Hydrogen Sulfide Improves the Endothelial Dysfunction in Renovascular Hypertensive Rats

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Short title: Hydrogen sulfide and renovascular hypertension

Summary

As a novel gasotransmitter, hydrogen sulfide (H_2S) has vasodilating and antihypertensive effects in cardiovascular system. Thus, we hypothesized that H_2S might have beneficial effects on thoracic endothelial function in two kidney one clip (2K1C) rats, a model of renovascular hypertension. Sodium hydrosulfide (NaHS, 56 μmol/kg/day) was administrated intraperitoneally from the third day after the 2K1C operation. Along with the development of hypertension, the systolic blood pressure (SBP) was measured before the operation and each week thereafter. The oxidative stress was determined by measurement of malondialdehyde (MDA) concentration, superoxide dismutase (SOD) activity and protein expression of oxidative stress-related proteins (AT_1R, NADPH oxidase subunits). Acetylcholine (ACh)-induced vasorelaxation and angiotensin II (Ang II)-induced vasocontraction were performed on isolated thoracic aorta. The SBP was significantly increased from the first week after operation, and was lowered by NaHS. NaHS supplementation ameliorated endothelial dysfunction. The protein expression of oxidative stress-related proteins were downregulated, while SOD activity upregulated. In conclusion, improvement of endothelial function is involved in the antihypertensive mechanism of H_2S. The protective effect of H_2S is attributable to suppression of vascular oxidative stress that involves inhibition of Ang II-AT_1R action, downregulation of oxidases, as well as upregulation of antioxidant enzyme.

Key words: Hydrogen sulfide • Angiotensin II • Endothelial dysfunction • Oxidative stress

Introduction

The novel gasotransmitter hydrogen sulfide (H_2S) possessed various physiological and pharmacological functions in cardiovascular system (Wang 2002). Nowadays, H_2S has been found to be produced by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and newly determined 3-mercaptopyruvate sulphurtransferase (3-MST) in concert with cysteine aminotransferase (CAT) (Tanizawa 2011). In particular, CSE is the main generating enzyme to be present in vascular bed and additionally located in vascular endothelial cells, which contributes to endothelium-dependent relaxation (Zhao *et al.* 2001). The vasorelaxing effect of H_2S has been appeared in various vascular tissues, such as rat aorta and mesenteric arteries (Wang *et al.* 2009). Moreover, CSE knockdown increased blood pressure (Yang *et al.* 2008), which might be eliminated by in vivo administration of H_2S (Mancardi *et al.* 2009). In addition, plasma level of H_2S was significantly decreased in spontaneously hypertensive rats (SHRs) (Zhao *et al.* 2008) and renovascular hypertensive rats (Lu *et al.* 2010). Thus, we hypothesized that there might be a correlation between H_2S and vascular function in hypertensive animal model.

Renovascular hypertension caused by partial renal-artery stenosis always leads to persistent high blood pressure and increased angiotensin II (Ang II) activity (Higashi *et al.* 2002, Takahashi and Smithies 2004). Ang II, acting via the angiotensin II type 1 receptor (AT_1R), increases the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which was proposed as a mechanism underlying the enhanced production of reactive oxygen species (ROS), especially superoxide anion ($O_2^{\cdot-}$) in a rat model of renovascular hypertension (Oliveira-Sales *et al.* 2009). The association between increased production of $O_2^{\cdot-}$ and endothelial dysfunction has been reported in both experimental and human renovascular hypertension(Cai and Harrison 2000, Dijkhorst-Oei *et al.* 1999, Taddei *et al.* 1993).

Vasoactive substances released by endothelium play an important role in regulating vascular tone and blood pressure (Bayraktutan 2002). The imbalance between endothelium-derived relaxing and contracting factors leads to endothelial dysfunction, which is related to cardiovascular mortality (Vanhoutte *et al.* 2009). In a large amount of experimental hypertensive models, such as two-kidney, one-clip (2K1C) renovascular, aortic coarctation, Dahl salt-sensitive, deoxycorticosterone acetate-salt and SHRs, the endothelium-dependent vasorelaxation is severely impaired (Choi *et al.* 2012). H_2S has been reported to be produced from vascular endothelial cells and be considered as a newly endothelial-dependent relaxing factor (EDRF) or endothelial-dependent hyperpolarized factor (EDHF), which plays a pivotal role in regulating endothelial function (Wang 2009). However, no studies show that H_2S could improve endothelial dysfunction in 2K1C model.

Sodium hydrosulfide (NaHS) is an H_2S donor. Although previous studies reported the antihypertensive

effect of this compound (Ahmad *et al.* 2014, Ford *et al.* 2013), the effects of NaHS on vascular function in renovascular hypertension remain poorly understood. In this study, we investigated the effect of NaHS on endothelial function in chronic 2K1C hypertensive rats, which is an ideal animal model of renovascular hypertension, with further elucidation of the underlying mechanisms by focusing on vascular oxidative stress.

Methods

Animals

All the rats used in present study were obtained from our local certified animal facility (Animal Research Center of Hebei Medical University, Hebei, China). 7-week old male Sprague-Dawley rats were randomly divided into 3 groups (n=6): Sham, 2K1C, 2K1C+NaHS. The rats were anesthetized with intraperitoneal injections of pentobarbital sodium (30 mg/kg). In the 2K1C and 2K1C+NaHS groups, a lumbar incision was made to provide access to the left renal artery. Cleared of the connective tissues, the left renal artery was clipped by a rigid U-shaped solid silver clip with an open slit of 0.25mm, resulting in partial occlusion of renal perfusion. The contralateral kidney was left untouched. Sham group underwent the same procedure, except for the clip placement. The rats were kept in cages after surgery, maintained on 12h light/dark cycle with free access to normal chow and tap water. 2K1C+NaHS group received NaHS 56 μmol/kg/day intraperitoneally. The treatment was started from the third day after the surgery and maintained for 4 weeks. Sham and 2K1C groups were received saline as vehicle.

Blood pressure measurement

Systolic blood pressure (SBP) was measured non-invasively by tail-cuff plethysmography (Chengdu Instrument Factory, Sichuan, China) in calm, conscious rats. Briefly, SBP was measured before and further at each week after surgery for 4 weeks. SBP measurement was always conducted between 9:00 and 12:00 AM and was calculated as the average value of 3 to 4 successive measurement.

Isometric force studies

The thoracic aorta was dissected, cleaned of connective tissue and cut into ring segments (-3mm length). The segments were then mounted between stainless steel triangles in a 10 ml organ bath filled with oxygenated (95% O_2 and 5% CO_2) Krebs-Henseleit solution (pH 7.3-7.5) (composition in mmol/l: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11) at 37℃. An optional baseline tone of 2 g was applied to all rings as a resting tension. After a 60 minute-equilibration, arteries were stimulated by 60 mmol/l KCl. After washing out, rings were pre-contracted by phenylephrine (Phe, 1 μmol/l). When the contraction was steady, acetylcholine (ACh, 10^{-8}~10^{-4} mol/l) or sodium nitroprusside (SNP, 10^{-9}~10^{-6} mol/l) were added respectively in a cumulative

manner to record the endothelial-dependent relaxation or endothelial-independent relaxation response curve. Ang II (10^{-10}~10^{-6} mol/l) was added cumulatively to conduct contraction curve. Further, AT_1R inhibitor losartan (10 μmol/l) was applied for 30 minutes before the Ang II-induced contraction was performed.

The extent of relaxation was expressed as percentage of relaxation from a submaximal phenylephrine-induced constriction. Vasocontraction was determined in relative values as the percentage of 60 mmol/l KCl contraction.

Measurement of plasma H_2S level

H_2S concentration in plasma was measured as previous described (Siegel 1965). Briefly, plasma (100 μl) were mixed with potassium phosphate buffer (pH 7.4, 2.5 ml) and zinc acetate (1% w/v, 500 μl), then further incubated with *N*,*N*-dimethyl-*p*-phenylenediamine sulfate (20 mmol/l, 500 μl) in 7.2 mol/l HCl and $FeCl_3$ (30 mmol/l, 400 μl) in 1.2 mol/l HCl. 10% trichloroacetic acid 1ml was added to terminate the reaction after 20-minute incubation. The absorbance of the mixture was measured at 665 nm. H_2S concentration was calculated against a calibration curve of NaHS (0.01 to 100 μmol/l). The results of H_2S concentration was expressed as μmol/l.

Measurement of plasma Ang II level

Ang II level in plasma was examined with an Ang II radioimmunoassay kit (Beijing Chemclin Biotecho Co. Ltd, Beijing, China). Briefly, blood samples were collected in tubes containing 20 μl EDTA in ice. After centrifugation at 1000×g for 15 minutes at 4℃, plasma (1 ml) were collected and mixed with enzyme inhibitor 1 (10 μl), enzyme inhibitor 2 (20 μl) following the instruction of the radioimmunoassay kit. Then aliquots (100 μl) were incubated with ^{125}I-AII (100 μl) and AII antibody (100 μl) for 15 hours at 2-8℃, followed by addition of separating reagent (including donkey anti-rabbit IgG serum and polyethylene glycol) 500 μl and incubated for 15 minutes at room temperature. The supernatant was discarded after centrifugation at 3,500 r/min for 20 minutes. The radioactive count was determined for 60 seconds. The results were expressed as pg/ml.

Western blot analysis

Thoracic aorta were homogenized with ice-cold RIPA lysing buffer and centrifuged at 20,000×g for 20 minutes. The supernatant was collected and analyzed for protein concentration using the bicinchoninic acid (BCA) method (Generay biotechnology, Shanghai, China). Aliquots (100 μg protein) were suspended in 6×sample buffer with 5% β-mercaptoethanol and denatured at 99℃ for 10 minutes. The protein samples were electrophoresed through 10% SDS-polyacrylamide gel and then transferred onto an immobilon-P polyvinylidene difluoride membrane using wet transfer at 100 V for 90 minutes at 4℃. The membrane was blocked with 0.05% Tween-20

Tris-buffered saline (TBST) containing 5% non-fat milk for 60 minutes at room temperature. The blots were then incubated overnight at 4℃ with anti-AT_1R (1:800, Abcam, Hongkong), anti-Nox2 (1:1000, Abcam, Hongkong), anti-Nox4 (1:1000, proteintech, Chicago, USA), anti-$p67^{phox}$ (1:1000, EPITOMICS, California, USA) and anti-superoxide dismutase (SOD)-1 (1:1000, proteintech, Chicago, USA) antibodies. All washes were performed in TBST. The membranes were then incubated with a horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:2000, proteintech, Chicago, USA) or HRP-conjugated rabbit anti-goat antibody (1:2000, proteintech, Chicago, USA) for 1 hour. Blots were developed with an enhanced chemiluminescence detection system (Sagecreation, Beijing, China). Densitometry was performed using lane-1 system (Sagecreation, Beijing, China).

Measurement of malondialdehyde (MDA) concentration

MDA concentration in aorta was measured by using thiobarbituric acid reactive substances (TBARS) assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) as previously described (Costa *et al*. 2009). Thoracic aorta were dissected out and snapped frozen at -80℃, then homogenized on ice and centrifuged at 10,000×g for 5 minutes to collect the supernatant. The supernatant was mixed with 10% trichloroacetic acid 1 ml and 0.67% thiobarbituric acid 1 ml, followed by 30-minute incubation in a boiling water bath. The absorbance of the mixture (532 nm) was measured. The results were expressed as nmol/mg protein.

Measurement of SOD activity

A SOD immunoassay kit was used to determine the total SOD activity in thoracic rings (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). Thoracic aorta was perfused with PBS to remove any red blood cells. Homogenized aorta on ice and centrifuged tissue lysate at 10,000×g for 5 minutes. The supernatant contains total SOD was then incubated with working solution for 20 minutes at 37°C. Read the absorbance at 450nm using a microplate reader. The results were expressed as U/mg protein.

Drugs

NaHS, Ang II, ACh, SNP were obtained from Sigma (Vienna, Austria). Losartan was obtained from ALEXIS (Switzerland). Phe was obtained from TCI (Japan). NaHS was freshly prepared and used to generate H_2S in solution according to previous paper (Qu *et al.* 2006).

Statistical Analysis

Data were expressed as the mean±SEM. The results were evaluated either by *t* test or 2-way ANOVA using Graphpad Prism. $p<0.05$ was considered significant. The dose response curves were analyzed by 2-way ANOVA, followed by vertical contrast with Bonferroni adjustment.

Results

Effect of NaHS on plasma H_2S, Ang II level and hypertension in 2K1C rats

The plasma H_2S level in 2K1C rats (26.8±4.8 μmol/l) was significantly lower than that in sham rats (50.6±6.9 μmol/l, $p<0.05$). 4 weeks of NaHS treatment elevated the plasma H_2S level to 56.4±9.2 μmol/l ($p<0.05$, Fig. 1A). However, Compared with sham rats, the plasma Ang II level was significantly higher in 2K1C rats (140.2±4.7 pg/ml vs. 101.1±1.9 pg/ml, $p<0.01$). Treatment with NaHS for 4 weeks lowered the Ang II level to 111.5±3.4 pg/ml (Fig. 1B).

SBP was measured before and weekly after 2K1C surgery. No differences were observed in SBP among the groups before surgery. At the end of the first week, the SBP in 2K1C rats was increased from 105.0±1.2 mmHg to 170.0±4.0 mmHg, and remained elevated throughout the 4-week study period. Sham operation did not affect SBP. Intraperitoneally administrated with NaHS 56 μmol/kg/day from the third day after surgery reduced the elevation of SBP (Fig. 1C) at the second week after surgery and remained lower than that of the non-treated 2K1C rats during the rest of the study period.

Effect of NaHS on Ang II-induced vasocontraction

The Ang II-induced vasocontraction was enhanced in the thoracic aorta of 2K1C rats. Such enhancement of vasocontraction was suppressed by the 4-week treatment of NaHS (Fig. 2A,B). In all the three groups including sham and 2K1C without or with NaHS treatment, the vasocontraction induced by Ang II was almost abolished by a 30-minute preincubation of the aorta with the losartan (10 μmol/l) (Fig. 2C).

Effect of NaHS on endothelium-dependent and-independent vasorelaxation

ACh-induced vasorelaxation was significantly reduced in Phe-precontracted aorta in 2K1C rats (E_{max} 50.8±3.7% vs. 100.2±0.3% in sham rats, $p<0.05$). Treatment with NaHS for 4 weeks improved ACh-induced endothelium-dependent relaxation (64.8±9.4%, Fig. 3A,B). By contrast, the SNP-induced endothelium -independent relaxation showed no differences among sham, 2K1C, and NaHS-treated 2K1C rats. (Fig. 3C,D).

Effect of NaHS on oxidative stress-related proteins

Western blot analysis showed that protein level of AT_1R, NADPH oxidase isoform $p67^{phox}$ and subunit Nox2、Nox4 was elevated in 2K1C rat thoracic aorta. The over-expression of AT_1R, $p67^{phox}$、Nox2 and Nox4 were reversed by chronic treatment with NaHS. (Fig. 4)

Effect of NaHS on MDA level

The MDA level in the thoracic aorta of 2K1C rats was significantly elevated (671.6±94.6 vs. 339.9±77.6 nmol/gprot in sham rats, $p<0.05$). Treatment with NaHS for 4 weeks normalized the tissue level of MDA (356.9±42.9 nmol/gprot) (Fig. 5).

Effect of NaHS on SOD

The tissue SOD activity in rat thoracic aorta was markedly decreased, whereas NaHS chronic treatment reversed the decreased SOD activity in 2K1C rats (Fig. 6A). The downregulated protein expression of SOD-1 was normalized by NaHS supplementation as well (Fig. 6B).

Discussion

The major findings of this study include: (1) NaHS reduces SBP in 2K1C hypertensive rats; (2) NaHS improves the endothelium-dependent relaxation and supresses Ang II-induced contraction; (3) This study is the first time to show that H_2S downregulates AT_1R protein expression and oxidative stress in 2K1C hypertension. Taken together, the results suggest that H_2S protect endothelial function by decreasing oxidative stress in 2K1C rats. Figure 7 is a signal transduction diagram depicting the possible mechanisms and assumed pathways.

Previous studies have demonstrated that endothelial function is impaired in 2K1C hypertensive rats (Callera *et al.* 2000) and NaHS exhibits anti-hypertensive effect in this animal model (Lu *et al.* 2010), however, the effect of NaHS on endothelial function in renovascular hypertension remains poorly studied. In this study, we demonstrated that NaHS treatment lowered blood pressure in 2K1C hypertensive rats, which is consistent to previous reports. Improvement of endothelium-dependent relaxation is a mechanism underlying the antihypertensive effect of NaHS. Suppression of vascular responsiveness to Ang II is also involved, evidenced by the attenuated contractile response of aorta to Ang II in rats treated with NaHS that is attributable to the lowered plasma level of Ang II and the downregulation of AT_1R in vascular cells.

The association between blood pressure elevation and vascular oxidative stress has been extensively reported in the development of hypertension, including essential, renovascular or malignant hypertension (Schulz *et al.* 2011). As a major source of ROS in vascular cells, NADPH oxidase can be activated by Ang II through acting on AT_1R (Drummond *et al.* 2011). Our results showed that NaHS treatment lowered plasma level of Ang II and downregulated aortic AT_1R protein expression in 2K1C hypertensive rats. As a consequence, the inhibition of Ang II-AT_1R action likely contributes to the inhibition of NADPH oxidases and the reduction of ROS production.

Nox proteins represent the catalytic subunits of NADPH oxidase. In various animal models of hypertension including Ang II infusion, deoxycorticosterone acetate-salt, and renovascular hypertension as well as SHRs, increased activity of Nox has been observed (Schulz *et al.* 2011). Ang II was reported to increase expression of several NADPH oxidase subunits, including Nox1 and Nox4 (Seshiah *et al.* 2002). In this study, we determined the expression of Nox2 and Nox4, the most important Nox isoforms in vascular cells (Brown and Griendling 2009), and found that in 2K1C rats, protein expressions of Nox2 and Nox4 were significantly increased in the

aortic tissue. 4-week treatment with NaHS normalized the expression of Nox2 and Nox4. NaHS treatment also lowered the protein level of $p67^{phox}$, the regulatory cytosolic subunit of NADPH, in the aorta of 2K1C rats. These results suggested the direct inhibitory effect of H_2S on NADPH oxidases. Furthermore, we showed that in NaHS-treated animals, the activity of the major antioxidant enzyme SOD is enhanced, accompanied by an upregulation of SOD-1 protein. Taken together, our results demonstrated the potent antioxidant effect of H_2S on vascular cells in renovascular hypertensive rats. The inhibition of vascular oxidative stress conferred by H_2S was further evidenced by the decreased MDA level in the aortic tissue.

NaHS is commonly used to deliver H_2S and increases H_2S concentrations rapidly (Li *et al.* 2008). NaHS incompletely dissociates into H_2S in Krebs solution, resulting in about 15-18.5% being present as H_2S, with 81.5-85% existing as hydrosulphide, HS^- and a trace of sulphide anion, S^{2-} (Dombkowski *et al.* 2004). Which form of H_2S (H_2S, HS^-, or S^{2-}) is physiologically active, or whether all three forms are active to varying extents are not known, however the active component is commonly termed as the chemical symbol "H_2S" (Al-Magableh *et al.* 2014). The physiological concentration of H_2S is difficult to measure and the measurement of H_2S not always gives the consistent values, varying from the previous micromolar range to the recent nanomolar range. Current literature reports several H_2S measurement methods, such as spectrophotometry, chromatography, ion-selective electrode and nanoparticles. The methylene blue method used in present study is the most common assay for detecting H_2S. This method revealed that human and rat serum contains 50-100 μM H_2S, which is in consistent with our results. However, this technique detects not only free H_2S but also other species such as HS^- and S^{2-}, the true plasma concentration of H_2S is probably to be lower than tens of micromolar range. Although the methylene method has limitation, the present result is able to indicate that NaHS treatment increased H_2S concentration in 2K1C rats.

Previous studies showed that NaHS > 50-100 μM is required to relax blood vessels in organ bath, 10-50 μmol/kg are needed to bring about significant falls in blood pressure. NaHS (56 μmol/kg/day, i.p.) treatment for 4 to 5 weeks decreased blood pressure and oxidative stress, inhibited atherogenesis and reduced atherosclerotic lesion size (Chen *et al.* 2011, Wang *et al.* 2009, Yan *et al.* 2004). Although lower doses of NaHS treatment (e.g. 10 or 30 μmol/kg/day, i.p.) were also effective in reducing blood pressure, a much longer treatment period (3 month) would be needed. Also, treatment with different doses of NaHS (10, 30 and 90 μmol/kg/day) in SHRs showed no obvious dose-related difference in blood pressure reduction (Shi *et al.* 2007). The present study showed the beneficial effect of NaHS in lowering blood pressure, improving endothelial function, reducing vascular superoxide production were all observed at a dose of 56 μmol/kg/day. However, the present study

demonstrates limitation that we used a fixed dose of NaHS without performing a dose-dependent study, thus may miss the potentially toxic or beneficial effects of NaHS.

In summary, improvement of endothelial function is involved in the antihypertensive mechanism of H_2S. The protective effect of H_2S is attributable to, at least in part, suppression of vascular oxidative stress that involves inhibition of Ang II-AT_1R action, downregulation of NADPH oxidases, as well as upregulation of antioxidant enzyme SOD. H_2S may have therapeutic potential for renovascular hypertension.

Conflict of Interest

There is no conflict of interest.

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Abbreviations: ACh: acetylcholine; Ang II :angiotensin II; AT_1R: angiotensin II type1 receptor; BCA: bicinchoninic acid; CBS: cystathionine β-synthase; CSE: cystathionine γ-lyase; CAT: cysteine aminotransferase; EDHF: endothelial-dependent hyperpolarized factor; EDRF: endothelial-dependent relaxing factor; HRP: horseradish peroxidase; H_2S: hydrogen sulfide; MDA: malondialdehyde; 3-MST: 3-mercaptopyruvate sulphurtransferase; NADPH: nicotinamide adenine dinucleotide phosphate; Phe: phenylephrine; ROS: reactive oxygen species; NaHS: sodium hydrosulfide; SNP: sodium nitroprusside; SHRs: spontaneously hypertensive rats; $O_2^{\cdot-}$: superoxide anion; SOD: superoxide dismutase; SBP: systolic blood pressure; TBST: tris-buffered saline; 2K1C: two-kidney, one-clip

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Figure Legends

Fig. 1 Administration of NaHS for 4 weeks increased the plasma H_2S level (A), reduced the plasma Ang II level (B), and attenuated the development of hypertension (C) in 2K1C rats. Results are expressed as mean±SEM. n=6, $^{*}p<0.05$, $^{**}p<0.01$ vs. Sham; $^{\#\#}p<0.01$ vs. 2K1C.

Fig. 2 The Ang II-induced contraction was attenuated by NaHS chronic treatment. NaHS chronic treatment ameliorated the contraction to Ang II (A,B). Pretreatment with losartan (10 μmol/l) for 30 minutes almost eliminated the Ang II-induced vasocontraction in rat thoracic aorta (C). Results are expressed as mean±SEM. n=6, $^{*}p<0.05$, $^{**}p<0.01$ vs. Sham; $^{\#\#}p<0.01$ vs. 2K1C.

Fig. 3 Effect of NaHS chronic treatment on endothelium-dependent and endothelium-independent relaxation. NaHS 4-week administration enhanced ACh-induced vasorelaxation in 2K1C rats (A,B). No differences were detected in SNP-induced endothelium-independent relaxation (C,D). Results are expressed as mean±SEM. n=6, $^{**}p<0.01$ vs. Sham; $^{\#}p<0.05$ vs. 2K1C.

Fig. 4 NaHS chronic treatment reduces the exaggerated protein expression of AT_1R (A), $p67^{phox}$ (B), Nox2 (C) and Nox4 (D) in thoracic aorta of 2K1C rats. Results are expressed as mean±SEM. n=4 or 5, $^{*}p<0.05$ vs. Sham; $^{\#}p<0.05$ vs. 2K1C.

Fig. 5 The MDA level in thoracic aorta was reduced by NaHS 4-week treatment. Results are expressed as mean±SEM. n=6, $^{**}p<0.01$ vs. Sham; $^{\#\#}p<0.01$ vs. 2K1C.

Fig. 6 Treatment with NaHS for 4 weeks remarkably enhanced the tissue activity of SOD (A) and protein expression (B) of SOD-1. Results are expressed as mean±SEM. n=4 or 5, $^{*}p<0.05$, $^{**}p<0.01$ vs. Sham; $^{\#}p<0.05$, $^{\#\#}p<0.01$ vs. 2K1C.

Fig. 7 Schematic diagram of mechanisms underlying the effect of NaHS against endothelial dysfunction in 2K1C rats. NaHS prevents endothelial dysfunction in thoracic aorta by inhibition of Ang II-AT_1R action and downregulation of NADPH oxidases, as well as upregulation of SOD.

Fig. 1

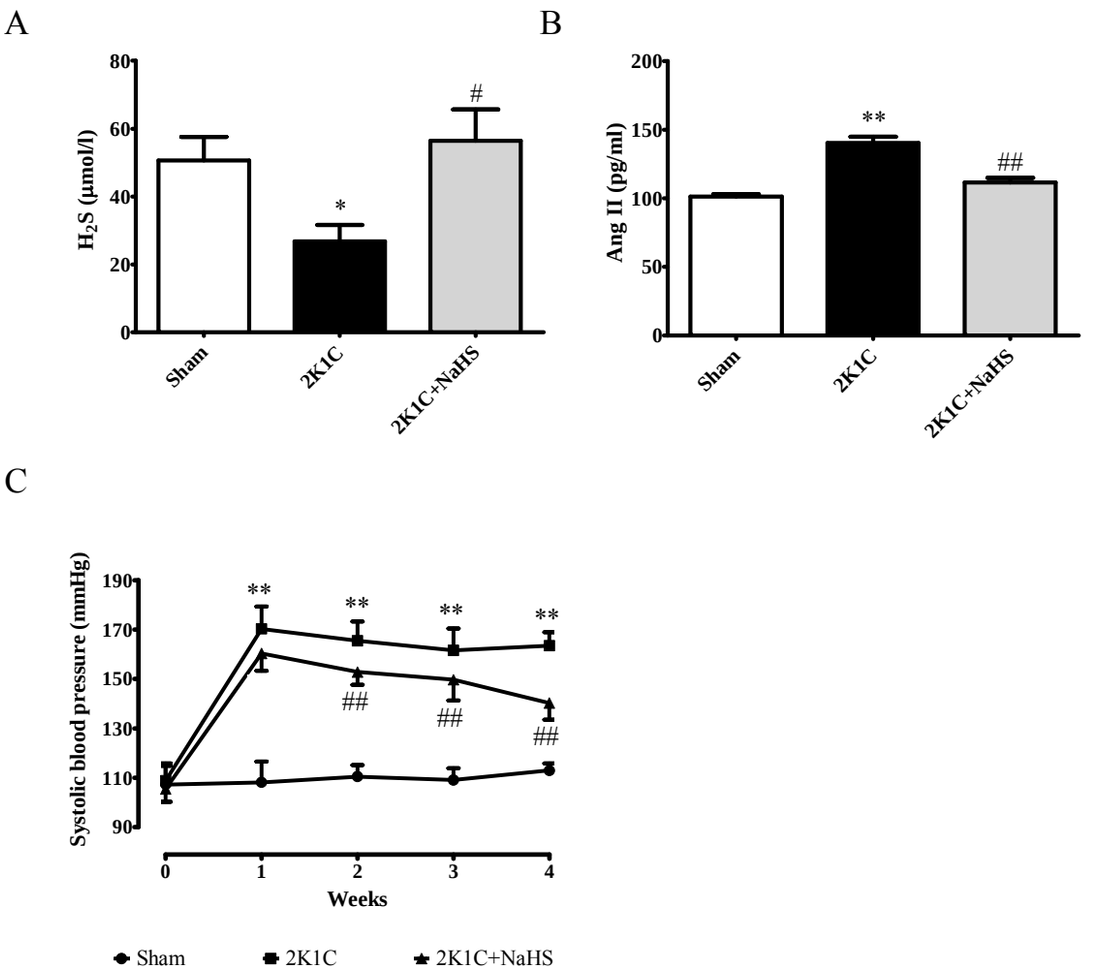

A
B
C
H_2S (μmol/l)
Ang II (pg/ml)
Systolic blood pressure (mmHg)
Weeks
Sham
2K1C
2K1C+NaHS
*
**
#
##

Fig. 2

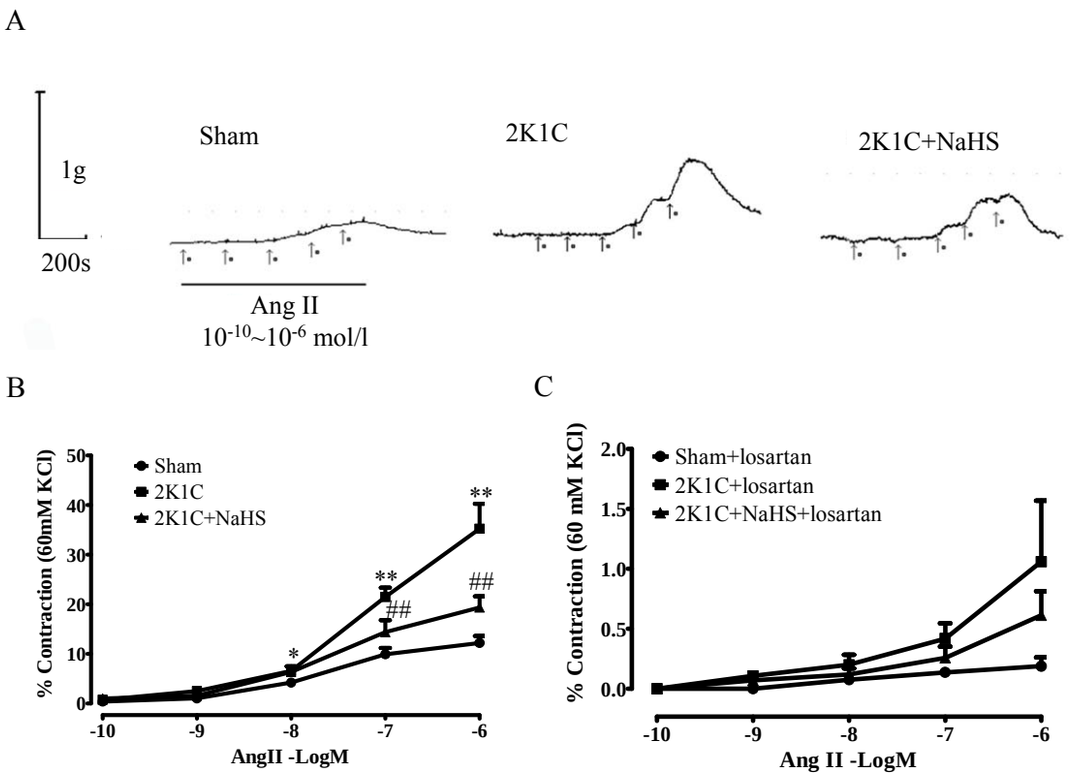

A
Sham
2K1C
2K1C+NaHS
1g
200s
Ang II
10-10~10-6 mol/l
B
Sham
2K1C
2K1C+NaHS
% Contraction (60mM KCl)
AngII -LogM
C
Sham+losartan
2K1C+losartan
2K1C+NaHS+losartan
% Contraction (60 mM KCl)
Ang II -LogM

Fig. 3

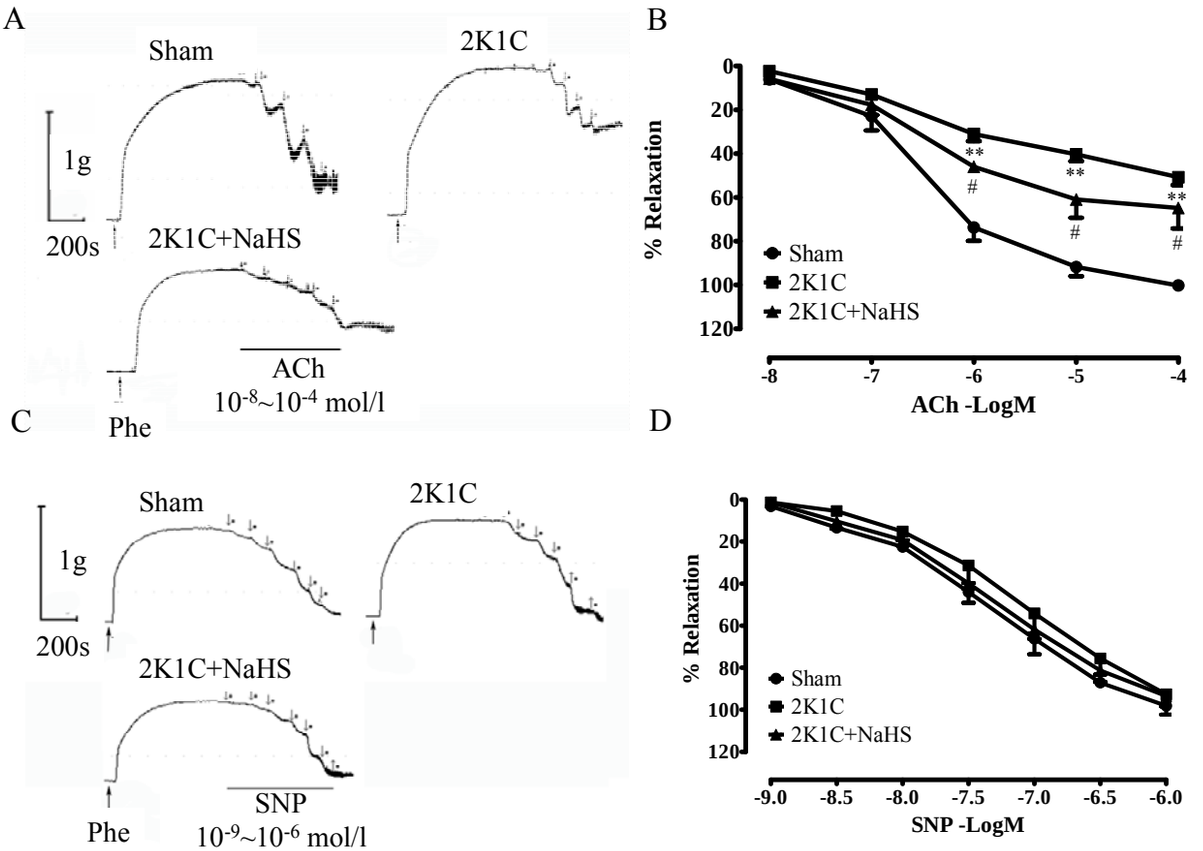

A
Sham
2K1C
1g
200s
2K1C+NaHS
ACh
10-8~10-4 mol/l
Phe
B
% Relaxation
0
20
40
60
80
100
120
Sham
2K1C
2K1C+NaHS
-8
-7
-6
-5
-4
ACh -LogM
C
Sham
2K1C
1g
200s
2K1C+NaHS
SNP
10-9~10-6 mol/l
Phe
D
% Relaxation
Sham
2K1C
2K1C+NaHS
-9.0
-8.5
-8.0
-7.5
-7.0
-6.5
-6.0
SNP -LogM

Fig. 4

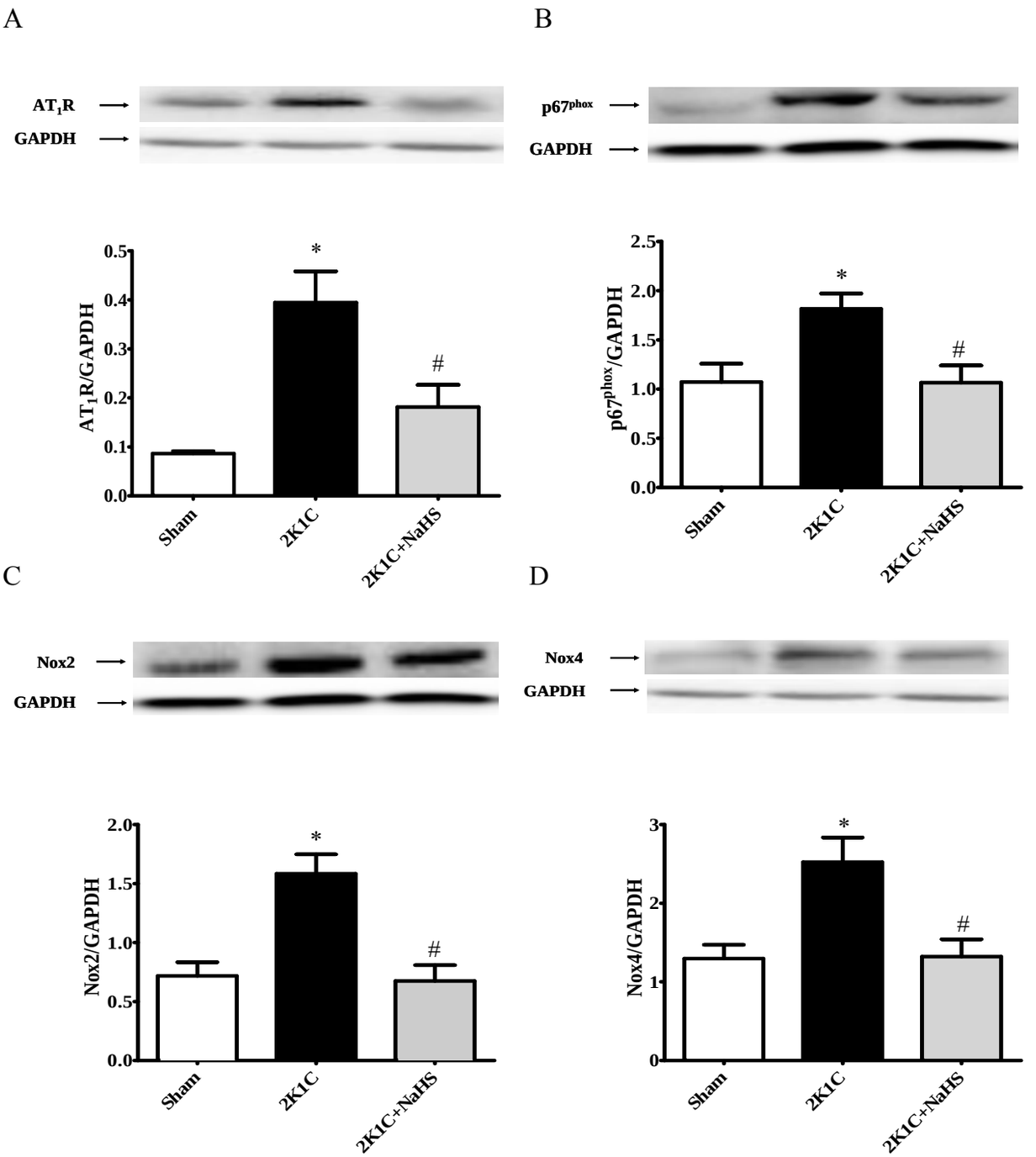

A
B
AT1R
GAPDH
p67phox
GAPDH
AT1R/GAPDH
p67phox/GAPDH
Sham
2K1C
2K1C+NaHS
C
D
Nox2
GAPDH
Nox4
GAPDH
Nox2/GAPDH
Nox4/GAPDH
Sham
2K1C
2K1C+NaHS

Fig. 5

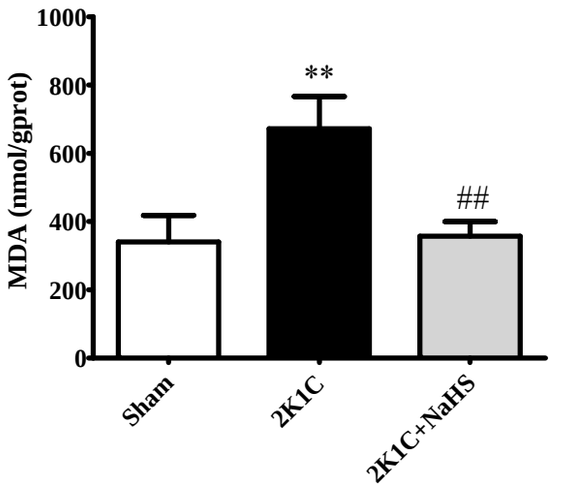


Fig.6

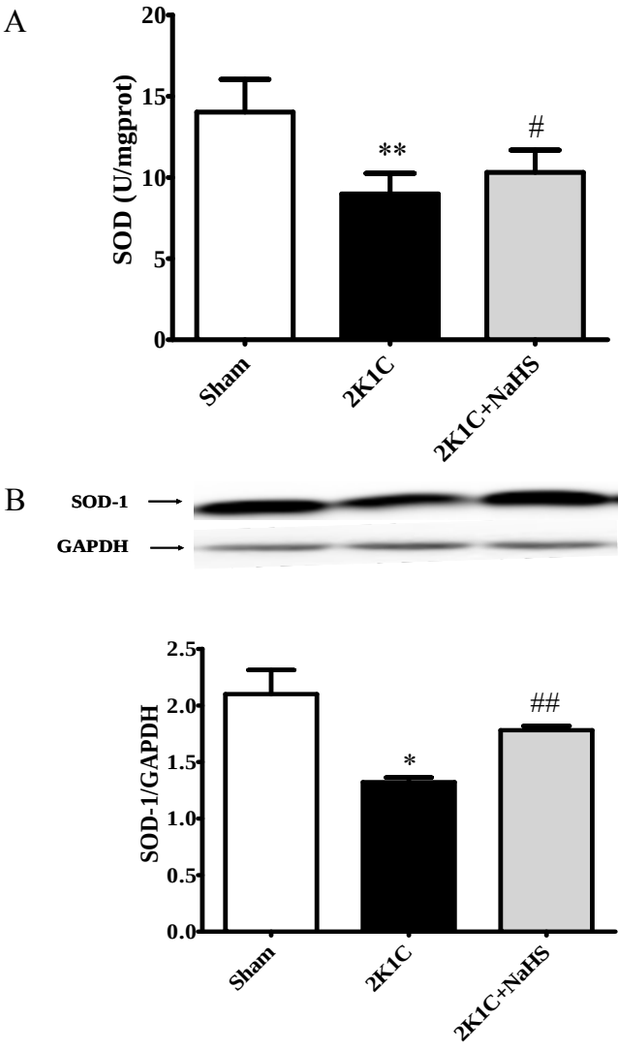
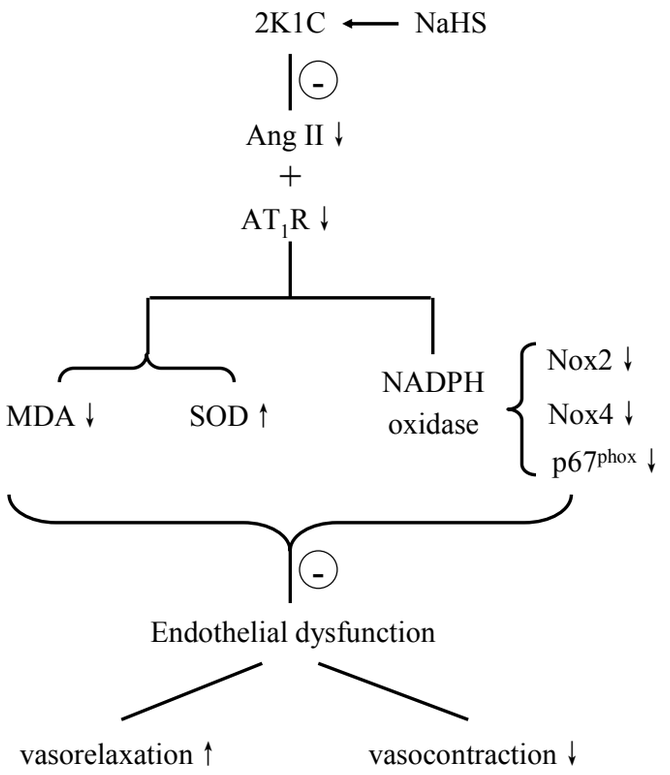

A
SOD (U/mgprot)
20
15
10
5
0
**
#
Sham
2K1C
2K1C+NaHS
B
SOD-1
GAPDH
SOD-1/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
*
##
Sham
2K1C
2K1C+NaHS

Fig. 7


2K1C
NaHS
Ang II ↓
AT1R ↓
MDA ↓
SOD ↑
NADPH oxidase
Nox2 ↓
Nox4 ↓
p67phox ↓
Endothelial dysfunction
vasorelaxation ↑
vasocontraction ↓