 45 days. Then, SMC count and contents of collagen and elastin were determined. $P <$ 0.05, vs. control group; **$p < 0.02$ vs. Cd group

In Cd-administered group, MMP-2 level increased to 53.54 %, as against 12.4 % in control mice. Administration of Cd elevated MMP-9 level to 60.4 %, relative to 20.3 % in control mice. However, NGF treatment significantly ($p < 0.05$) reduced the Cd-induced enhancement in MMP's level. The Cd-induced increases levels of MMP's in mice were reduced close to those of control mice, on treatment with NGF at a dose of 10 mg/kg.
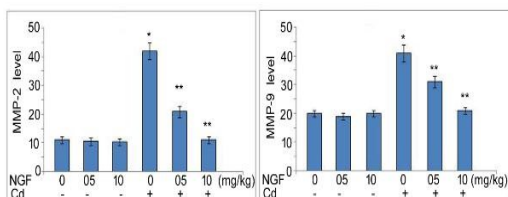
Figure 4: Effect of NGF on Cd-induced changes in expression levels of MMP-2 and MMP-9. Mice administered Cd were treated with NGF for 45 days. Then, MMP-2 and MMP-9 expressions in aorta were determined with western blotting. *$P < 0.05$, vs. control group; **$p < 0.02$ vs. Cd group

DISCUSSION

The induction of hypertension by Cd exposure is associated with elevated arterial blood pressure, and it constitutes a major risk to human health [2,7,12]. Exposure to Cd impairs the functioning of the vascular system and perturbs integrity of vascular supply [7,11]. Reports have shown that Cd exposure initiates oxidative stress, leading to endothelium damage and abnormalities in the vascular system [10-12]. In the present study, Cd administration significantly elevated systolic as well as diastolic blood pressures of mice. Moreover, Cd administration caused marked

enhancement in MAP in mice, when compared to normal mice.

However, NGF treatment significantly and dose-dependently attenuated the Cd-induced increase in blood pressure in mice. In the group treated with NGF at a dose of 10 mg/kg, the Cd-induced increase in blood pressure was successfully reduced to normal values. The Cd-induced elevation in MAP in mice was also reduced on treatment with NGF at doses of 5 and 10 mg/kg. Exposure to Cd promotes antagonism to Ca^{2+} channels, a phenomenon which is responsible for loss of Phe-induced vascular contractility [13]. The production of NO by endothelial cells enhances vasodilation, muscular relaxation, anti-oxidative properties, anti-thrombogenic potential and anti-inflammatory activities [14]. The bioavailability of NO is reduced by suppression of eNOS expression and deficiency of eNOS cofactors [15]. In Cd-exposed animals, vascular response to Ach is suppressed through reduction in eNOS expression and decrease in vaso-relaxation via endothelium-dependent pathway [9].

Tumor necrosis factor alpha (TNF-α), a mediator of inflammation, induces the activation of iNOS, resulting in increased levels of NO and several disorders [16]. In the current study, mice administered Cd had impaired vasodilating and vasconstricting properties, when compared to the control group, as was indicated by hypo-responsiveness to Phe, ACh and SNP. However, treatment with NGF enhanced vascular responsiveness in Cd-administered mice in a dose-based manner. Extracellular matrix reorganization via synthesis and degradation of proteins is an important feature of vascular remodeling during hypertension [17]. It has been demonstrated that activation of MMP through NADPH oxidase-mediated generation of ROS enhances mechanical stretch of arteries [18]. Exposure to Cd upregulates the expressions of MMP's, resulting in arterial wall inflammation [18]. The present study found enhanced expressions of arterial MMP'sin mice on exposure to Cd. However, NGF markedly reversed the Cd-induced upregulations in MMP-2 and MMP-9 levels. The Cd-induced higher levels of MMP's in mice were reduced close to those of control mice on treatment with NGF at a dose of 10 mg/kg.

CONCLUSION

The present study has demonstrated that NGF increased vascularity and arterial stiffness, and mitigated Cd-induced hypertension in mice. Moreover, NGF elevated eNOS expression,

suppressed iNOS level and inhibited MMP expression in mice exposed to Cd. Thus, NGF has beneficial anti-hypertensive potential which may be clinically useful.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hao Sun, Xinchun Yang, Meili Zheng, Jiamei Liu, Zongsheng Guo, Linying Shi, Boqia Xie, Yuan Zhang and Xin Wang performed the experimental work, carried out the literature survey, analysed and compiled the data. Lefeng Wang designed the study and wrote the paper. Both the authors read the paper thoroughly and approved it for publication.

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