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Treatment with NaHS reduces systolic blood pressure and ameliorates oxidative stress in DOCA-Salt hypertensive rats

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Abstract

Background: Hypertension is one of the most serious cardiovascular diseases due to its end organ complications on the heart, kidney, and brain. Hydrogen sulfide gas was shown to be synthesized in vivo and it was found to play multiple physiologic and pathophysiologic roles on different organ systems; However, its role in regulation of blood pressure is not well-understood; Thus, the aim of this study is to investigate the effects of the H_2S donor, NaHS on systolic blood pressure and oxidative stress in a chronic model of hypertension. **Materials and methods**: Hypertension was induced in male Sprague Dawley rats by unilateral nephrectomy followed by injection of deoxycorticosterone acetate (DOCA) (20 mg/kg)in olive oil s.c. for 4 weeks in addition; drinking water was replaced by 1 % NaCl solution. Briefly animals were divided into three groups, 4 animals of each; sham control, hypertensive non-treated (HTN) and hypertensive treated with NaHS (HTN-NaHS) 56 µmole/kg/day in normal saline, i.p for 4 weeks. Blood pressure was measured by a non-invasive tail cuff method before induction of hypertension, after induction and after treatment with NaHS. At the end of experiment blood and aortic tissue samples were collected for assessment of malondialdehyde (MDA) and catalase enzyme activity. **Results:** Treatment with NaHS caused significant decrease in systolic blood pressure, decrease of MDA level in serum and aortic tissue and increase of catalase enzyme activity in aortic tissue. **Conclusion:** NaHS treatment reduced blood pressure and ameliorated oxidative stress in hypertensive rats.

Key words

Hypertension, DOCA, Hydrogen sulfide, NaHS, Oxidative stress.

1. Introduction

Hypertension is defined as a sustained elevation of arterial blood pressure above 120/80 mmHg. It is considered as a major risk factor for cardiovascular complications such as ischemic heart disease, congestive heart failure and other complications including stroke and chronic kidney disease.[1, 2] Control of blood pressure is a quite complex physiologic process that involves contributions from the nervous system, hormonal control, in addition to the renal system. Similarly, the pathogenesis of hypertension involves multiple dysregulations of the controlling mechanisms. Overactivation of the sympathetic nervous system, [3] oversecretion of vasoconstrictor hormones and peptides such as angiotensin II and endothelin [4, 5], impairment of renal excretory functions [6] in addition to endothelial dysfunction [7, 8] represent major pathways implicated in the development of hypertension. Moreover, increased oxidative stress, a hallmark of hypertension, [9] was found to contribute in the development of the previously mentioned pathophysiologic conditions. [8] Reduction of blood pressure in hypertensive patients is of great importance in order to lower the risk of cardiovascular and other complications. [10] Despite the variety of antihypertensive drugs available and the deep understanding of pathogenesis of hypertension, there is still a need for new agents for controlling hypertension and ameliorating its underlying pathologies.

Hydrogen sulfide; the previously classified as a biological hazard gas, has been shown to be synthesized in the brain, blood vessels, heart and other organs from homocysteine and L-cysteine by the action of one of two key enzymes;cystathionine –β-synthase (CBS) and cystathionine-γ-lyase (CSE) as illustrated in **(Figure 1)** [11,13].

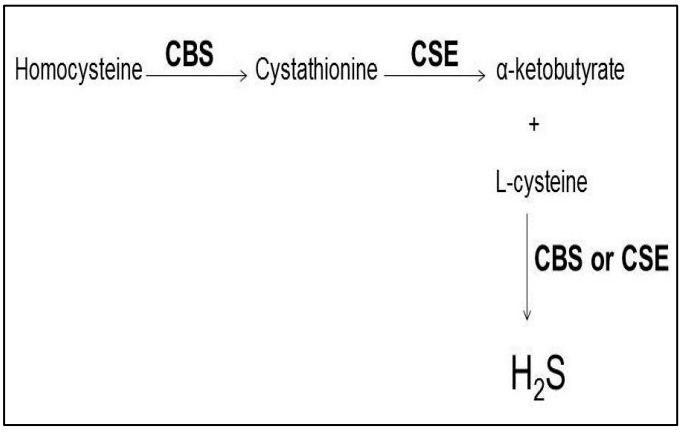

Figure 1: Synthesis of hydrogen sulfide in mammalian tissues. CBS: \ cystathionine-β-sybthase, CSE:cystathionine-γ-lyase.(1)

Functionally, H_2S was found to have a direct smooth muscle relaxant effect in vitro [14] Moreover, exogenous hydrogen sulfide was found to protect from ischemia reperfusion injury in the heart. [15] In addition, H_2S was found to protect against

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diabetic nephropathy; [16,17] Thus, it represents a potential target to study in chronic hypertension.

The aim of this study is to investigate the effect chronic administration of the H_2S donor; NaHS on Blood pressure and oxidative stress in a chronic model of hypertension.

2. Materials and methods

Chemicals: Deoxycorticosterone acetate (DOCA) and Sodium hydrogen sulfide (NaHS) were purchased from Sigma-Aldrich, USA. Xylazine solution for injection (20 mg/ml), ADWIA Company, Egypt, ketamine vials (50 mg/ml), Sigma Pharmaceutical Industries, Egypt, and thiopental sodium 500 mg vials, EIPICO, Egypt. All other chemicals were of analytical grade.

Animals: 12 male Sprague Dawley rats were purchased from the Experimental Animal Facility of Nahda University in Beni Suef, Egypt. Rats were kept under constant environmental conditions, exposed to dark/light cycle of 14:10 hours and had free access to standard commercial rat chow and tap water *ad libitum*. Before the start of the experiment, rats were allowed to acclimatize for two weeks. All experiments were approved by the Research Ethics Committee for the Ethical Principles and Guidelines of the Care and Use of Laboratory Animals, Faculty of Pharmacy, Minia University (Ethical approval number: MPH-02/18).

3. Induction of hypertension & Experimental design

For unilateral nephrectomy rats weighing 250-300 g were anaesthetized with a combination of xylazine 10 mg/kg and ketamine 80 mg/kg [18], fixed on a surgical plate on their ventral side and a 2 cm dorsal incision just below the end of thorax and perpendicular to the spinal cord was made to expose the kidney. Kidney was freed from surrounding fat, and the ureter, renal artery and renal vein were ligated with silk surgical suture then, the kidney was removed and the incision was sutured **(Figure 2 illustrates the steps of nephrectomy)**. [19] Nephrectomized rats were kept isolated and given ampicillin 50 mg/kg i.m for 5 days for complete healing then, injected with DOCA 20 mg/kg in olive oil twice weekly for 4 weeks accompanied by 1 % NaCl in drinking water. [19] Sham-operated animals were subjected to the same surgical procedure except for the step of nephrectomy and were injected with the vehicle; olive oil twice weekly for 4 weeks. At the end of induction period, hypertensive animals were subdivided into 2 groups: hypertensive group treated with NaHS 56 µmole/kg/day i.p. [20] for 4 weeks (HTN-NaHS) and hypertensive non-treated group (HTN) injected with equivalent volumes of normal saline.

4. Measurement of Blood Pressure

Blood pressure was measured before induction, after induction and at the end of treatment by non-invasive tail cuff method (Model LE 5001 pressure meter, Panlab, Harvard Apparatus, Spain).Rats were trained for 3 consecutive days prior to measurement to ensure the reliability of readings according to manufacturer recommendations. Systolic blood pressure 160 mmHg and above was considered successfully induced hypertension. [19]

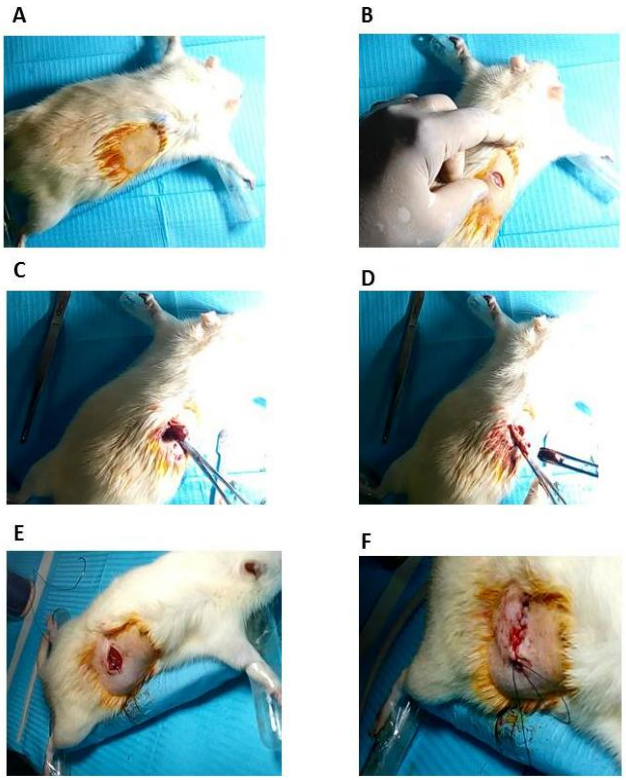

Figure 2: Steps of unilateral nephrectomy [19]
A: Shaving and sterilization with povidone iodine. **B:** incision, **C:** Exposure of kidney and ligation of renal artery, renal vein and ureter, **D:** Removal of kindney, **E:** suturing of muscle laver, F: suturing of skin

5. Blood collection

At the end of study rats were fasted for 12 hours, and then anaesthetized with thiopental 50 mg/kg i.p. Blood samples were collected in Eppendorff tubes by exsanguination, allowed to coagulate and centrifuged at 10000 rpm for 10 minutes to separate serum. Serum samples were collected and stored at – 20 °C till the time of analysis.

6. Tissue isolation and preparation

Aortae were harvested immediately and freed from perivascular advential tissue using stereoscope (Leica MS 5) as previously described by El-Daly et al, 2014, [21] flash frozen in liquid nitrogen and stored at – 20 °C for biochemical analysis.

7. Evaluation of oxidative stress parameters

1. Malondialdehyde (MDA) content in serum and aortic tissue homogenate (10 % in PBS) was determined colorimetrically as thiobarbituric acid reactive species (TBARS) by the method described by Buege and Aust 1978. [22]
2. Activity of catalase (CAT) enzyme in aortic tissue homogenate (10 % in PBS) was measured by colorimetric assay following the manufacturer instructions (Biodiagnostics, Egypt) [23].

8. Statistical analysis

Data are represented as mean ± SEM. Comparisons were done by One-way ANOVA followed by Tukey`s multiple comparisons test using GraphPad Prism version 7.04 for Windows, GraphPad Software, La Jolla California USA, *"www.graphpad.com"* and *p* values less than 0.05 were considered significant.

9. Results

9.1. NaHS reduces Systolic Blood Pressure (SBP) in hypertensive rats

9.1.1. Effect of Doca-Salt-Nephrectomy on SBP

After induction period, nephrectomized-DOCA-treated rats showed significant elevation in systolic blood pressure (123.2 ± 1.37 and 166.8 ± 3.97 mmHg. $p < 0.05$)while sham-operated rats, injected with olive oil showed no significant change (122.10 ± 0.66 and 124.3 ± 1.57 mmHg, $p < 0.05$). The elevation in SBP in induced rats was significantly different from sham animals ($p < 0.05$). **(Figure 3)** A shows systolic blood pressure before and after the induction period and figure 3 B illustrates the % change in SBP at the end of induction period.

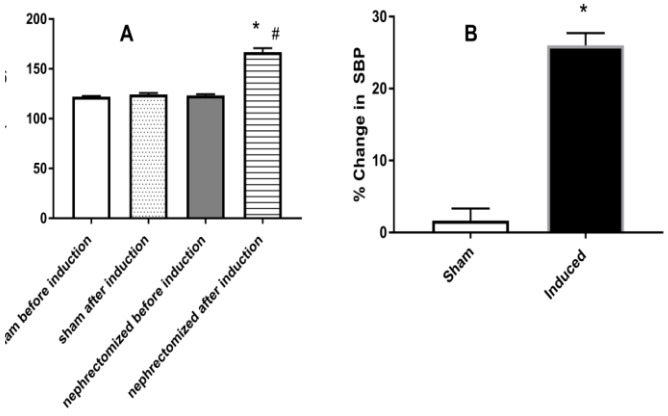

Figure 3: (A) Systolic blood pressure before and after induction by DOCA-salt nephrectomy model. Sham operated rats, injected with olive oil, show no significant change in SBP while nephrectomized rats, given DOCA and salt, show significant increase in SBP. * Significant difference from sham group *($p < 0.05$)*, # significant difference from before induction *($p < 0.05$)*. **B:** % change in SBP before and after induction period. * Significant difference from sham group *($p < 0.05$)*. Data represents mean ± SEM of 4 observations

9.1.2. Effect of NaHS treatment on SBP

Treatment with NaHS (56 µmole/kg/day i.p for 4 weeks) caused significant decrease in SBP compared to hypertensive non-treated animals (142.58 ± 0.54 mmHg vs. 174.33 ± 1.46 mmHg, $p < 0.05$) **(Figures 4)**.

9.2. NaHS reduces MDA level in serum and aortic tissue

HTN rats showed significant increase in serum MDA level compared to sham rats (22.78 ± 1.72 vs. 15.50 ± 0.47nmole/ml respectively. $p < 0.05$). On the other hand treatment with NaHS 56 µmole/kg/day i.p for 4 weeks significantly reduced serum MDA compared to HTN and sham groups (9.10 ± 0.34 and vs. 22.78 ± 1.72 and 15.50 ± 0.47 nmole/ml respectively. $p < 0.05$) (**Figure 5 A)**.

Hypertension caused significant elevation in aortic tissue MDA level compared to sham animals while treatment with NaHS significantly decreased MDA level (23.33 ± 0.6692, 15.1 ± 0.9048 and 17.33± 1.63nmole/g tissue respectively. $p < 0.05$). **(Figure 5 B)**

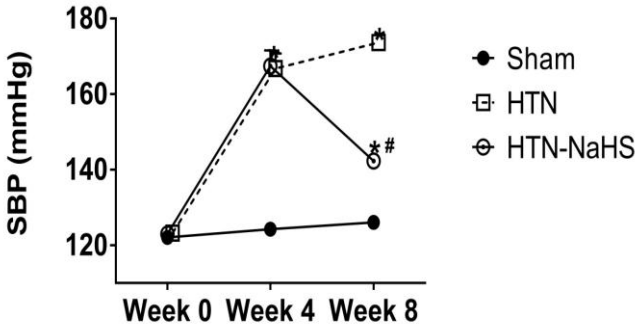

Figure 4: SBP before induction (week 0), after induction (week 4) and after treatment (week 8). * Significant difference from sham group at corresponding week *($p < 0.05$)*, # significant difference from HTN group at corresponding week *($p < 0.05$)*.Data represents the mean ± SEM of 4 observations.

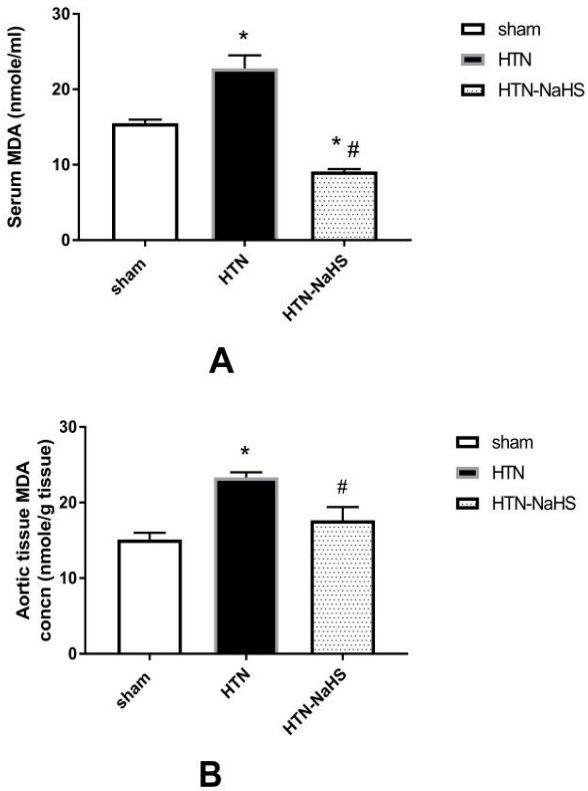

Figure 5: A) Effect of hypertension and treatment with NaHS (56 µmole/kg/day i.p for 4 weeks) on serum MDA level. **B)** Effect of hypertension and treatment with NaHS (56 µmole/kg/day i.p for 4 weeks) on aortic tissue MDA level. * Significant difference from sham group, $p < 0.05$, # significant difference from HTN group, $p < 0.05$.Data represents the mean ± SEM of 4 observations.

9.3. NaHS enhances endogenous catalase activity in aortic tissue

Hypertension was associated with significant reduction in aortic tissue catalase activity compared to sham animals (7.13 ±

0.48vs. 14.17 ± 0.23 U/g tissue, respectively. $p < 0.05$) while treatment with NaHS caused significant elevation in catalase activity compared to HTN animals (17.07± 1.024 vs. 7.13 ± 0.48 U/g tissue, respectively. $p < 0.05$). **(Figure 6)**.

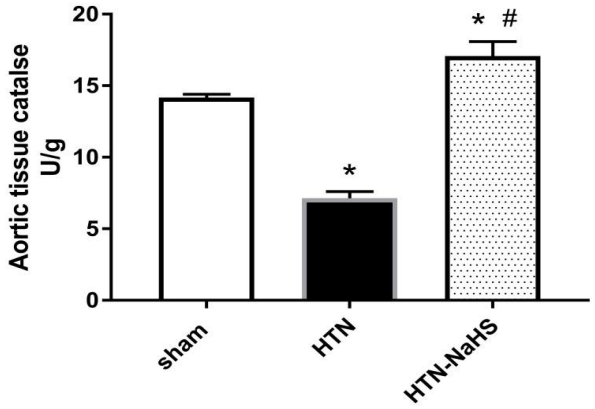

Figure 6: Effect of hypertension and treatment with NaHS 56 µmole/kg/day i.p for 4 weeks on aortic tissue catalase activity. * Significant difference from sham group, $p < 0.05$, # significant difference from HTN group, $p < 0.05$.Data represents the mean ± SEM of 4 observations.

10. Discussion

Hypertension is a condition of sustained elevation of systolic blood pressure that develops as a result of neural, hormonal, vascular, renal and other mechanisms. [1, 2, 6, 8, 9] Most of these mechanisms involves or induces oxidative stress. [9] Hydrogen sulfide has found a place as an endogenous gaseous mediator with a wide range of biological functions. [24] On the heart, H_2S has negative inotropic effects in vitro and in vivo, and protects against injury following coronary artery ligation, ischemia-reperfusion and lipopolysaccharide injection. [25,27] Also, H_2S has direct vasodilator effects on blood vessels *in vitro* probably through opening of K_{ATP} channel [28]. Examples include the isolated rat aorta and portal vein, [28,30] rabbit corpus cavernosum, [31] and the perfused rat mesenteric [32] and hepatic, [33] but not coronary, [15] vascular beds. In intact animals, intravenous injection of NaHS caused short-lived but dose-dependent decrease in mean arterial blood pressure. [28, 30] Not only blood vessels that respond to H_2S with relaxation; but other in vitro smooth muscle preparations as gastrointestinal [34] and airways [35] smooth muscle preparations are relaxed by H_2S as well. The previously mentioned studies showed that H_2S has a vasorelaxant effect and a short-lived hypotensive response; however, these studies were conducted either in vitro on isolated vessels and vascular beds or on anaesthetized normotensive animals. Thus, the hypothesis of this study was that in vivo treatment with NaHS in a chronic model of hypertension may cause reduction of systolic blood pressure. To test this hypothesis, a well-established model of chronic hypertension, DOCA-Salt nephrectomy model, [5, 19, 36, 37] was applied and the results showed that the model caused a significant and persistent elevation of systolic blood pressure. In addition, NaHS was given as an H_2S donor [38] to hypertensive rats. In the NaHS - treated hypertensive rats, approximately a 15 % reduction in SBP was found at the end of treatment while the hypertensive non-treated and sham rats showed minor elevation in SBP (approximately, 2.3 % and 1 %, respectively). This indicates that treatment with an H_2S donor significantly reduced systolic blood pressure on the other hand; the hypertensive non-treated rats showed no spontaneous reduction in SBP so that, it is quite possible to correlate this reduction in blood pressure to H_2S effect. Oxidative stress contributes to the pathogenesis of hypertension through vascular dysfunction, vascular smooth muscle remodeling, and atherosclerotic plaque formation, in addition, ROS mediates the vasoconstrictor effects of angiotensin II. [8, 9] The cellular damage of ROS is mainly through lipid peroxidation leading to structural changes of biological membranes.MDA is a result of lipid peroxidation that can reliably reflect the oxidative stress status of hypertension. [39,41] Besides, catalase enzyme represents an important endogenous antioxidant defense that also was found to be down regulated in hypertension. [41, 42] The results showed that, in comparison with sham control group, the hypertensive non-treated group showed significant elevation in MDA level in serum and aortic tissue moreover, catalase activity was significantly reduced in aortic tissue while NaHS treatment ameliorated the increase in MDA and improved the activity of catalase enzyme. Although the marked improvement of oxidative stress parameters measured in this study, further assessment of other oxidative stress parameters is required for complete evaluation of the antioxidant activity of H_2S. Nonetheless, the marked reduction in blood pressure and in the measured oxidative stress parameters indicates that H_2S may represent a potential target for treatment of chronic hypertension.

Conclusion:

The findings of this study showed that treatment with the H_2S donor, NaHS, significantly reduced systolic blood pressure, some reduced the oxidative stress marker MDA and improved endogenous antioxidant catalase enzyme. Whether, the hypotensive effect was attributed to the partial amelioration of oxidative stress, or to other mechanisms requires more future work.

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