Original Research

Sulfur dioxide derivatives improve the vasorelaxation in the spontaneously hypertensive rat by enhancing the vasorelaxant response to nitric oxide

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Abstract

The present study was designed to explore the role of sulfur dioxide (SO_2) in the regulation of vasorelaxation in the spontaneously hypertensive rat (SHR). Twenty-two Wistar rats and 15 SHRs were divided randomly into the following groups: Wistar-Kyoto (WKY) control ($n = 8$), WKY+Na_2SO_3/$NaHSO_3$ ($n = 8$), WKY+L-aspartic acid-β-hydroxamate (HDX) ($n = 6$), SHR control ($n = 8$) and SHR+Na_2SO_3/$NaHSO_3$ ($n = 7$). Their blood pressure *in vivo* was measured by tail plethysmography. The vasorelaxant response of the thoracic aorta to acetylcholine (Ach) and sodium nitroprusside (SNP) in all rats was tested, respectively, in the experiment. At the same time, the SO_2 content of the WKY aorta after incubation with HDX and the vasorelaxant response to Ach after incubation with HDX were quantified. Nitric oxide (NO) production in the aorta of all rats was determined. We also measured the vasorelaxant responses of WKY aorta to different concentrations of SO_2 after incubation with the NO inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME). The blood pressure decreased significantly in SHRs treated with SO_2 derivatives ($P < 0.05$). Reduction of endogenous SO_2 in WKY vessels resulted in a decrease in the vasorelaxation induced by Ach. Vasorelaxation in response to both Ach and SNP increased in SHRs treated with SO_2 derivatives compared with SHR controls, but decreased in WKY given HDX compared with WKY controls ($P < 0.05$). The NO level in arterial tissues increased in SHRs treated with SO_2 derivatives ($P < 0.05$). However, the vasorelaxant response to SO_2 derivatives in the presence of L-NAME decreased markedly compared with WKY controls. The results suggest that SO_2 reduced blood pressure and increased vasorelaxation in SHR arteries via enhancing the vasorelaxant response to NO in isolated aortic rings and increasing the NO level of aortic tissues.

Keywords: sulfur dioxide, nitric oxide, vasorelaxation, spontaneously hypertensive rat

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Introduction

Hypertension is the most common cardiovascular disease, but our understanding of the cellular processes involved is limited. Elucidating the physiological mechanisms responsible for hypertension is one of the most important issues in the field of medical science today. Historically, great progress has been made in research on hypertension concerning, for example, neurohumoral factors,[1] the renin–angiotensin system,[2] vasoactive peptides[3] and central nervous system control,[4] but the mechanisms of hypertension remain unclear.

Sulfur dioxide (SO_2) is considered a waste gas and air pollutant.[5–7] However, biochemical metabolic studies indicate that SO_2 can be derived endogenously from sulfur-containing amino acids *in vivo*.[8] L-cysteine is first oxidized to L-cysteine sulfinate and then, through transamination by aspartate aminotransferase, is converted to β-sulfinylpyruvate, which decomposes spontaneously to pyruvate and SO_2.[9] SO_2 is further metabolized to form sulfite *in vivo*, which is then oxidized by sulfite oxidase to sulfate and excreted in urine.[10–12] Endogenous SO_2 is hypothesized to be a physiological gasotransmitter in the regulation of cardiovascular function.[13] Our previous research demonstrated that the SO_2 level in plasma is decreased in spontaneously hypertensive rats (SHRs), and that endogenous SO_2 and its derivatives have a vasorelaxant function.[14,15] Consequently, we hypothesize that SO_2 might play a role in modulating hypertension.

Nitric oxide (NO) is the first gasotransmitter to be discovered in the body, and plays an important role in the pathogenesis of hypertension. Previous studies have indicated that SO_2 regulates smooth muscle tone, in synergy with NO, in the aorta of Wistar rats.[16] It has also been demonstrated that SO_2 increases the activity of endothelial nitric oxide synthase (eNOS) and increases expression of the eNOS gene at the transcription and translation levels in Wistar rat aorta.[17] However, the interaction between SO_2 and NO in hypertensive rats has not been defined. This study was designed to explore the possible role of SO_2 in the regulation of vasorelaxation in SHRs and to elucidate the possible mechanisms.

Materials and methods

Preparation of the animal model

The study protocol was approved by the Animal Research Committee of Peking University (Beijing, China).

In the *in vivo* part of the study, 22 male four-week-old Wistar-Kyoto (WKY) rats and 15 SHRs were divided randomly into a WKY control group ($n = 8$), a WKY+Na_2SO_3/$NaHSO_3$ group ($n = 8$), a WKY+L-aspartic acid-β-hydroxamate (HDX) group ($n = 6$), a SHR control group ($n = 8$) and a SHR+Na_2SO_3/$NaHSO_3$ group ($n = 7$). Na_2SO_3/$NaHSO_3$, a SO_2 derivative, was dissolved in physiological saline (0.9%) at 0.54 mmol/kg:0.18 mmol/kg body weight, every day, and was then administered to the animals by intraperitoneal injection. HDX, an inhibitor of the enzyme responsible for endogenous generation of SO_2, was given by intraperitoneal injection, at a dose of 3.7 mg/kg per day. An identical volume of physiological saline was injected into the rats in the other groups.

In the *in vitro* part of the study, isolated thoracic aortic rings from WKY rats weighing 220–250 g and male SHRs weighing 320–350 g, all aged 12 weeks, were used to analyse the interaction between SO_2 and NO. N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO, was administered at a dose of 100 μmol/L into the aortic rings of the WKY rats.

Chemicals and solution preparation

Sodium sulfite and sodium bisulfite (Na_2SO_3/$NaHSO_3$, SO_2 derivatives), HDX, noradrenaline (NE), acetylcholine (Ach), sodium nitroprusside (SNP) and monobromobimane (mBrB) were purchased from Sigma (St Louis, MO, USA). Other chemicals and reagents were of analytical grade. Levels of NO were measured using an NO detection kit (nitrate reductase method), which was purchased from NIBI (Nanjing, China). Krebs–Ringer bicarbonate (pH 7.4) incubation medium contained (mmol/L): 120 NaCl, 5.5 KCl, 2.5 $CaCl_2$, 1.2 MgSO4, 1.2 NaH_2PO_4, 20 $NaHCO_3$, 0.03 EDTA-Na_2 and 10 D-glucose.

Measurement of blood pressure

The blood pressure of rats in the WKY control group, the WKY+Na_2SO_3/$NaHSO_3$ group, the WKY+HDX group, the SHR control group and the SHR+Na_2SO_3/$NaHSO_3$ group was measured after eight weeks of treatment by tail plethysmography (Beijing, China) while the rats were awake.

Preparation of isolated aortic rings

In the *in vitro* part of the study, isolated thoracic aortic rings were prepared following the procedure described in previous publications.[18,19] Briefly, male WKY rats ($n = 22$) and SHRs ($n = 15$) were anesthetized, and their thoracic aorta removed immediately and stripped of fat and connective tissues. The aortas were then cut into rings of length of approximately 3 mm. The rings were placed in a bath of Krebs buffer at pH 7.4 and 37°C under a resting optimal tension of 1 g, while 95% O_2 and 5% CO_2 was bubbled through the buffer from the bottom of the tube. The tension was recorded with a JZ101 Force Transducer (Beijing, China), connected to a BL-420S Data Acquisition System (Chengdu, China) during the experiment. The aortic rings in the bath tubes were equilibrated for 60 min before beginning the experiment, and the Krebs buffer was changed every 15 min. The endothelial viability and integrity of the artery were tested by the dilatory response of the rings to Ach (1 μmol/L).

Vasorelaxation experiments *in vitro*

Isolated thoracic aortic rings from rats of the WKY control group, the WKY+Na_2SO_3/$NaHSO_3$ group, the WKY+HDX group, the SHR control group and the SHR+Na_2SO_3/$NaHSO_3$ group were pre-treated with a submaximal dose of NE (1 μmol/L) to initiate contraction and then incubated with Ach (1 μmol/L) or SNP (1 μmol/L).

To explore the effect of endogenous SO_2 on the vasorelaxation response to Ach, aortic rings from WKY rats were first incubated for 30 min with HDX (40, 100 or 160 μmol/L). Then, Ach (10^{-9}–10^{-6} mol/L) was added cumulatively to the bath solution until the vasoconstriction induced by 10^{-6} mol/L NE reached a plateau at maximal tension.

Assay of NO production in rat arterial tissues

Aortic samples from rats of the WKY control group, the WKY+Na_2SO_3/$NaHSO_3$ group, the WKY+HDX group, the SHR control group and the SHR+Na_2SO_3/$NaHSO_3$ group were used to measure NO production. Aortic tissue NO levels were measured using an NO detection kit according to the manufacturer's protocol. The absorbance was determined at 550 nm with a UV 2100 spectrophotometer (Shimadzu, Kyoto, Japan).

Determination of SO_2 content in rat arterial tissues

To measure the SO_2 content of the aortic rings, the rings were first incubated with HDX (40, 100 or 160 μmol/L), and then their sulfite concentration, which indirectly represents SO_2 content, was determined by high-performance liquid chromatography (HPLC) with fluorescence detection (Agilent series 1100; Agilent Technologies, Karlsruhe,

Germany).[20] In brief, 100 μL of sample was mixed with 70 μL of 0.212 mol/L sodium borohydride in 0.05 mol/L Tris-HCl (pH 8.5) and incubated at room temperature for 30 min. The sample was then mixed with 10 μL of 70 mmol/L mBrB in acetonitrile, incubated for 10 min at 42°C and mixed with 40 μL of 1.5 mol/L perchloric acid. Protein precipitates in the mixture were removed by centrifugation at 12,400 $\times$ *g* for 10 min at 23°C. The supernatant was immediately neutralized by adding 10 μL of 2 mol/L Tris-HCl (pH 3.0) and centrifuged at 12,400 $\times$ *g* for 10 min. The neutralized supernatant was used for HPLC. The column (4.6 $\times$ 150 mm C18 reverse-phase column, Agilent series 1100; Agilent Technologies) was first equilibrated with a buffer (methanol:acetic acid:water, 5.00:0.25:94.75 by volume; pH 3.4). The sample loaded onto the column was resolved by a gradient of methanol at a flow rate of 1.0 mL/min over the following time periods: 0–5 min, 3%; 5–13 min, 3–35%; 13–30 min, 35–62%; 30–31 min, 62–100%; 31–39 min, 100%; 39–40 min, 100–3%; and 40–45 min, 3%. Sulfitebimane was measured by excitation at 392 nm and emission at 479 nm. Sodium sulfite concentration was standardized for quantification and the tissue sulfite content was expressed as μmol/g protein.

Statistical analysis

Results were expressed as mean $\pm$ SD. Statistical analysis was performed using SPSS 11.5 (SPSS, Chicago, IL, USA). Student's *t*-test for unpaired samples was used to compare results between two groups. For multiple group comparisons, analysis of variance followed by a *post hoc* analysis (Student–Newman–Keuls test) was used. Statistical significance was set at $P < 0.05$.

Results

SO_2 derivatives decreased blood pressure *in vivo*. First, we investigated the significance of SO_2 in blood pressure control in SHRs. The results showed that the blood pressure of the rats in the WKY+Na_2SO_3/$NaHSO_3$ group was decreased compared with that of the WKY control group ($P < 0.05$). The blood pressure of the rats in the SHR control group was significantly increased compared with that of the WKY control group ($P < 0.05$). The blood pressure of the rats in the SHR+Na_2SO_3/$NaHSO_3$ group was significantly decreased compared with that of the SHR control group ($P < 0.05$) (Table 1).

SO_2 increased the vasorelaxant response to Ach in isolated aortic rings

We examined the impact of SO_2 on the vasorelaxant response to Ach in isolated aortic rings. The response in the WKY+HDX group was lower than that in the WKY control group ($P < 0.05$), and was markedly decreased in the SHR group compared with the WKY control group ($P < 0.05$). However, the vasorelaxant response to Ach was significantly increased in the SHR+Na_2SO_3/$NaHSO_3$ group, compared with the SHR control group ($P < 0.05$) (Table 2).

Table 1 The change of blood pressure in 12-week rats (mean $\pm$ SD)

Group	*N*	BP (mmHg)
WKY	8	120 $\pm$ 3
WKY+Na_2SO_3/$NaHSO_3$	8	116 $\pm$ 2*
WKY+HDX	6	121 $\pm$ 5
SHR	8	182 $\pm$ 4*
SHR+Na_2SO_3/$NaHSO_3$	7	138 $\pm$ 2**

WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat; HDX, L-aspartic acid-β-hydroxamate; BP, blood pressure
*Compared with WKY group, $P < 0.05$
**Compared with SHR group, $P < 0.05$

Next, we tested the vasorelaxant response to Ach after pre-incubation with HDX *in vitro*. Figure 1 shows the vasorelaxant response of aortic rings from WKY rats to Ach (10^{-9}–10^{-4} mol/L) after pre-incubation with HDX (40, 100 or 160 μmol/L). The response decreased significantly following pre-incubation with 40 and 100 μmol/L HDX, with an EC_{50} of 0.42 $\pm$ 0.17 and 1.09 $\pm$ 0.45 μmol/L, respectively, compared with that of the control group (EC_{50} 0.08 $\pm$ 0.01 μmol/L, $P < 0.05$). However, the EC_{50}

Table 2 The vasorelaxation to Ach and SNP (mean $\pm$ SD)

Group	*n*	Relaxation to Ach	Relaxation to SNP
WKY	8	0.34 $\pm$ 0.03	0.66 $\pm$ 0.09
WKY+Na_2SO_3/$NaHSO_3$	8	0.39 $\pm$ 0.09	0.68 $\pm$ 0.20
WKY+HDX	6	0.21 $\pm$ 0.09*	0.46 $\pm$ 0.12*
SHR	8	0.16 $\pm$ 0.06*	0.45 $\pm$ 0.09*
SHR+Na_2SO_3/$NaHSO_3$	7	0.26 $\pm$ 0.05**	0.60 $\pm$ 0.09**

Ach, acetylcholine; SNP, sodium nitroprusside; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat; HDX, L-aspartic acid-β-hydroxamate
*Compared with WKY group, $P < 0.05$
**Compared with SHR group, $P < 0.05$

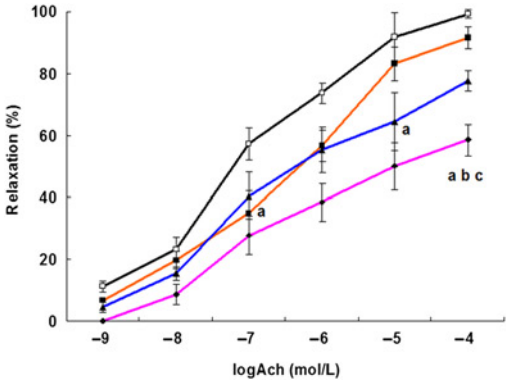

Figure 1 The vasorelaxation to Ach after incubation with HDX. The aortic rings were pre-incubated without HDX (black, -□- control) ($n = 8$), and with 40 μmol/L HDX (orange, -■-) ($n = 7$), 100 μmol/L HDX (blue, -▲-) ($n = 8$), 160 μmol/L HDX (pink, -◆-) ($n = 7$) for 30 min and then in buffer containing 1 μmol/L NE to induce its constriction. Ach (10^{-6}~10^{-9} mol/L) was cumulatively added to the bath solution when the vasodilator curve of the ring reached a plateau phase. [a]Compared with control, $P < 0.05$; [b]compared with HDX 40 μmol/L group, $P < 0.05$; [c]compared with HDX 100 μmol/L group, $P < 0.05$. Ach, acetylcholine; HDX, L-aspartic acid-β-hydroxamate; NE, noradrenaline. (A color version of this figure is available in the online journal)

showed no significant difference between the above two concentrations ($P > 0.05$). The vasorelaxant response to Ach following pre-incubation with 160 μmol/L HDX was significantly reduced, with an EC_{50} of 16.42 $\pm$ 12.45 μmol/L, compared with pre-incubation with 40 μmol/L or 100 μmol/l HDX ($P < 0.05$).

Figure 2 shows SO_2 levels in rat aortas after pre-incubation with 40, 100 or 160 μmol/L HDX. At all three concentrations of HDX, SO_2 levels were significantly reduced compared with those of the control group ($P < 0.05$), but there was no significant difference in SO_2 levels between 40 and 100 μmol/L HDX ($P > 0.05$). SO_2 levels in arteries pre-incubated with 160 μmol/L HDX were decreased significantly compared with those in arteries pre-incubated with 40 or 100 μmol/L HDX ($P < 0.05$).

SO_2 increased vasorelaxant response to SNP in isolated aortic rings from rats of different groups

To understand how SO_2 increased the vasorelaxant response in isolated aortic rings, we first tested whether SO_2 increased the vasorelaxant response to SNP. The results showed that the vasorelaxant response to SNP was lower in the WKY + HDX group than in the WKY control group ($P < 0.05$) and was markedly decreased in the SHR group compared with the WKY control group ($P < 0.05$). However, the vasorelaxant response to SNP was notably enhanced in the SHR+Na_2SO_3/$NaHSO_3$ group compared with the SHR control group ($P < 0.05$) (Table 2).

Role of NO in SO_2-induced vasorelaxation in rat arterial tissues

We next investigated whether SO_2 affects the generation of NO in arterial tissues. Figure 3 shows that the NO level in aortic tissues from the WKY+HDX group was decreased significantly compared with that in the WKY control group ($P < 0.05$). However, the NO level in the aortic tissues of the SHR+Na_2SO_3/$NaHSO_3$ group was increased significantly compared with that of the SHR control group ($P < 0.05$).

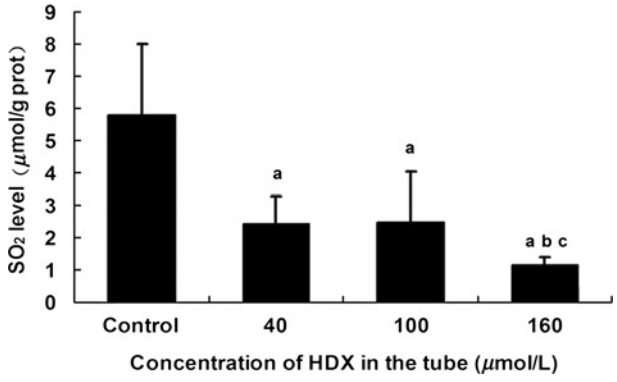

Figure 2 SO_2 level in rat artery after pre-incubating with 40 μmol/L HDX ($n = 6$), 100 μmol/L HDX ($n = 6$), 160 μmol/L HDX ($n = 6$) and in the artery of control group ($n = 8$). [a]Compared with control, $P < 0.05$; [b]compared with HDX 40 μmol/L group, $P < 0.05$; [c]compared with HDX 100 μmol/L group, $P < 0.05$. SO_2, sulphur dioxide; HDX, L-aspartic acid-β-hydroxamate

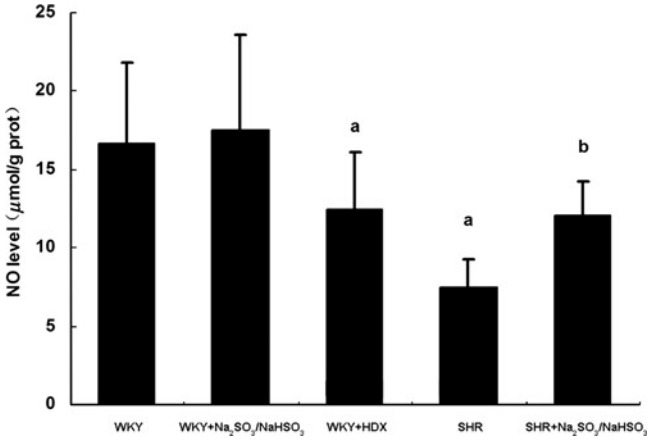

Figure 3 NO levels in artery from rats of WKY control group ($n = 8$), WKY+Na_2SO_3/$NaHSO_3$ group ($n = 8$), WKY+HDX group ($n = 6$), SHR control group ($n = 8$) and SHR+Na_2SO_3/$NaHSO_3$ group ($n = 7$). [a]Compared with WKY group, $P < 0.05$; [b]compared with SHR group, $P < 0.05$. NO, nitric oxide; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat; HDX, L-aspartic acid-β-hydroxamate

We also measured the SO_2-induced vasorelaxant response after incubation with L-NAME, an inhibitor of NOS. Compared with the WKY control group, the vasorelaxant response to SO_2 derivatives at concentrations of 1, 2, 4, 6, 8 and 10 mmol/L, in the presence of L-NAME, was significantly decreased ($P < 0.05$; Figure 4).

Discussion

NO, carbon monoxide, hydrogen sulfide and SO_2 have been identified as toxic environmental pollutants. However, they can be synthesized in the body from arginine, glycine and cysteine, respectively, and serve as important signaling molecules in animals and humans.[16] The reference range for

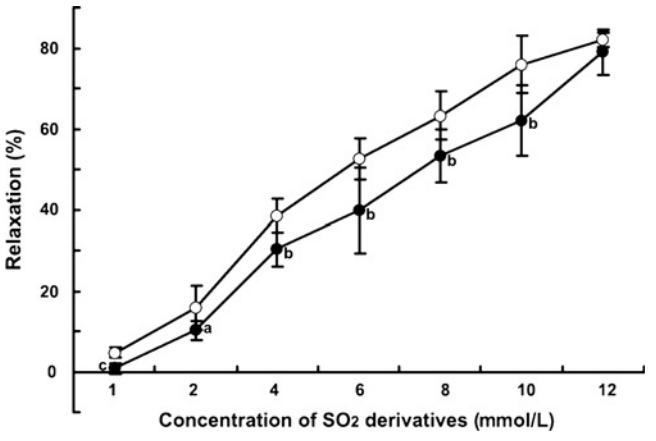

Figure 4 The vasorelaxation to SO_2 derivative on aortic rings after incubation with L-NAME in Wistar rats. The aortic rings were pre-incubated without L-NAME (○; $n = 10$) and with 100 μmol/L L-NAME (•; $n = 6$) for 60 min and then in buffer including 1 μmol/L NE to induce their constriction. Different concentrations of SO_2 derivatives (1, 2, 4, 6, 8, 10 and 12 mmol/L) were added to the bath solution, respectively, when the vasodilator curve of the rings reached a plateau phase. [a]Compared with the control group, $P < 0.05$; [b]compared with the control group, $P < 0.01$; [c]compared with the control group, $P < 0.001$. SO_2, sulphur dioxide; L-NAME, N^G-nitro-L-arginine methyl ester; NE, noradrenaline

total serum sulfite (SO_2 derivative) is 0–10 μmol/L in healthy individuals.[21] It was reported that SO_2 derivatives (sodium sulfite and sodium bisulfite, about 3 and 1 mol/L, respectively, pH 7.0) administered via intraperitoneal injection decreased the blood pressure of male Wistar rats.[22] There have been studies concerning the vasodilatory effect of SO_2 derivatives on isolated aortic rings from WKY rats *in vitro*.[23,24] Our previous study demonstrated that vascular endogenous SO_2 was reduced in rats with hypertension.[14] In this study, we found that the blood pressure of SHRs was significantly decreased when SO_2 derivatives were given by intraperitoneal injection for eight weeks, which further suggests that SO_2 plays a role in the development of hypertension.

Abnormal vasorelaxation and vascular structural remodeling are the principal components of the pathogenesis of hypertension.[25,26] Imai *et al.* reported the reaction of dimerized NO with SO_2 in a restricted slit-shaped micropore,[27] which suggested a possible interaction between NO and SO_2 in the regulation of vasorelaxation in SHRs.

Ach could activate M carinic receptors on endothelial cells, resulting in NO release and a consequent decrease in the tension of vascular smooth muscle cells. HDX, as an inhibitor of SO_2-producing enzymes, inhibits the generation of endogenous SO_2. When investigating the possible mechanisms by which SO_2 regulates vasorelaxation, we found that the vasorelaxant response to Ach in the WKY+HDX and SHR groups was significantly decreased compared with that in the WKY control group. However, the vasorelaxant response to Ach in the SHR+Na_2SO_3/$NaHSO_3$ group was significantly increased compared with the SHR control group. We also used isolated aortic rings from WKY rats to investigate the vasorelaxant response to Ach after pre-incubation with HDX; this was markedly decreased, indicating that HDX significantly reduced SO_2 production in the aortic rings.

SNP is a NO donor that directly relaxes blood vessels. We found that the vasorelaxant response to SNP in the WKY+HDX group was significantly decreased compared with both the WKY control group and the SHR group. However, the vasorelaxant response to SNP in the SHR+Na_2SO_3/$NaHSO_3$ group was significantly increased compared with the SHR control group. These results suggested that SO_2 possibly improved NO-induced vasorelaxation in SHRs.

In previous studies by Li *et al.*,[17] it was found that SO_2 could increase NO production in Wistar rats. The vascular endothelium can produce a wide array of vasoactive substances, including gaseous NO, that modulate the tone of vascular smooth muscle cells.[28,29] We therefore investigated whether SO_2 can promote the generation of NO in the blood vessels of SHRs and whether SO_2 can relax those vessels by increasing NO production. Our results showed that the NO level in the aorta decreased notably in the WKY+HDX and SHR groups compared with the WKY controls. However, the NO level was reversed in aortas from the SHR+Na_2SO_3/$NaHSO_3$ group. These results suggested that SO_2 increased NO production, which in turn facilitated vasorelaxation in the rats in the SHR+Na_2SO_3/$NaHSO_3$ group.

We also examined vasorelaxation in aortic rings from Wistar rats after pre-incubation with the NO inhibitor L-NAME. Compared with controls, the vasorelaxation induced by SO_2 derivatives after incubation with L-NAME was significantly decreased, suggesting that the vasorelaxation depended on the promotion of NO production by SO_2.

In summary, our study indicates that SO_2 reduces blood pressure and increases vasorelaxation in the arteries of SHRs. SO_2 could produce these effects by enhancing the vasorelaxant response to NO in isolated aortic rings and increasing the NO level in aortic tissues.

Author contributions: WL participated in the research design, writing of the paper and data analysis. YS participated in the writing of the paper and performance of the research. She also contributed analytic techniques and participated in data analysis. CT participated in the research design and writing of the paper. TO participated in the research design, writing of the paper and data analysis. JQ participated in the research design and writing of the paper. She also contributed analytic techniques and participated in data analysis. HJ participated in the research design and writing of the paper. She also contributed analytic techniques and participated in data analysis. JD participated in the research design and writing of the paper. He also participated in data analysis and result interpretation. WL and YS contributed equally to this work.

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REFERENCES

1 Julius S. Effect of sympathetic overactivity on cardiovascular prognosis in hypertension. *Eur Heart J* 1998;**19**(Suppl. F):F14–18
2 Johns EJ. Role of angiotensin and the sympathetic nervous system in the control of renal function. *J Hypertens* 1989;**7**:695–701
3 Dvoráková MC. Cardioprotective role of the VIP signaling system. *Drug News Perspect* 2005;**18**:387–91
4 Chalmers J, Pilowsky P. Brainstem and bulbospinal neurotransmitter systems in the control of blood pressure. *J Hypertens* 1991;**9**:675–94
5 Meng Z. Oxidative damage of sulfur dioxide on various organs of mice: sulfur dioxide is a systemic oxidative damage agent. *Inhal Toxicol* 2003;**15**:181–95
6 Meng Z, Qin G, Zhang B, Bai J. DNA damaging effects of sulfur dioxide derivatives in cells from various organs of mice. *Mutagenesis* 2004;**119**:465–8
7 Meng Z, Liu Y. Cell morphological ultrastructural changes in various organs from mice exposed by inhalation to sulfur dioxide. *Inhal Toxicol* 2007;**19**:543–51
8 Liu D, Jin H, Tang C, Du J. Sulfur dioxide: a novel gaseous signal in the regulation of cardiovascular functions. *Mini Rev Med Chem* 2010;**10**:1039–45
9 Stipanuk MH. Metabolism of sulfur containing amino acids. *Annu Rev Nutr* 1986;**6**:179–209
10 Stipanuk MH, Dominy JE Jr, Lee JI, Coloso RM. Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. *J Nutr* 2006;**136**(Suppl.):1652S–9S

11 Ubuka T, Yuasa S, Ohta J, Masuoka N, Yao K, Kinuta M. Formation of sulfate from L-cysteine in rat liver mitochondria. *Acta Med Okayama* 1990;**44**:55–64
12 Griffith OW. Cysteinesulfinate metabolism altered partitioning between transamination and decarboxylation following administration of beta-methyleneaspartate. *J Biol Chem* 1983;**258**:1591–8
13 Sun Y, Tian Y, Prabha M, Liu D, Chen S, Zhang R, Liu X, Tang C, Tang X, Jin H, Du J. Effects of sulfur dioxide on hypoxic pulmonary vascular structural remodeling. *Lab Invest* 2010;**90**:68–82
14 Zhao X, Jin HF, Tang CS, Du JB. Effects of sulfur dioxide, on the proliferation and apoptosis of aorta smooth muscle cells in hypertension: experiments with rats. *Zhonghua Yi Xue Za Zhi* 2008;**88**:1279–83
15 Du SX, Jin HF, Bu DF, Bu DF, Zhao X, Geng B, Tang CS, Du JB. Endogenously generated sulfur dioxide and its vasorelaxant effect in rats. *Acta Pharmacol Sin* 2008;**29**:923–30
16 Li J, Meng Z. The role of sulfur dioxide as an endogenous gaseous vasoactive factor in synergy with nitric oxide. *Nitric Oxide* 2009;**20**:166–74
17 Li J, Li R, Meng Z. Sulfur dioxide upregulates the aortic nitric oxide pathway in rats. *Eur J Pharmacol* 2010;**645**:143–50
18 Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. *EMBO J* 2001;**20**:6008–16
19 Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 1997;**237**:527–31
20 Mitsuhashi H, Ikeuchi H, Yamashita S, Kuroiwa T, Kaneko Y, Hiromura K, Ueki K, Nojima Y. Increased levels of serum sulfite in patients with acute pneumonia. *Shock* 2004;**21**:99–102
21 Ji AJ, Savon SR, Jacobsen DW. Determination of total serum sulfite by HPLC with fluorescence detection. *Clin Chem* 1995;**41**:897–903
22 Meng Z, Geng H, Bai J, Yan G. Blood pressure of rats lowered by sulfur dioxide and its derivatives. *Inhal Toxicol* 2003;**15**:951–9
23 Meng Z, Zhang H. The vasodilator effect and its mechanism of sulfur dioxide derivatives on isolated aortic rings of rats. *Inhal Toxicol* 2007;**19**:979–86
24 Meng Z, Li Y, Li J. Vasodilatation of sulfur dioxide derivatives and signal transduction. *Arch Biochem Biophys* 2007;**467**:291–6
25 Mulvany MJ. Vascular growth in hypertension. *J Cardiovasc Pharmacol* 1992;**20**:S7–11
26 Gohlke P, Lamberty V, Kuwer I, Bartenbach S, Schnell A, Unger T. Vascular remodeling in systemic hypertension. *Am J Cardiol* 1993;**71**:2E–7E
27 Imai J, Souma M, Ozeki S, Suzuki T, Kaneko K. Reaction of dimerized NOx (x =1 or 2) with SO_2 in a restricted slit-shaped micropore space. *J Phys Chem* 1991;**95**:9955–60
28 Becker BF, Heindl B, Kupatt C, Zahler S. Endothelial function and hemostasis. *Z Kardiol* 2000;**89**:160–7
29 Ignarro LJ, Buga GM, Wood KS, Byms RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987;**84**:9265–9

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