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Hydrogen sulfide: A novel mechanism for the vascular protection by resveratrol under oxidative stress in mouse aorta

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HYDROGEN SULFIDE: A NOVEL MECHANISM FOR THE VASCULAR PROTECTION BY
RESVERATROL UNDER OXIDATIVE STRESS IN MOUSE AORTA

Running title: Resveratrol targets H₂S to provide cardiovascular protection

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Abstract

Reactive oxygen species (ROS) decreases bioavailability of nitric oxide (NO) and impairs NO-dependent relaxations. Like NO, hydrogen sulfide (H₂S) is an antioxidant and vasodilator; however, the effect of ROS on H₂S-induced relaxations is unknown. Here we investigated whether ROS altered the effect of H₂S on vascular tone in mouse aorta and determined whether resveratrol (RVT) protects it via H₂S. Pyrogallol induced ROS formation. It also decreased H₂S formation and relaxation induced by L-cysteine and in mouse aorta. Pyrogallol did not alter sodium hydrosulfide (NaHS)-induced relaxation suggesting that the pyrogallol effect on L-cysteine relaxations was due to endogenous H₂S formation. RVT inhibited ROS formation, enhanced L-cysteine-induced relaxations and increased H₂S level in aortas exposed to pyrogallol suggesting that RVT protects against “H₂S-dysfunctions” by inducing H₂S formation. Indeed, H₂S synthesis inhibitor AOAA inhibited the protective effects of RVT. RVT had no effect on Ach-induced relaxation that is NO dependent and the stimulatory effect of RVT on H₂S-dependent relaxation was also independent of NO. These results demonstrate that oxidative stress impairs endogenous H₂S-induced relaxations and RVT offers protection by inducing H₂S suggesting that targeting endogenous H₂S pathway may prevent vascular dysfunctions associated by oxidative stress.

Keyword: Oxidative Stress, Resveratrol, Hydrogen Sulfide, Aorta, Relaxation

Introduction

Atherosclerosis, hypertension, diabetes mellitus and even preeclampsia are all associated with increased reactive oxygen species (ROS) [1]. Nitric oxide (NO) protects against myocardial infarction [2; 3] and ROS decreases bioavailability of NO. Hydrogen sulfide (H₂S) also possesses cardiovascular protective properties. Levels of H₂S is decreased in aging [4], hyperglycaemia [5; 6], diabetes [7] and preeclampsia [8]. The enzymes responsible for the generation of endogenous H₂S include cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and mitochondrial 3-mercaptopyruvate sulfurtransferase (3MPST). The production of H₂S through the MPST pathway is decreased by pro-oxidant H₂O₂ *in vitro* [6], however the role of oxidants on H₂S formation and vasorelaxation induced by L-cysteine is unknown. The aim of this study was to investigate the effects of oxidative stress induced by pyrogallol on H₂S-induced relaxation in mouse aorta.

The antioxidant Resveratrol (RVT), a polyphenol present in red wine, offers protection in vascular pathologies such as diabetes, erectile dysfunction, hypertension and metabolic syndrome [9] and preeclampsia [10]. Interestingly, the beneficial effect of RVT is attributed to activation of eNOS and inhibition of NADPH oxidase [11; 12] via SIRT [12; 13] in vascular system. Hydrogen sulfide activates eNOS [14; 15] and SIRT [16] and inhibits NADPH oxidase [17; 18; 19]. Also nuclear factor erythroid 2-related factor 2 (Nrf-2) is enrolled in the antioxidant effect of both RVT [20] and H₂S [7; 21; 22]. These common links led us to further investigate the role of H₂S in the protective effects of RVT in vascular oxidative stress in the regulation of vascular tone and ROS formation in mouse aorta.

Method

Animals

The present study was approved by the Animal Experiment Local Ethical Committee of Ege University (No: 2015-070) in agreement with European guidelines for animal care. All experiments were conducted on 10 to 12 weeks of age male CD1 mice (n=50) obtained from the Breeding Center of Experimental Animals in Ege University (ARGEFAR). The animals were stunned by inhalation of CO₂, sacrificed by decapitation and exsanguinated.

Drugs and treatments

For ROS/H₂S formation, isolated aortic rings in HEPES buffer or homogenates in phosphate buffer were incubated respectively with either cystathionine gamma lyase/cystathionine beta synthase (CSE/CBS) inhibitor (AOAA, 2 mM), CSE inhibitor PAG (10 mM) or the eNOS inhibitor L-NNA (0.1mM) for 30 minutes. Thereafter, without washing, RVT (0.01 mM) was added for 30 min in the presence or absence of the inhibitors. Pyrogallol was then added (0.1mM) for 5 min prior to assaying (Fig 1).

The same order of incubation without washing in KREBS Buffer was used for isolated organ bath experiments to get dose response curves after confirmation of the presence of the endothelium. In some experiments to measure H₂S or ROS level, NAHS (1mM) was added to the homogenates or isolated aortic rings for 10 minutes followed by pyrogallol for 5 min without washing.

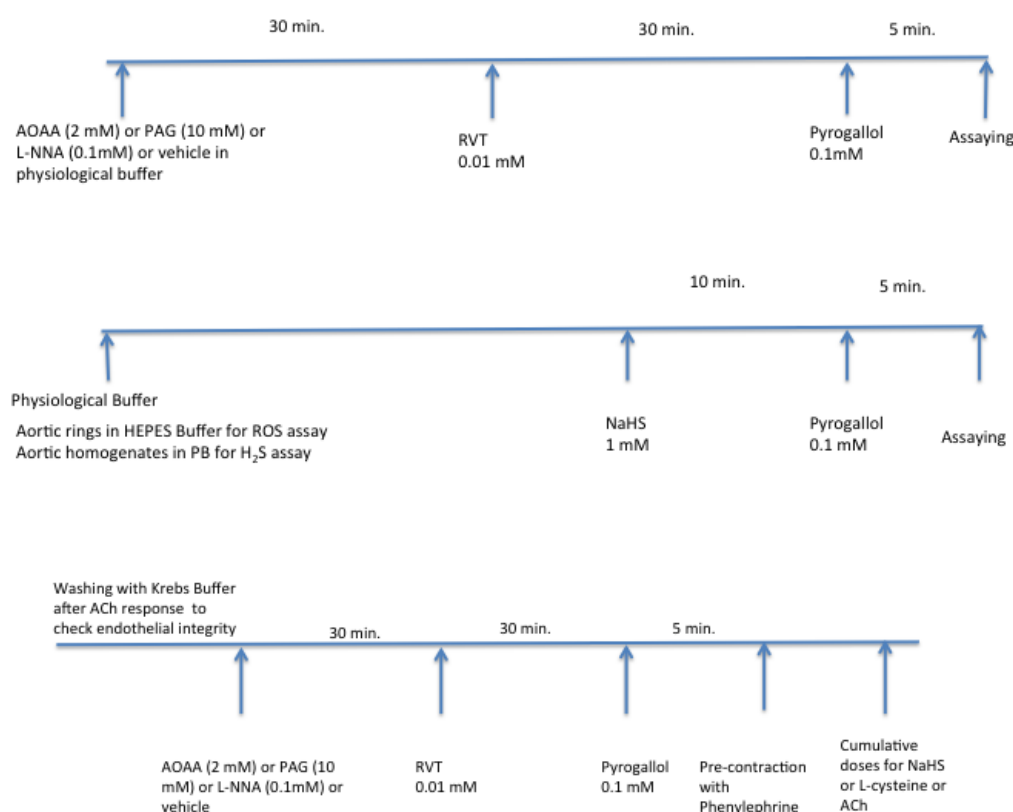


Fig. 1 Protocol for the treatments used in the study

RVT were acquired from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Unless otherwise stated, all other chemicals were obtained from Sigma.

Measurement of reactive oxygen species formation by chemiluminescence

Luminol and lucigenin chemiluminescences was measured as indicators of ROS formation according to the modified method described by Munzel et al [23]. The assay was performed with aortic rings in 1 ml HEPES-buffered physiological solution (pH 7.4). After incubations with pharmacological agents as given above lucigenin or luminol (5 $\mu\text{mol/L}$) were added to measure ROS level. This lower concentration of lucigenin was demonstrated not to be involved in redox cycling and to specifically indicate superoxide anion levels in intact vascular tissue. ROS were quantified using a multi-plate reader (VarioskanTM Flash multimode reader, Thermo Scientific, USA). Luminol detects a group of reactive species, i.e., $\cdot\text{OH}$, H_2O_2 , HOCl , and lucigenin is selective for O_2^- [24]. Counts were obtained at 1 min intervals for a counting period of 5 min. and the results were given as the area under the curve (AUC) of relative light units (rlu)/mg of wet tissue.

Isolated organ bath experiments

The aorta was isolated and cut into two 4 mm-long rings after removal of loose connective tissue. The individual aortic rings were separated, mounted in 5 ml organ bath of DMT 610 ring myograph for isometric force recording (Danish Myograph Technology, Aarhus, Denmark) coupled to a PowerLab 8/SP data acquisition system (Chart 5.0 software; ADInstruments, Colorado Springs, CO) and bathed in carboxygenated (95% O_2 ; 5% CO_2) modified Krebs–Ringer solution NaCl, 130 mM; NaHCO_3 , 14.9 mM; dextrose, 5.5 mM; KCl, 4.7 mM; KH_2PO_4 , 1.18 mM; $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 1.17 mM and $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$, 1.6 mM) at 37 °C. Tissues were allowed to equilibrate for 90 min. under a resting tension of 20 mN. Experiments were done in rings with endothelium as confirmed by relaxation more than 40 % to ACh (1 μM) after contraction with phenylephrine (Phe, 10 μM). One concentration-response curve was obtained in each ring. After confirmation of presence of the endothelium, relaxant responses to either ACh (10^{-9} - 10^{-4} M) or exogenous H_2S donor NaHS (10^{-6} - $3\cdot 10^{-2}$ M) or endogenous H_2S donor L-cysteine (10-20-40-80 mM) was obtained after pre-contraction by phenylephrine. The concentration of phenylephrine is altered in some vessels to get equal pre-contraction. Aortic rings were exposed to pyrogallol to induce oxidative stress.

Measurement of H₂S level by Methylene blue assay

To assess H₂S formation by CSE/CBS pathway in aorta, H₂S determination was evaluated according to Stipanuk and Beck [25]. Since the mouse aorta is thin and small, at least 3-4 mice aorta were combined, homogenized with lysis buffer. After incubation with pharmacological agents as given above, the same protocol with our previous study was used to measure H₂S formation in aortic homogenates [26]. The optical absorbance of the resulting solution was measured after 10 min. at a wavelength of 650 nm. All samples were assayed in duplicate. The absorbance values obtained in the absence of homogenates under different treatments were subtracted from reel absorbance to get net absorbance to calculate H₂S concentration in samples against a calibration curve of NaHS (3.9–250 μM). Data were calculated as nmol per milligram of protein.min⁻¹.

Statistics

Data are expressed as % relaxation of the Phe-induced tone. Concentration–response curves were fitted by sigmoid curves using the least squares method. All calculations were determined using a standard statistical software package (Prism5, Graphpad, San Diego, California, USA). Significance was accepted at p<0.05. The data were computed as means ± SEM and evaluated statistically using the One-Way ANOVA or Two-Way ANOVA when appropriate. If there is an interaction between concentrations and treatments Bonferroni post hoc test were used after ANOVA. “n” is the number of tissues.

RESULTS

Reactive oxygen species

Pyrogallol treatment (0.1 mM, 5 min.) induced both superoxide and other reactive oxygen species (ROS) generation in mouse aorta (Figure 2a and 2b, respectively, p<0.01, One-Way ANOVA, Bonferroni post hoc test, n=6). Both RVT (0.01 mM, 30 min.) and exogenous H₂S donor NaHS significantly inhibited ROS generation (Figure 2a and 2b, p<0.01, p<0.05, respectively, One-Way ANOVA, Bonferroni post hoc test, n=7). CBS and CSE inhibitor AOAA or the CSE inhibitor PAG completely reversed the antioxidant effect of RVT (Figure 2a and b, p<0.05, One-Way Anova,

Bonferroni post hoc test, n=7). However eNOS inhibitor L-NNA did not alter RVT-induced inhibition of superoxide or other ROS formation significantly.

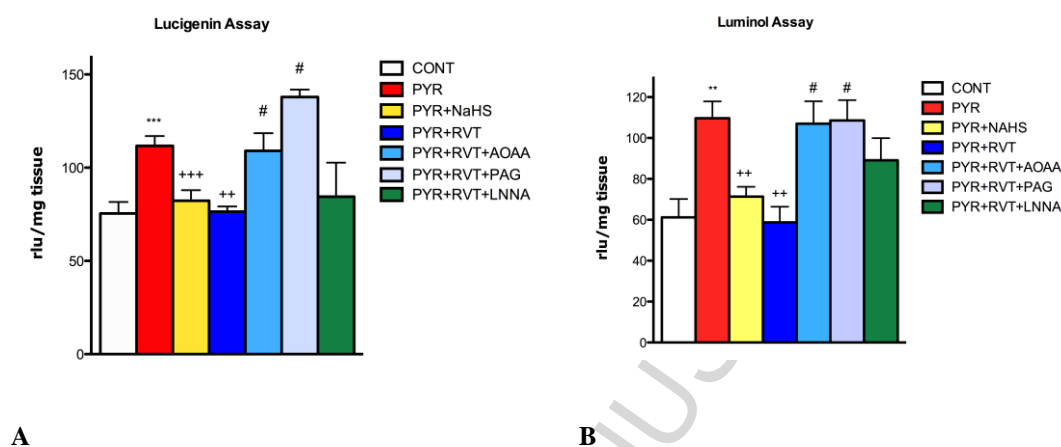


Fig. 2 The effects of RVT on A) superoxide B) other reactive oxygen species induced by pyrogallol in mouse aorta

Pyrogallol (0.1mM) increased superoxide (O_2^-) and ROS generation, as measured by lucigenin and luminol assay, respectively. RVT (0.01 mM) or NaHS (1mM) significantly inhibited O_2^- and ROS formation induced by pyrogallol. H_2S inhibitor AOAA (2 mM) or PAG (10 mM), reversed these effects significantly. Although there is a trend, L-NNA (0.1mM) could not reverse the antioxidant effect of RVT significantly. ** $p < 0.01$, *** $p < 0.001$ compared to control, ++ $p < 0.01$, +++ $p < 0.001$, compared to pyrogallol (PYR), # $p < 0.05$ compared to pyrogallol+resveratrol (PYR+RVT), One-Way Anova, n=7.

ACh relaxations

ACh caused relaxation in the mouse aorta in a concentration-dependent manner. Pyrogallol decreased the ACh-induced relaxation (Figure 3, $p < 0.05$, compared to control, Two-Way ANOVA, n=5). RVT (0.01 mM, 30 min.) did not alter NO-dependent relaxations in normal conditions or in the presence of oxidative stress (Figure 3, Two way Anova, n=5). Pyrogallol reduced pD_2 values significantly (Table 1, $p < 0.01$, One way Anova, n=5) and tends to decrease maximal relaxation to ACh however it did not reach a significant level. RVT did not alter pyrogallol-induced decrease in pD_2 values or tendency to reduced maximal relaxation to ACh

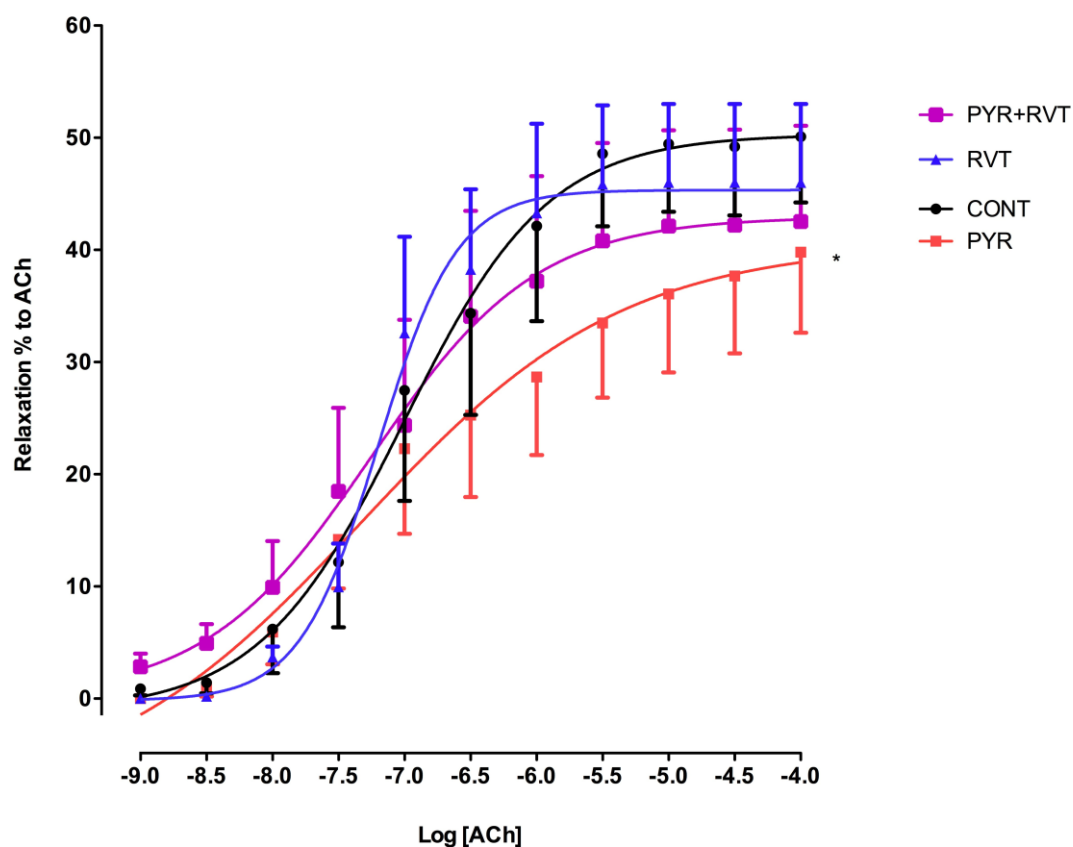


Fig. 3 The effect of RVT on ACh relaxation in pyrogallol-induced oxidative stress in mouse aorta

Pyrogallol (0.1mM) slightly decreased ACh relaxations in mouse aorta. RVT (0.01 mM) did not alter NO-dependent relaxations in normal conditions but restored impaired relaxations in the presence of pyrogallol-induced oxidative stress. * $p < 0.05$, control vs. pyrogallol, Two-Way Anova, $n = 5$.

| | CONT | PYR | RVT | RVT+PYR |
|-------------------|-------------------|----------------------|-------------------|-------------------|
| pD_2 for ACh | 6.921 ± 0.256 | 6.791 ± 0.329 ** | 7.131 ± 0.122 | 6.996 ± 0.212 |
| E_{Max} for ACh | 50.10 ± 5.876 | 39.79 ± 7.174 | 46.02 ± 6.996 | 42.52 ± 8.539 |

Table 1 The effect of RVT on pD_2 values and maximal relaxation of ACh relaxation in the presence or absence of pyrogallol-induced oxidative stress in mouse aorta. Pyrogallol (0.1mM)

significantly decreased pD_2 values without changing maximal relaxation to ACh relaxations in mouse aorta. RVT (0.01 mM) did not alter maximal relaxations or pD_2 values. ** $p < 0.01$, control vs. pyrogallol, One-Way Anova, $n = 5$.

L-cysteine relaxations

L-cysteine caused a concentration dependent slight contraction at the very first concentrations and then relaxation in mouse aorta. Endogenous H₂S-relaxation was significantly diminished in the presence of oxidative stress (Figure 5, $p < 0.001$, Two-Way Anova, $n = 9-6$, respectively). Despite, RVT (0.01 mM, 30 min.) enhanced relaxation to L-cysteine and this effect was inhibited by the CSE inhibitor PAG or CBS/CSE inhibitor AOAA at the same rate but not by eNOS inhibitor L-NNA significantly (Figure 4, $p < 0.001$ compared to control and $p < 0.001$, compared to RVT, Two-Way Anova, $n = 5-11$).

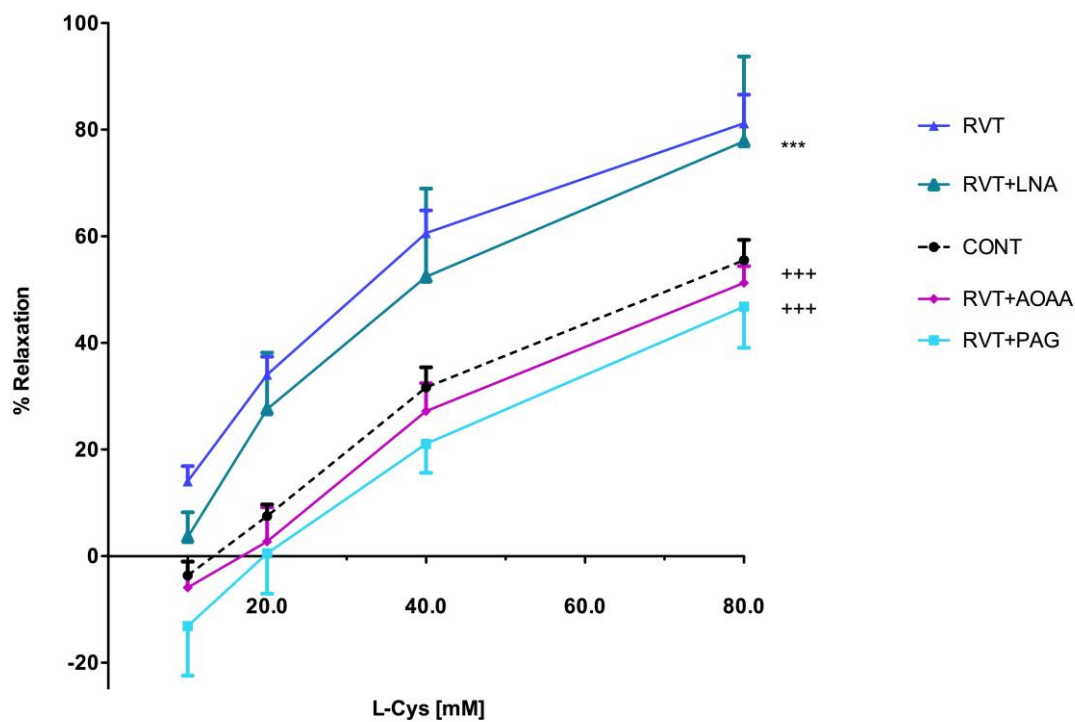


Fig. 4 The effect of RVT on L-cysteine induced relaxations in mouse aorta

RVT (0.01 mM) increased L-cysteine relaxation significantly. This effect of RVT were inhibited by CBS/CSE inhibitor AOAA (2 mM) or the CSE inhibitor PAG (10 mM) significantly but not by eNOS inhibitor L-NNA (0.1mM). *** $p < 0.001$ compared to control (CONT), +++ $p < 0.001$ compared to resveratrol (RVT), Two Way Anova, $n = 5-11$.

The stimulatory effect of RVT was still dominant under oxidative stress. Since both PAG and AOAA inhibited RVT-induced augmentation of L-cysteine relaxation, the effect of PAG as CSE inhibitor has been tested to investigate the role of H₂S in the effect of RVT to restore impaired relaxation to L-cysteine under oxidative stress. RVT could not rescue disrupted endogenous H₂S-dependent relaxations when H₂S synthesis is inhibited by PAG, suggesting H₂S was enrolled in the protective effect of RVT under oxidative stress (Figure 5, $p < 0.001$, compared to PYR, $p < 0.05$ compared to PYR+RVT, Two-Way Anova $n=5$, respectively).

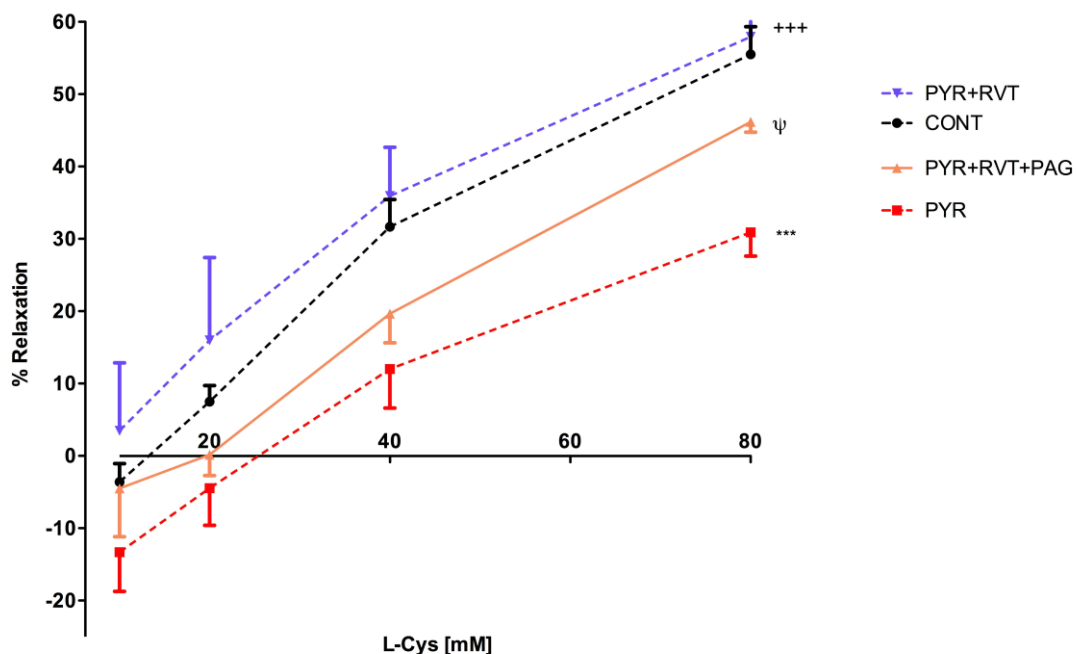


Fig. 5 The effect of resveratrol on L-cysteine induced relaxations in the presence of pyrogallol-induced oxidative stress in mouse aorta

Pyrogallol (0.1mM) decreased L-cysteine relaxation significantly. RVT (0.01 mM) restored the diminished endogenous H₂S-dependent relaxations in oxidative stress. H₂S synthesis inhibitor PAG (10 mM) inhibited RVT-induced protection on L-cysteine relaxations. *** $p < 0.001$ compared to control (CONT), +++ $p < 0.001$, compared to Pyrogallol (PYR), ψ $p < 0.05$ compared to pyrogallol+resveratrol (PYR+RVT) Two Way Anova, $n=5-9$.

NaHS relaxations

Alterations in downstream mechanisms of H₂S were tested in response to the exogenous H₂S donor (NaHS). NaHS caused a concentration-dependent relaxation in the mouse aorta. Pyrogallol-induced oxidative stress did not alter relaxation to NaHS (Figure 6, $p > 0.05$, compared to control, Two-Way Anova, $n = 5$).

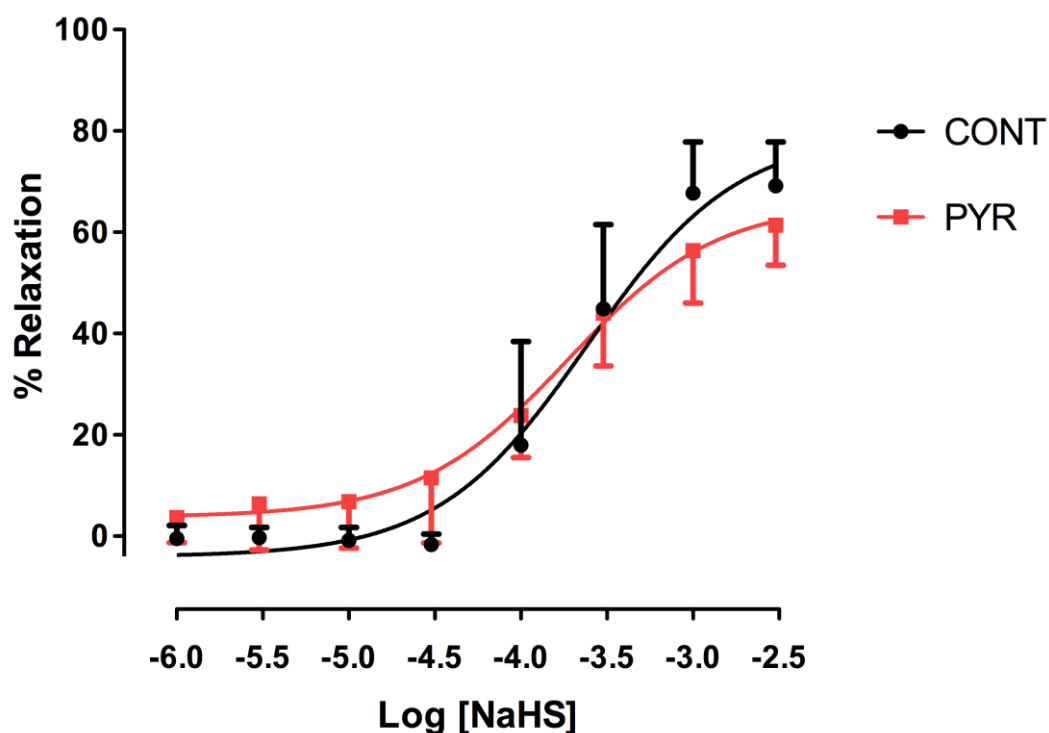


Fig. 6 The effect of pyrogallol on NaHS induced relaxations in mouse aorta

Exogenous H₂S donor NaHS induced concentration dependent relaxation in mouse aorta. Pyrogallol (0.1mM) did not significantly change exogenous H₂S dependent relaxations. $p > 0.05$ compared to control (CONT), Two-Way Anova, $n = 5$.

H₂S formation

H₂S formation in mouse aortic rings was measured by methylene blue assay, to investigate if oxidative stress alters H₂S formation and the role of H₂S in the protective effect of RVT under oxidative stress, RVT incubation caused a significant increase in H₂S levels in both basal and L-cysteine stimulated

conditions. RVT-induced augmentation of H₂S level was inhibited by AOAA. Pyrogallol decreased L-cysteine-stimulated H₂S formation significantly. (Figure 7, $p < 0.001$, One-Way Anova, $n = 7$). RVT reversed this impaired H₂S formation in the presence of oxidative stress (Figure 7, $p < 0.01$, One-Way Anova, $n = 7$).

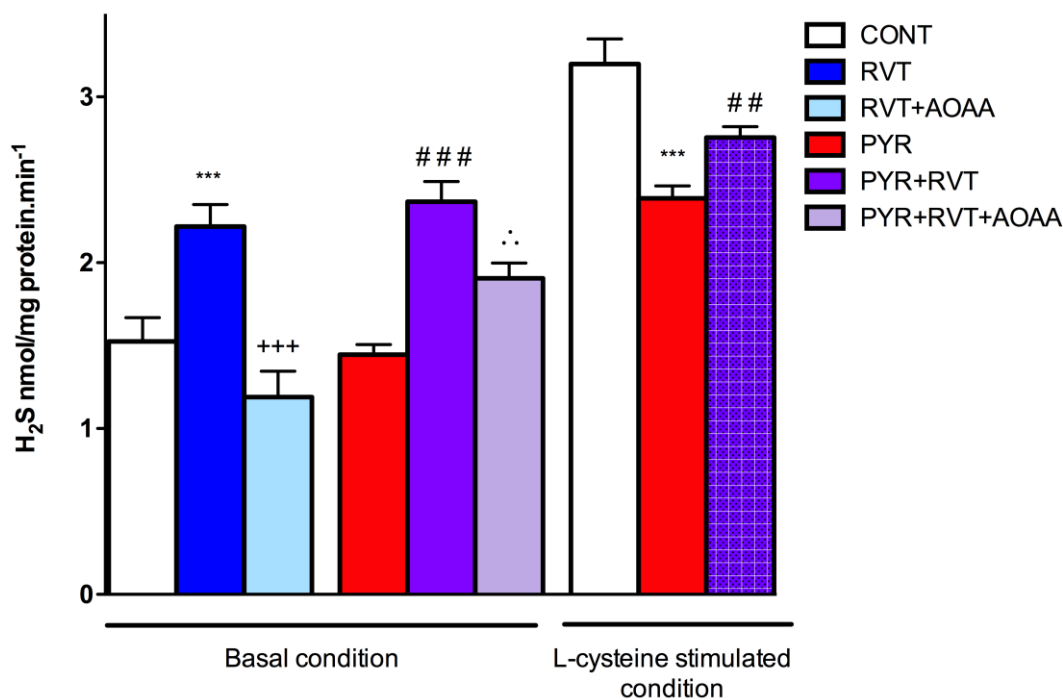


Fig. 7 The effect of RVT and oxidative stress on H₂S formation in mouse aorta

RVT (0.01 mM) significantly increased basal H₂S formation in the presence or absence of oxidative stress and this stimulatory effect was reversed by H₂S synthesis inhibitor AOAA (2 mM). Pyrogallol (0.1mM) decreased the L-cysteine (10 mM) stimulated H₂S formation and RVT restored it slightly. *** $p < 0.001$, compared to control (CONT), +++ $p < 0.001$ compared to RVT, # $p < 0.05$, ## $p < 0.001$ compared to pyrogallol (PYR), ∴ $p < 0.01$ compared to pyrogallol+resveratrol (PYR +RVT). One-Way ANOVA, $n = 7$.

Discussion

This study shows that ROS generated by pyrogallol significantly decreases H₂S formation, which resulted in disruption to the endogenous H₂S-dependent relaxations. Furthermore, the antioxidant effect of RVT requires H₂S but not NO. RVT stimulates H₂S formation through the CBS/CSE pathways and

restores impaired endogenous H₂S-dependent relaxations in the presence of oxidative stress. These results support the therapeutic value of RVT and demonstrate significance of H₂S dysfunction in vascular oxidative stress.

Oxidative stress inhibits NO bioavailability, which is accepted as the main cause of endothelial dysfunction and cardiovascular diseases. H₂S have some similarities to NO to provide protection for cardiovascular system as a vasodilator, antioxidant, anti-inflammatory gasotransmitter, depends on concentration as in NO. There are accumulated evidence showing impaired H₂S level in hyperglycaemia [27], diabetes [28] [29], atherosclerosis [30] and hypercholesterolemia [3] where oxidative stress is accompanied. Although a recent study shows acute oxidative stress by H₂O₂ causes a decrease in MPST-induced H₂S formation [6], there is no study investigating the role of vascular oxidative stress on the CSE/H₂S -dependent regulation of ROS formation and vascular tonus yet. We found that pyrogallol causes both superoxide radical and other ROS formation (Figure 2a and 1b) and oxidative stress induced by pyrogallol significantly decreases L-cysteine stimulated endogenous H₂S formation without altering basal H₂S formation in mouse aorta (Figure 7). To our knowledge this is the first study reporting impaired L-cysteine induced H₂S formation and relaxation in oxidative stress. Several studies showed that L-cysteine, the substrate of CSE and CBS, induces relaxations through H₂S, since CBS/CSE inhibitor AOAA [31] or CSE inhibitor PAG [32] inhibits this relaxation in bladder [33] corpus cavernosum [26; 34] as well as aorta [31] [35]. In our study, L-cysteine caused a relaxation in mouse aorta (Figure 4 and 5) confirming previous studies [35; 36]. The major findings of our study showed that acute oxidative stress inhibits endogenous H₂S-dependent relaxation induced by L-cysteine (Figure 5). Further we found that the impairment in L-cysteine-induced relaxation was not depending on downstream mechanism of vasorelaxation through H₂S, since exogenous H₂S donor NaHS-induced relaxations were not decreased by oxidative stress (Figure 6). Thus we conclude that disrupted L-cysteine induced relaxation in oxidative stress was dependent on decreased endogenous H₂S formation. These data suggest that decreased H₂S may contribute to the regulation of vascular tonus in cardiovascular disease where oxidative stress accompanies.

H₂S-targeting treatments have protective effects in endothelial dysfunctions [3] including diabetes [27], myocardial infarction [37], myocardial ischemia/reperfusion [38], hypertension [39], atherosclerosis

[29; 30] and erectile dysfunction [40]. Interestingly RVT is also protective in these cardiovascular diseases [41]. RVT and H₂S share some mechanisms such as SIRT activation, PDE inhibition and KATP activation. Recently we have showed RVT induce H₂S formation in penile tissue [26]. These observations had led us to investigate whether H₂S contribute to the beneficial effect of RVT in aorta. RVT induced CSE/CBS dependent H₂S formation in mouse aorta in the presence or absence of oxidative stress (Figure 7), suggesting the effect of RVT on H₂S formation was not limited to pathological conditions or penile tissue. Beside RVT enhanced the endogenous H₂S dependent relaxation (Figure 4) via promoting H₂S formation in mouse aorta as in mouse corpus cavernosum (MCC) [26]. We now further showed that CBS/CSE inhibitor AOAA or the CSE inhibitor PAG reversed the augmentation of L-cysteine relaxations in mouse aorta in a same rate (Figure 4), emphasize the role of CSE/H₂S pathway rather than CBS in protective effect of RVT. Further this effect was not returned by eNOS inhibitor L-NNA in mouse aorta as in MCC [26], suggesting NO-independent regulation of endogenous H₂S-relaxation by RVT. Although vascular protective effects of RVT have been mostly attributed to eNOS activation [42; 43; 44; 45], we showed in this study that RVT also protects impaired endogenous H₂S dependent relaxations under oxidative stress. CSE/H₂S pathway contributes to the protective effect of RVT, since PAG reversed the enhanced relaxation to L-cysteine by RVT (Figure 4). We should note that although the inhibition rate by PAG was around 56%, but still it was not complete. The reason for not full inhibition may result from the activation of H₂S formation by RVT and a counterbalance effect of PAG to inhibit H₂S formation. Alternatively this could be due to a lack of efficacy of PAG in inhibiting CBS/CSE or the contribution of an additional enzyme (MPST) or additional mechanisms. However the contribution of the third enzyme MPST to RVT-induced H₂S synthesis and relaxation could not be determined, since there are no specific inhibitors of MPST. On the other side oxidative stress depressed endogenous H₂S induced relaxation more than NO-dependent relaxations in mouse aorta (Figure 5 and 3, respectively). Impaired relaxation to ACh was not restored by RVT in mouse aorta (Figure 3). However improvement of endothelial relaxations by chronic RVT treatment in aorta from spontaneous hypertensive conditions but not normotensive conditions was reported in rat [46]. This discrepancy may result from differences between pathological conditions or species. Nevertheless the protective effect of RVT on L-cysteine relaxation was much more pronounced than from ACh-induced relaxations.

H₂S have also significant role in vessels via regulating ROS formation, which is an important signalling molecule in inflammation, apoptosis and proliferation processes that ultimately regulate vascular disease such as atherosclerosis. Interestingly recent studies revealed that SIRT activation is linked to NADPH inhibition [12] and RVT as a known SIRT activator [13] have a capacity to inhibit NADPH [11] in vascular system as in reported by H₂S [17; 19]. Beside other antioxidant mechanisms of RVT and H₂S are similar such as activation of eNOS [1] [14], SOD [47] [48] and HO-1 [10; 49] as well as Nrf-2 translocation [50] [7]. Because of these similarities we investigated the role of H₂S in the antioxidant effect of RVT in mouse aorta. RVT (0.1 mM, 30 min) significantly inhibited superoxide radicals and other ROS formation induced by pyrogallol in mouse aorta (Figure 2) as NaHS (1 mM, 10 min.) did. H₂S synthesis inhibitor AOAA or PAG completely reversed it significantly, showing a strong role of H₂S pathway in antioxidant effect of RVT (Figure 2). Since inhibition of CSE alone or together with CBS did reverse the effect of RVT on superoxide formation at same level, we suggest that CSE is involved in these effects rather than CBS. Supporting this, it was reported that CSE overexpression inhibits ROS formation and restores impaired ACh-relaxations due to high glucose in mouse aorta, whereas CSE inhibition causes an augmentation in mitochondrial ROS formation [27]. On the other hand, in our study L-NNA could not cause a significant decrease in antioxidant effect of RVT, suggesting pronounced effect of H₂S pathway on the effects of antioxidants. Thus we suggest that H₂S may contribute as an upstream mechanism of protective effect of RVT in oxidative stress.

We should note that the effect of RVT on ROS formation, endogenous H₂S dependent relaxation or H₂S level was returned by AOAA or PAG partly but not completely. This could be due to the contribution of 3-mercaptopyruvate sulphurtransferase (MPST), the third enzyme that enrolled in H₂S formation as well as CBS and CSE, since AOAA is a CBS/CSE inhibitor but a specific pharmacological inhibitor of MPST is not known yet. Moreover Czabo et al. already showed that H₂S production through MPST pathway is reduced in H₂O₂ induced oxidative stress [6].

Conclusion

In summary, as PAG or AOAA decreased the effect of RVT on inducing H₂S formation and inhibiting ROS formation as well as l-cysteine-induced relaxation, we suggest that CSE/H₂S pathway can contribute to the beneficial effect of RVT under oxidative stress. However we can not rule out the role

of other possible mechanisms, since the inhibition of the beneficial effect of RVT by PAG or AOAA was not full and we should note that we are suggesting that CSE/H₂S pathway may not be the only pathway for the beneficial effect of RVT but it contributes to the protective effect of RVT. There are already several clinical trials investigating the effect of RVT in cardiovascular diseases in different doses. Our study may be important to show the potential of RVT as an H₂S-targeting drugs to treat H₂S pathway related physiological effects in cardiovascular diseases. As H₂S requires of smooth muscles cells instead of endothelial cell to produce the gasotransmitter to relax the vessel, targeting H₂S may be a better option in endothelial dysfunctions associated with oxidative stress where NO-dependent relaxations is decreased. We conclude that acute oxidative stress impairs H₂S dependent protection in the aorta. RVT provides protection by limiting ROS formation and promoting vascular relaxation through production of H₂S under vascular oxidative stress. These data suggest that H₂S targeting molecules such as RVT may offer an additional supplementation against cardiovascular disease where oxidative stress are accompanied with decreased H₂S dependent regulation of vascular tone and antioxidant systems are impaired.

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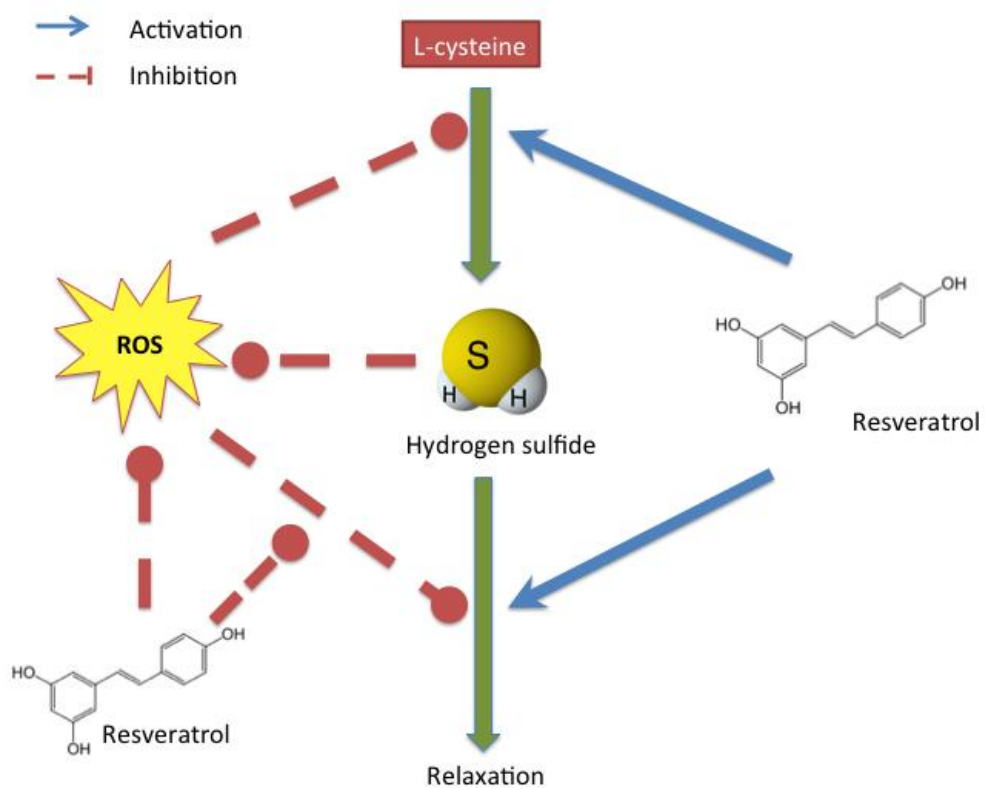
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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest



Graphical abstract

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