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Original Article

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Hydrogen sulfide improves vascular endothelial function in hypertensive states through SIRT6 anti-inflammatory signaling

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Abstract

Hypertension is commonly accompanied by endothelial dysfunction, characterized by an imbalance between vasodilatation and constriction, increased levels of the proinflammatory factors interleukin-6 (IL-6) and intercellular adhesion molecule-1 (ICAM-1), and decreased nitric oxide (NO) bioavailability. Using an angiotensin II (Ang II)-induced endothelial dysfunction model, we show that treatment with the hydrogen sulfide (H_2S) donor GYY4137 significantly reverses Ang II-induced damage. GYY4137 restores sirtuin 6 (SIRT6) expression, suppresses inflammation, and improves vasodilatory function. Furthermore, endothelial-specific cystathionine-γ-lyase (CSE)-deficient mice exhibit inflammation and endothelial dysfunction in blood vessels, which is reversed by H_2S supplementation. Critically, SIRT6 inhibitors block the protective effects of H_2S in the endothelium. This study demonstrates that H_2S protects vascular endothelial function by activating the SIRT6 anti-inflammatory pathway.

Key words hydrogen sulfide, angiotensin II, endothelial cells, inflammation, SIRT6

Introduction

Hypertension has been identified as the most important risk factor for death worldwide, with approximately 9.4 million people dying of hypertension every year globally [1]. In China, hypertension remains the leading cause of cardiovascular morbidity and mortality; therefore, effective management of hypertension is needed [2]. Endothelial dysfunction plays a key role in the pathogenesis of hypertension [3]. In particular, endothelial dysfunction can enhance contraction and vascular remodeling, which increases peripheral resistance and leads to hypertension. Angiotensin II (Ang II), a critical component of the renin–angiotensin–aldosterone system, controls blood pressure by impairing endothelial function and inducing smooth muscle cell contraction [4]. Recent studies have shown that both salt-induced and Ang II-mediated hypertension are associated with increased inflammation and cytokine production, as well as endothelial dysfunction [5]. In addition to its role as a potent vasoconstrictor and regulator of blood pressure and fluid homeostasis, Ang II is involved in the development of vascular lesions in cardiovascular disease through the activation of various immune cells and cellular inflammation [6]. Ang II has been shown to facilitate the adhesion and infiltration of inflammatory cells into the aortic wall [7]. Numerous studies have demonstrated that inflammation affects nitric oxide (NO) synthesis and vascular smooth muscle function. Specifically, the influence of inflammation on endothelium-dependent vasodilation is mediated by delaying NO synthesis and degrading NO, promoting an increase in the levels of vasoconstrictive factors and a decrease in the levels of diastolic factors. Despite extensive research on the etiology and pathogenesis of hypertension, new therapeutic mechanisms remain underexplored.

SIRT6, a member of the mammalian sirtuin family, is a protease with multiple catalytic activities in the nucleus, including histone deacetylation, ADP-ribosylation, and lysine deacylation. SIRT6 plays a critical role in maintaining genome stability and DNA repair

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[8] and participates in numerous physiological regulatory mechanisms, such as cell proliferation, differentiation, and aging. SIRT6 overexpression inhibits various inflammatory responses, such as collagen-induced arthritis and hypoxia-induced inflammation [9]. Studies have also shown that SIRT6 reduces endothelial cell inflammation induced by tiny cholesterol crystals and reverses endothelial dysfunction by increasing the level of angiotensin-converting enzyme 2 [10].

H_2S, the third gas transmitter discovered after NO and carbon monoxide, has attracted considerable attention because of its ability to preserve blood vessel function, regulate vascular tone, and inhibit cell proliferation [11]. Studies have shown that H_2S restores endothelial NO-dependent vascular function in patients with hyperglycemia via a cAMP response element-binding (CREB)-dependent pathway [12], attenuates disturbed flow-induced vascular remodeling by inhibiting LDHB-mediated autophagic flux [13], stimulates NO production and protects human umbilical vein endothelial cells (HUVECs) by inducing the protein kinase B (Akt) endothelial nitric oxide synthase (eNOS) signaling pathway [14]. Moreover, H_2S has been found to preserve cellular function through SIRT6. In particular, exogenous H_2S inhibits cellular senescence through the SIRT6/adenosine monophosphate-activated protein kinase (AMPK) signaling pathway, thereby alleviating myocardial fibrosis associated with diabetes [15]. Furthermore, H_2S significantly inhibits ischemia/reperfusion-induced brain endothelial injury by upregulating the expression of SIRT6 [16]. Another study revealed that H_2S inhibits inflammation and protects neurons from microglial activation-induced neuronal death by increasing the expression of SIRT6, enhancing the interaction between SIRT6 and H3K9ac, and downregulating the expression of Notch homolog 1 (Notch1) [17]. However, the mechanisms by which H_2S and SIRT6 exert endothelial protection remain unclear.

Therefore, we hypothesized that H_2S reduces Ang II-induced endothelial dysfunction by increasing SIRT6 protein expression and inhibiting the expression of inflammatory factors.

Materials and Methods

Animals and experimental methods

All animal experiments were performed in compliance with the Management Rule of the Ministry of Health, China (Document No. 55, 2001) and the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011), and were approved by the Laboratory Animal Ethical and Welfare Committee of Hebei Medical University (Approval No. IACUC-Hebmu-2024015). All the mice were on a C57BL/6J background. The endothelial-specific *CSE*-knockout mouse model was generated using Cre/LoxP technology. The CSE gene was floxed via homologous recombination in fertilized eggs, yielding positive floxed heterozygous mice ($CSE^{f/+}$). Floxed homozygous mice ($CSE^{f/f}$) were subsequently produced by intercrossing $CSE^{f/+}$ animals. To obtain endothelial-specific CSE-deficient mice ($CSE^{f/f}Cdh5^{Cre}$, designated $CSE^{endo^{-/-}}$), $CSE^{f/+}$ mice were crossed with $Cdh5^{Cre}$ mice to generate $CSE^{f/+}$ $Cdh5^{Cre}$ offspring, which were then backcrossed to $CSE^{f/f}$ mice. The $CSE^{f/+}$ and $Cdh5^{Cre}$ founder lines were procured from Shanghai Southern Model Biotechnology Co., Ltd (Shanghai, China). The genotyping of all the mice was performed via PCR: for the detection of the CSE flox allele, the primers F1 (5′-CTCAATCCCAGACTGCAGGG-3′) and R1 (5′-TACAGGCCCATCC CACCATA-3′) were used to generate amplification products of 393 bp for the wild-type allele and 463 bp for the floxed allele; for Cre gene detection, the primers F2 (5′-TCGATGCAACGAGTGAT GAG-3′) and R2 (5′-TCCATGAGTGAACGAACCTG-3′) were used to specifically amplify a 400 bp target band.

The animals were procured from the Animal Center of Hebei Medical University and were randomly assigned to cages with ad libitum access to food and water. The humidity within the cages was maintained between 45% and 55%, the temperature was set at 20°C to 25°C, and the light-dark cycle was set at 12 h:12 h (lights on from 8:00 to 20:00). The cages and drinking bottles were regularly cleaned and disinfected, and the bedding was sterilized by autoclaving.

Aortic isolation and incubation

The animals were euthanized via carbon dioxide asphyxiation. All surgical instruments and animal body surfaces were sterilized with 75% ethanol. Thoracic aortas were carefully dissected to ensure that they were free of surrounding adipose tissue, rinsed in sterile phosphate-buffered saline (PBS), and sectioned into 2-mm rings. Mouse aortic rings were incubated in a 5% CO_2 atmosphere at 37°C for 48 h in low-glucose Dulbecco's Modified Eagle Medium (Gibco, Grand Island, USA) supplemented with 10% fetal bovine serum (Gibco) and 1% antibiotics. For the experimental treatments, the aortic rings were exposed to Ang II (100 μM; Sigma Aldrich, St Louis, USA) and GYY4137 (200 μM; Cayman Chemical, Ann Arbor, USA). An equal volume of PBS was added to the control group.

Cell culture and passage

HUVECs were purchased from ScienCell Research Laboratories (San Diego, USA) and cultured in complete endothelial cell medium (ECM; ScienCell Research Laboratories) supplemented with 5% fetal bovine serum (FBS), 1% endothelial cell growth supplement (ECGS), and 1% penicillin/streptomycin (P/S). The SIRT6 inhibitor OSS-128167 (200 μM) was obtained from MCE (Monmouth Junction, USA). All cell culture supplements were obtained from ScienCell Research Laboratories. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. Cells at passages 4–6 were used for experimentation when they reached 80%–90% confluence.

Western blot analysis

Frozen thoracic and abdominal aorta tissues stored at −80°C were weighed and homogenized in complete RIPA lysis buffer (containing protease and phosphatase inhibitors) at a 1:1 (w/v) ratio (1 mL of buffer per 1 g of tissue), while HUVECs were directly lysed in the same buffer. The homogenates were centrifuged at 12,000 *g* for 20 min at 4°C, after which the supernatant was collected for protein quantification via the bicinchoninic acid assay. The supernatant was then mixed with 5× loading buffer at a 1:4 volume ratio, and equal amounts of protein samples were separated via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride membranes. After being blocked with 5% non-fat milk for 1 h at room temperature, the membranes were incubated overnight at 4°C with primary antibodies targeting CSE (1:1000; Santa Cruz Biotechnology, Dallas, USA), interleukin-6 (IL-6) (1:1000; Proteintech, Rosemont, USA), SIRT6 (1:1000; Proteintech), intercellular adhesion molecule-1 (ICAM-1) (1:1000; Proteintech), and GAPDH (1:5000; Proteintech) as loading controls, followed by incubation with appropriate HRP-

conjugated secondary antibody. After visualization via enhanced chemiluminescence (ECL) reagents (Merck, Darmstadt, Germany), protein signals were detected via the ChemiScope 6100 imaging system (CLiNX, Shanghai, China), and quantified via analysis of band intensities via ImageJ software.

Vascular function measurements

Male wild-type C57BL/6J mice were euthanized via carbon dioxide asphyxiation, after which their arteries were promptly transferred to a small dish containing Krebs-Hensleit's (K-H) maintained at 4°C. Subsequently, the surrounding adipose tissues were rapidly and meticulously removed, and the arteries were incised into 2-mm segments. The rings were then inserted into the myograph using two stainless steel wires fixed to the jaw. The transducer was positioned within a DMT (Danish Myo Technology A/S, Hinnerup, Denmark) bath containing 5 mL of K-H solution. The temperature of the water bath was maintained at 37°C, and 95% oxygen was continuously supplied to the K-H solution. The vessels were adjusted to an optimal preload, equilibrated for 40 min, and then treated with 60 mM KCl. Once the contractions of the aortas had reached a plateau, the aortas were flushed with K-H solution three times, each occurring after 5- to 10-min intervals. After each vessel was initially contracted with phenylephrine (Phe, 1 μM; TCI Development Co., Ltd, Tokyo, Japan), a gradient of acetylcholine (ACh, 0.003–10 μM; Sigma-Aldrich) was employed to elicit an endothelium-dependent diastolic response. The non-endothelium-dependent diastolic response was induced by the addition of N-nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich) at a concentration of 100 μM for 30 min, followed by Phe (1 μM) and a gradient of sodium nitroprusside (SNP, 3 pM–0.1 mM; Sigma Aldrich). The responses of each vascular segment were then documented.

Immunofluorescence staining

Vessel samples were cryo-embedded in optimal cutting temperature (O.C.T.) compound (Sakura, Torrance, USA) and sliced into 6-μm-thick sections. After being blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature, the sections were incubated overnight at 4°C in a humidified chamber with a mixture of primary antibodies diluted in 5% BSA and 1% Triton X-100, including anti-CSE (1:100; Santa Cruz Biotechnology), anti-IL-6 (1:100; Proteintech), and anti-CD31 (1:400; Servicebio, Shanghai,China) antibodies. The next day, the slides were warmed to room temperature for 1 h, washed three times with PBS, and incubated for 2 h at room temperature with the following fluorescence-conjugated secondary antibodies: Cy3-labeled goat anti-rabbit IgG (1:400) and Alexa Fluor®488-labeled goat anti-mouse IgG (1:400; Servicebio). After incubation, the unbound antibodies were removed by washing with PBS under low light conditions. The nuclei were stained with DAPI-containing mounting medium, and the slides were shielded from light and stored at room temperature for 24 h prior to imaging. Fluorescence images were acquired via a fluorescence microscope.

Statistical analyses

GraphPad Prism Version software (GraphPad Software Inc., San Diego, USA) was used for analyses. Comparisons between two groups were performed via Student's *t* test, whereas comparisons between more than two groups were performed via one-way ANOVA followed by the Bonferroni post hoc test. The experimental data are expressed as the mean ± standard error of the mean. $P < 0.05$ was considered statistically significant.

Results

Ang II induces endothelial injury, elevates the expression of inflammatory factors, and reduces the expression of SIRT6 and CSE in mouse aortas

Aortas incubated with Ang II (100 μM) for 24 h showed a significant decrease in endothelial-dependent relaxation (EDR) compared with those in the control group, whereas those treated with Ang II for 6 and 12 h showed no obvious changes in EDR compared with those in the control group (Figure 1A). However, Ang II treatment did not significantly affect SNP-mediated nonendothelial diastolic function between the groups (Figure 1B). Therefore, Ang II treatment for 24 h was used in the subsequent experiments. Elevated expression of ICAM-1 and IL-6, which are indicators of inflammatory status, was observed in the vessels of the Ang II group (Figure 1C–E). Compared with the control group, the Ang II group showed decreased expression of eNOS, SIRT6, and CSE (Figure 1F–I). These data suggest that Ang II-treated aortas exhibit increased production of inflammatory molecules, impaired endothelial function, and decreased eNOS, SIRT6, and CSE expression.

Exogenous hydrogen sulfide ameliorates Ang II-induced vascular endothelial injury, reduces the expression of inflammatory factors, and increases SIRT6 level

Compared with the control group, EDR of the aorta was significantly impaired in the Ang II group. Notably, exogenous supplementation with GYY4137 (200 μM, 24 h), a slow-release hydrogen sulfide donor, significantly ameliorated the impaired EDR in Ang II-treated aortas (Figure 2A). However, no significant difference in endothelium-independent relaxation of the aorta was observed between the Ang II and GYY4137 groups (Figure 2B). Ang II-treated aortas showed upregulated protein expression of ICAM-1 and IL-6 and significantly downregulated expression of eNOS, SIRT6, and CSE, which was reversed by the administration of GYY4137 (Figure 2C–I).

CSE deletion increases the expression of inflammatory factors and decreases eNOS, SIRT6, and CSE levels

In mammals, the primary enzymes that catalyze the generation of H_2S include CSE, cystathionine-synthesizing enzyme (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST). These three enzymes are expressed in a tissue-specific manner [18], with CSE playing a major role in the vascular endothelium. Our previous study revealed that H_2S synthase CSE was reduced in D-gal-treated HUVECs, whereas the expression of CBS and 3-MST did not change with D-gal treatment [19]. To further investigate the beneficial effects of endogenous H_2S on the vascular endothelium, endothelial CSE-specific knockout mice were constructed, and the production of inflammatory molecules and eNOS in the aorta was evaluated. Compared with those of the $CSE^{endo+/+}$ aortas, the $CSE^{endo-/-}$ aortas showed a notable reduction in CSE levels (Figure 3A). Compared with the $CSE^{endo+/+}$ aortas, the $CSE^{-/-}$ aortas presented significantly greater IL-6 levels (Figure 3B). ICAM-1 and IL-6 expression levels were greater in $CSE^{endo-/-}$ aortas than in $CSE^{endo+/+}$ aortas (Figure 3C–E). We also found that the expression of eNOS, SIRT6, and CSE was significantly lower in the $CSE^{endo-/-}$ aortas than in the $CSE^{endo+/+}$ aortas (Figure 3F–I). These data suggest that $CSE^{endo-/-}$ aortas

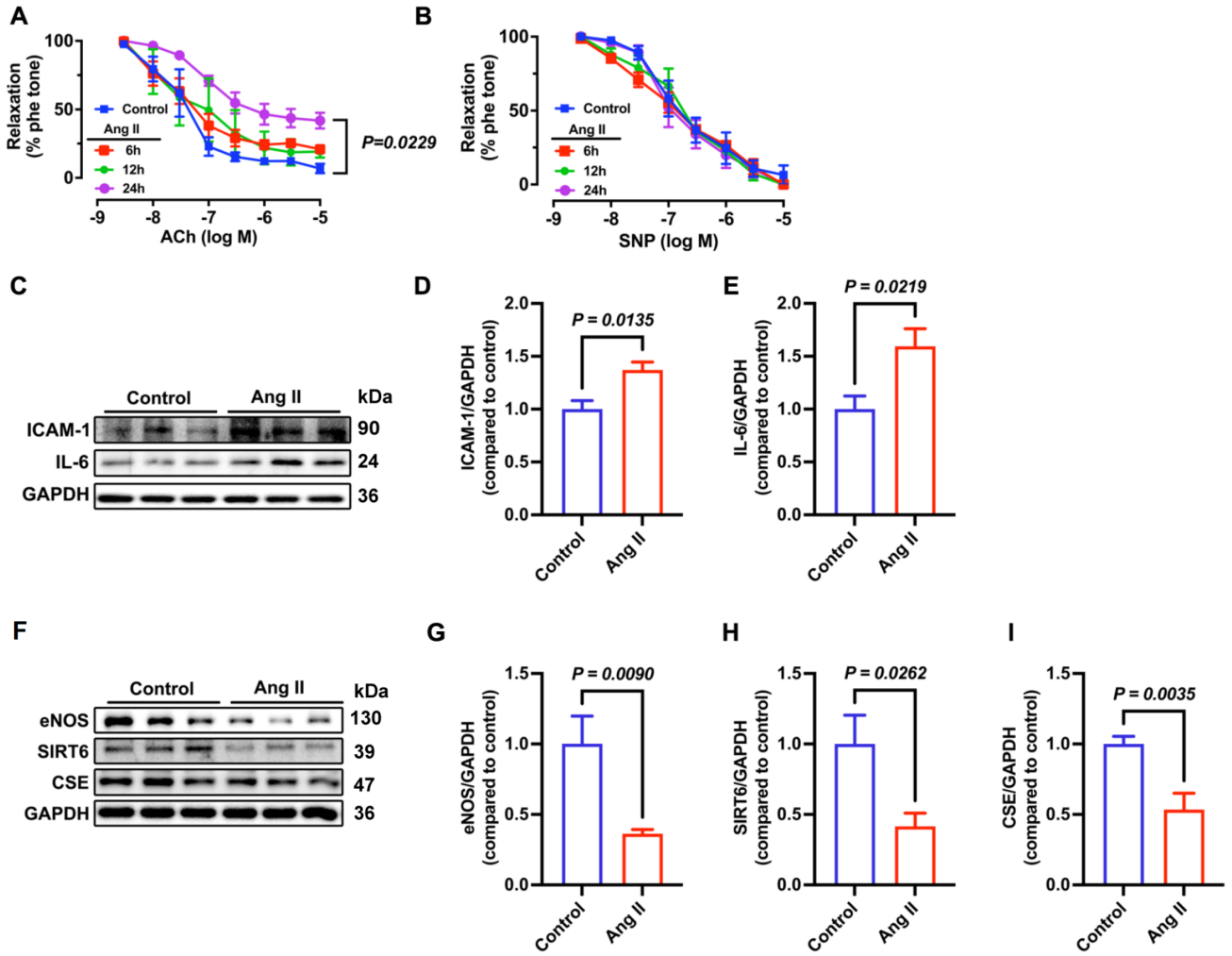


Figure 1. Ang II damages endothelial-dependent relaxation, aggravates inflammation, and reduces the expression of SIRT6 and CSE in the aorta (A) Measurement of endothelium-dependent relaxation in aortas. (B) Measurement of endothelium-independent relaxation in aortas. (C) Expression of ICAM-1 and IL-6 in aortas detected by western blotting, with GAPDH as the internal control. (D,E) Quantitative analysis for ICAM-1 and IL-6 expression. (F) Expression of eNOS, SIRT6, and CSE in the aortas detected via western blotting. GAPDH was used as the internal control. (G–I) Quantitative analysis for eNOS, SIRT6, and CSE expression. Results are presented as the mean ± standard error of the mean (n = 6).

display increased production of inflammatory molecules, impaired endothelial function, and decreased SIRT6 expression.

H_2S supplementation reverses the elevated levels of inflammatory factors and decreases SIRT6 level in $CSE^{endo-/-}$ aortas

The production of ICAM-1 and IL-6 was greater in $CSE^{endo-/-}$ aortas than in $CSE^{endo+/+}$ aortas, which was reversed by GYY4137 (Figure 4A–C). Moreover, the expression of eNOS and SIRT6 was reduced in $CSE^{endo-/-}$ aortas, which was reversed by GYY4137 (Figure 4D–F).

H_2S ameliorates Ang II-induced endothelial inflammation by activating SIRT6

Treatment with the SIRT6 inhibitor OSS-128167 (200 μM) increased the levels of ICAM-1 and IL-6 but decreased the levels of eNOS and SIRT6. OSS-128167 treatment did not elicit any detectable alterations in CSE. However, the administration of GYY4137 failed to reverse the OSS-128167-induced changes in HUVECs (Figure 5A–G).

Discussion

The present study revealed that Ang II decreased the expression of CSE and SIRT6, increased the levels of inflammatory factors, and induced endothelial dysfunction in the aorta. CSE deletion increased inflammatory factor production and impaired endothelial function. In contrast, H_2S supplementation ameliorated endothelial dysfunction in mice by increasing SIRT6 production and inhibiting inflammatory factor expression in the aortas of $CSE^{endo-/-}$ mice.

Increasing evidence suggests that inflammation plays a crucial role in the pathophysiology of hypertension [20]. Multiple factors that induce hypertension, such as Ang II, aldosterone, and high salt intake, promote the release of proinflammatory cytokines, such as interleukin 17, interleukin 6, and interferon, and the secretion of autoantibodies, which cause vascular injury [2]. In addition, Ang II leads to the overproduction of reactive oxygen species, which promotes endothelial dysfunction by impairing eNOS function and disrupting NO bioavailability [21]. To investigate the optimal duration of Ang II-induced endothelial injury, time points of 6 h, 12 h, and 24 h were used to study the appropriate timing of Ang II-

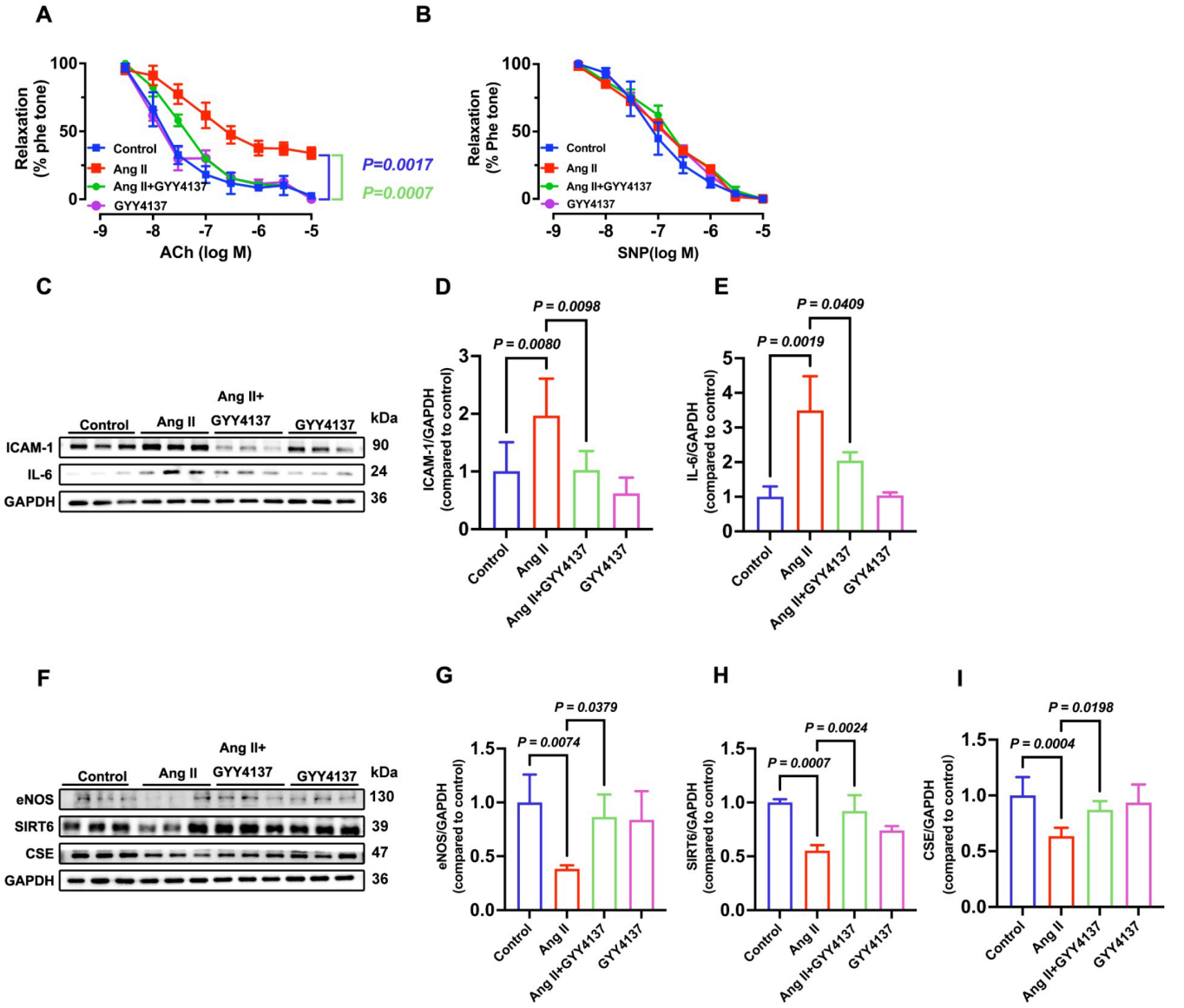


Figure 2. GYY4137 ameliorates Ang II-induced vascular endothelial injury, reduces the expression of inflammatory factors, and increases SIRT6 level (A) Measurement of endothelium-dependent relaxation in aortas. (B) Measurement of endothelium-independent relaxation in aortas. (C) Expression of ICAM-1 and IL-6 in aortas detected via western blotting, with GAPDH as the internal control. (D,E) Quantitative analysis for ICAM-1 and IL-6 expression. (F) Expression of eNOS, SIRT6, and CSE in the aortas detected via western blotting, with GAPDH as the internal control. (G–I) Quantitative analysis for eNOS, SIRT6, and CSE expression. Results are presented as the mean ± standard error of the mean (n = 6).

stimulated inflammation. Notably, vascular injury was shown to be most pronounced after 24 h of Ang II stimulation, which is consistent with the findings of a previous study [22]. Therefore, we selected a duration of 24 h as the processing time for Ang II stimulation of blood vessels.

Studies have shown that endothelial-specific deletion of SIRT6, a highly conserved NAD^+-related deacetylase, significantly enhances DOCA/salt-induced and Ang II-induced blood pressure in mice. SIRT6 expression is reduced in Ang II-treated endothelium, whereas SIRT6 overexpression reduces endothelial cell permeability and improves vascular endothelium-dependent diastolic function and NO bioavailability [23]. SIRT6 attenuates oxidative stress in endothelial cells by regulating protein processing in the endoplasmic reticulum [24], suggesting the importance of endothelial SIRT6 in the maintenance of endothelial function [23]. Our experiments also revealed that decreased SIRT6 was accompanied by increased inflammation. SIRT6 primarily binds to chromatin and is closely associated with chromatin remodeling, which plays a crucial role in regulating H3K9 and H3K56 deacetylation [25]. SIRT6 prevents endothelial inflammation by preventing H3K9 acetylation, thereby triggering Nkx3.2-GATA5 signaling under pathophysiological conditions [23]. Another study also revealed that SIRT6 prevents vascular inflammation through its deacetylation of the Nrf2-dependent signaling pathway [26]. In our experiments, elevated SIRT6 level decreased the expression of inflammatory factors, possibly through the deacetylation of inflammatory molecules.

H_2S counteracts cardiovascular damage caused by L-NAME-induced hypertension in rats via the NO/eNOS pathway [27].

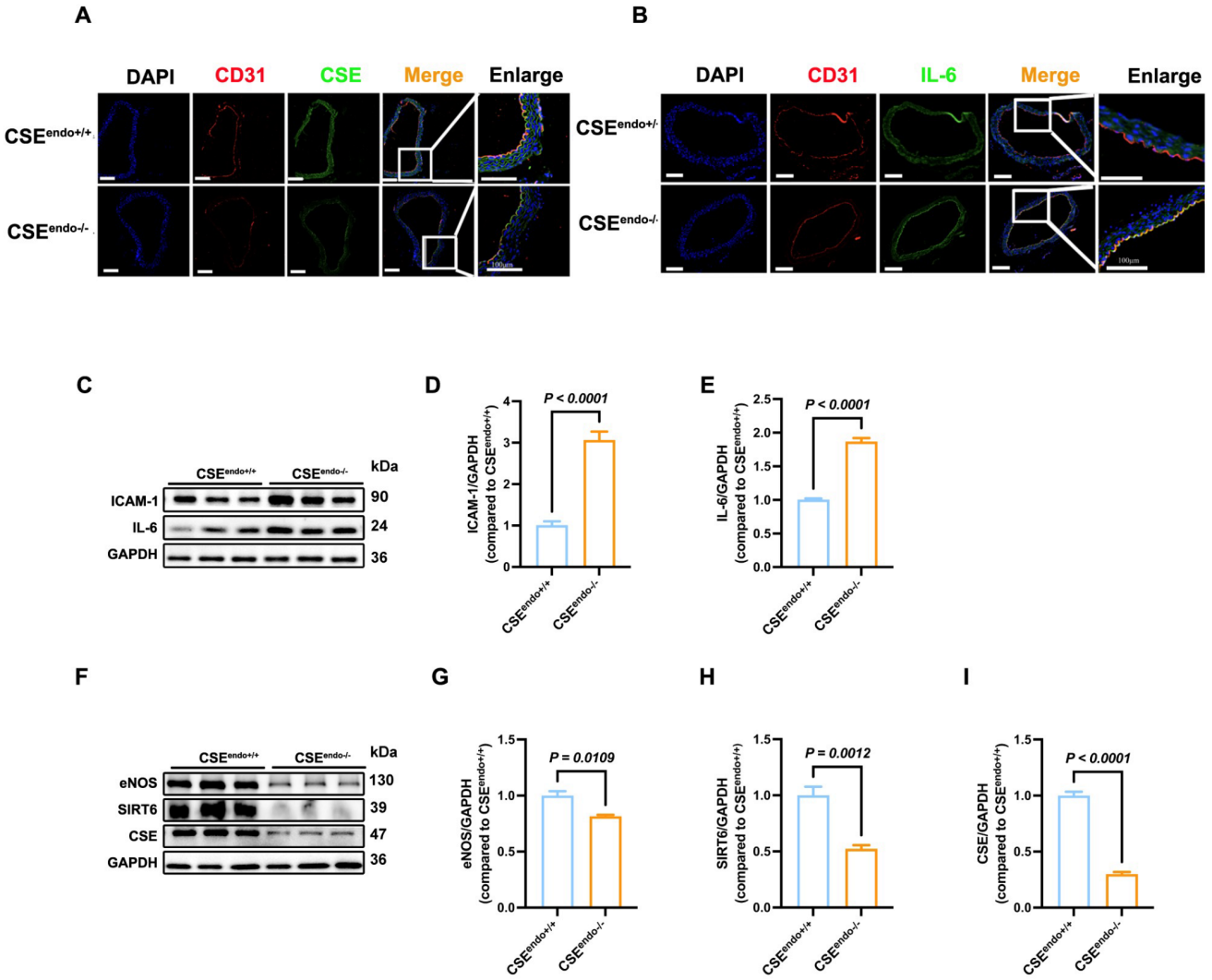


Figure 3. Decreased endogenous hydrogen sulfide increases the expression of inflammatory factors and decreases the levels of eNOS, SIRT6, and CSE (A) Immunofluorescence staining of CD31 and CSE in the aortas. (B) Immunofluorescence staining of CD31 and IL-6 of aortas. Scale bar: 100 μm. (C) Expression of ICAM-1 and IL-6 in the aortas detected via western blotting, with GAPDH as the internal control. (D,E) Quantitative analysis for ICAM-1and IL-6 expression. (F) Expression of eNOS, SIRT6, and CSE in the aortas detected via western blotting, with GAPDH as the internal control. (G–I) Quantitative analysis for eNOS, SIRT6, and CSE expression. Results are presented as the mean ± standard error of the mean (n = 6).

Another study revealed that H_2S inhibits inflammation by modulating SIRT6 and is able to reduce hypertrophy in the PFC and BV2 cells in mice with type 1 diabetes [17]. In mammals, several key enzymes are responsible for catalyzing the production of H_2S. Among them, CSE, CBS, and 3-MST are the primary enzymes, with all three having tissue-specific expression patterns. Specifically, CSE plays a dominant role in H_2S generation in the vascular endothelium [28]. Circulating and plaque H_2S levels are decreased in mice after carotid artery ligation and in humans with atherosclerosis. CSE deficiency damaged the anti-adhesive function of the endothelium, attracted more monocytes, caused endothelial dysfunction, and accelerated atherosclerosis. However, exogenous H_2S reversed these effects [29]. $CSE^{endo-/-}$ mice showed decreased level of circulating H_2S, thickened endocardium, increased heart fibrosis, and impaired cardiovascular function. In contrast, CSE overexpression in endothelial cells increased H_2S, reduced endothelial-to-mesenchymal transition and fibrosis, and preserved cardiac and endothelial functions [30]. Thus, the $CSE^{endo-/-}$ model is ideal for studying the impact of H_2S on cellular inflammation and cardiovascular diseases. Our study also revealed that endothelium-specific knockdown of *CSE* increased ICAM-1 and IL-6 levels but decreased eNOS, SIRT6, and CSE levels, which were reversed by GYY4137 supplementation.

H_2S has been shown to prevent endothelial dysfunction by modulating multiple intracellular signaling pathways, which reduce the levels of inflammatory factors, including IL-1β, TNF-α, and IL-6, in endothelial cells [31]. In the present study, we found that GYY4137 supplementation reversed the elevated expression of IL-6 and ICAM-1 and the damage to endothelial function caused by Ang II and *CSE* knockdown, suggesting that H_2S ameliorated Ang II-induced endothelial dysfunction by suppressing the production of inflammatory molecules. H_2S inhibits the inflammatory response

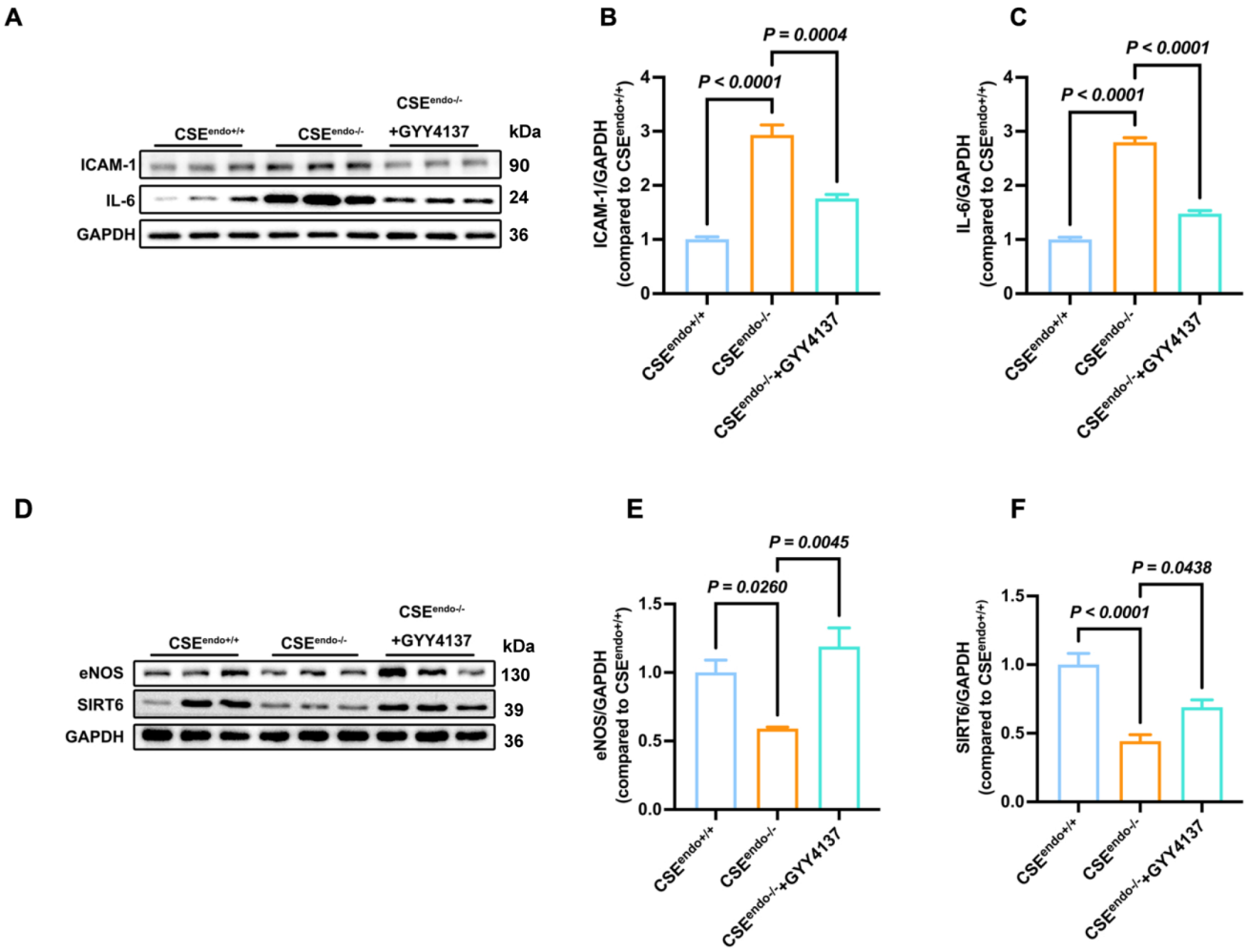


Figure 4. Exogenous H_2S supplementation reduces inflammatory factor expression and improves SIRT6 expression in $CSE^{endo-/-}$ aortas (A) Expression of ICAM-1 and IL-6 in aortas detected via western blotting, with GAPDH as the internal control. (B,C) Quantitative analysis for ICAM-1 and IL-6 expression. (D) Expression of eNOS and SIRT6 in the aortas detected via western blotting, with GAPDH as the internal control. (E,F) Quantitative analysis for eNOS and SIRT6 expression. Results are presented as the mean ± standard error of the mean (n = 6).

and protects neurons from microglial activation-induced death by upregulating SIRT6 expression, enhancing the interaction between SIRT6 and H3K9ac, and downregulating Notch1 expression [17]. Our experiments revealed that SIRT6 levels were reduced in Ang II-treated aortas. Administration of a SIRT6 inhibitor to HUVECs increased the expression of the inflammatory factor IL-6 and decreased the production of eNOS, CSE, and SIRT6, which could not be ameliorated by GYY4137.

Nevertheless, the present study has several limitations. We did not directly examine H_2S level in the endothelium. Previous studies conducted by our research group demonstrated a positive correlation between H_2S levels and CSE expression in tissues [32]. SIRT6 also protects the endothelium by deacetylating MRTF-A [33]. Consequently, the specific mechanism by which SIRT6 inhibits inflammation remains to be investigated. In future studies, we plan to determine whether SIRT6 exerts its vascular protective function through deacetylating proteins.

In summary, the present study revealed that aortas cultured with Ang II presented significantly elevated expression of IL-6 and ICAM-1 and impaired endothelial function. Moreover, endothelial CSE-specific knockout mice presented elevated levels of inflammatory molecules and endothelial dysfunction, which were reversed by GYY4137 supplementation. These findings suggest that H_2S ameliorates Ang II-induced endothelial dysfunction by inhibiting inflammatory responses. As such, H_2S supplementation may be a new therapeutic approach for the treatment of Ang II-induced hypertension through its ability to inhibit inflammation in the endothelium.

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Conflict of Interest

The authors declare that they have no conflict of interest.

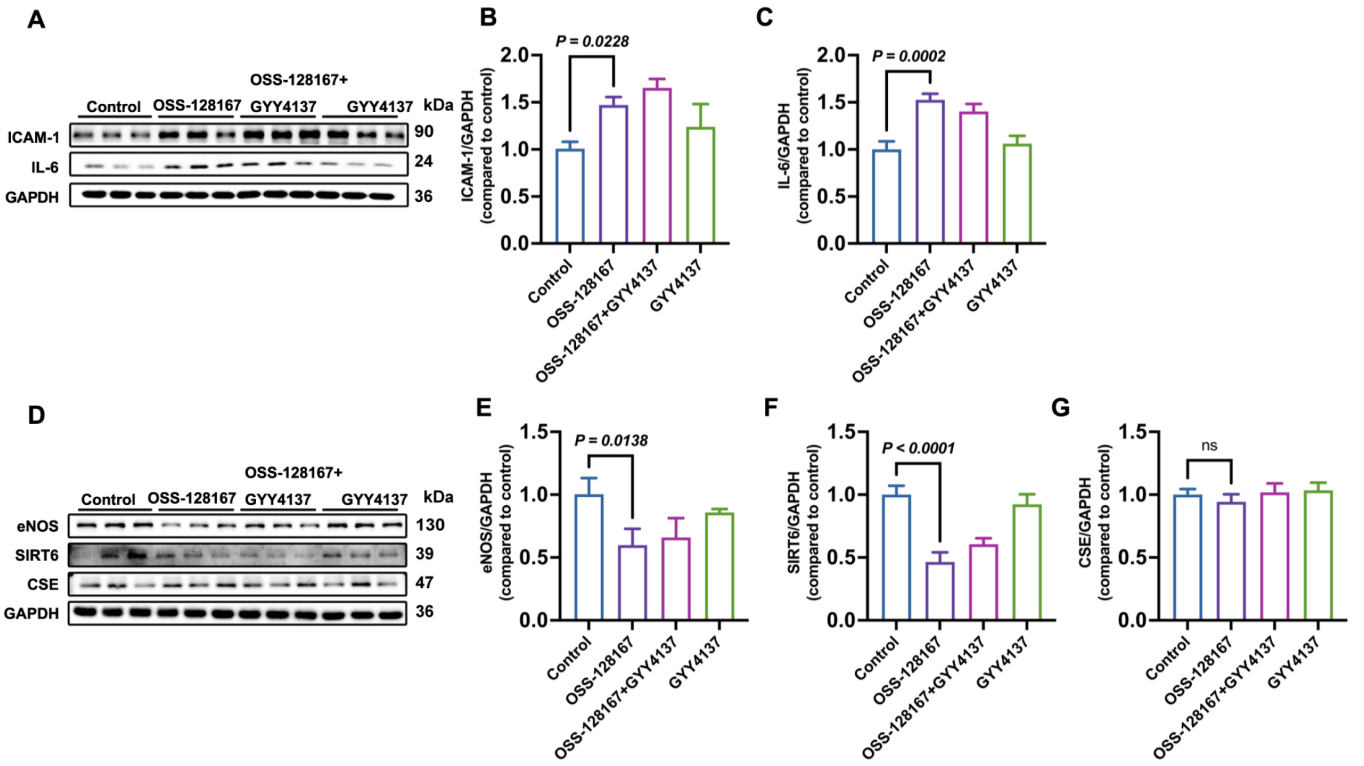


Figure 5. Hydrogen sulfide ameliorates Ang II-induced HUVEC injury via SIRT6 (A) Expression of ICAM-1 and IL-6 in HUVECs detected via western blotting, with GAPDH as the internal control. (B,C) Quantitative analysis for ICAM-1 and IL-6 expression. (D) Expression of eNOS, SIRT6, and CSE in HUVECs detected via western blotting, with GAPDH as the internal control. (E–G) Quantitative analysis for eNOS, SIRT6, and CSE expression. Results are presented as the mean ± standard error of the mean (n = 6). ns, not significant.

References

1. Crouch SH, Soepnel LM, Kolkenbeck-Ruh A, Maposa I, Naidoo S, Davies J, Norris SA, *et al.* Paediatric hypertension in africa: a systematic review and meta-analysis. *eClinicalMedicine* 2022, 43: 101229
2. Li X, Krumholz HM, Yip W, Cheng KK, De Maeseneer J, Meng Q, Mossialos E, *et al.* Quality of primary health care in China: challenges and recommendations. *Lancet* 2020, 395: 1802–1812
3. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000, 87: 840–844
4. Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, Scalia R, *et al.* Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. *Physiol Rev* 2018, 98: 1627–1738
5. Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction. *Circ Res* 2008, 102: 488–496
6. Piqueras L, Sanz MJ. Angiotensin II and leukocyte trafficking: new insights for an old vascular mediator. Role of redox-signaling pathways. *Free Radical Biol Med* 2020, 157: 38–54
7. Jeong J, Lee J, Lim J, Cho S, An S, Lee M, Yoon N, *et al.* Soluble RAGE attenuates AngII-induced endothelial hyperpermeability by disrupting HMGB1-mediated crosstalk between AT1R and RAGE. *Exp Mol Med* 2019, 51: 1–15
8. Chen J, Liu Z, Wang H, Qian L, Li Z, Song Q, Zhong G. SIRT6 enhances telomerase activity to protect against DNA damage and senescence in hypertrophic ligamentum flavum cells from lumbar spinal stenosis patients. *Aging* 2021, 13: 6025–6040
9. Lee HS, Ka SO, Lee SM, Lee SI, Park JW, Park BH. Overexpression of sirtuin 6 suppresses inflammatory responses and bone destruction in mice with collagen-induced arthritis. *Arthritis Rheumatism* 2013, 65: 1776–1785
10. Zheng Z, Wang B, Lv X, Yao F, Gao H, Jin Z, Liu Y, *et al.* Protective effect of SIRT6 on cholesterol crystal-induced endothelial dysfunction via regulating ACE2 expression. *Exp Cell Res* 2021, 402: 112526
11. Polhemus DJ, Lefer DJ. Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. *Circ Res* 2014, 114: 730–737
12. Montanaro R, Vellecco V, Torregrossa R, Casillo GM, Manzo OL, Mitidieri E, Bucci M, *et al.* Hydrogen sulfide donor AP123 restores endothelial nitric oxide-dependent vascular function in hyperglycemia via a CREB-dependent pathway. *Redox Biol* 2023, 62: 102657
13. Wang X, Huang X, Zhang Y, Huo H, Zhou G, Shen L, Li L, *et al.* Hydrogen sulfide attenuates disturbed flow-induced vascular remodeling by inhibiting LDHB-mediated autophagic flux. *Redox Biol* 2024, 79: 103456
14. Cui J, Zhuang S, Qi S, Li L, Zhou J, Zhang W, Zhao Y, *et al.* Hydrogen sulfide facilities production of nitric oxide via the Akt/endothelial nitric oxide synthases signaling pathway to protect human umbilical vein endothelial cells from injury by angiotensin II. *Mol Med Rep* 2017, 16: 6255–6261
15. Li Y, Liu M, Song X, Zheng X, Yi J, Liu D, Wang S, *et al.* Exogenous hydrogen sulfide ameliorates diabetic myocardial fibrosis by inhibiting cell aging through SIRT6/AMPK autophagy. *Front Pharmacol* 2020, 11: 1150
16. Hu Y, Li R, Yang H, Luo H, Chen Z. Sirtuin 6 is essential for sodium sulfide-mediated cytoprotective effect in ischemia/reperfusion-stimulated brain endothelial cells. *J Stroke Cerebrovasc Dis* 2015, 24: 601–609

17. Wang Y, Wang S, Xin Y, Zhang J, Wang S, Yang Z, Liu C. Hydrogen sulfide alleviates the anxiety-like and depressive-like behaviors of type 1 diabetic mice via inhibiting inflammation and ferroptosis. *Life Sci* 2021, 278: 119551
18. Combi Z, Potor L, Nagy P, Sikura KÉ, Ditrói T, Jurányi EP, Galambos K, ***et al.*** Hydrogen sulfide as an anti-calcification stratagem in human aortic valve: altered biogenesis and mitochondrial metabolism of H2S lead to H2S deficiency in calcific aortic valve disease. *Redox Biol* 2023, 60: 102629
19. Tian D, Meng J, Li L, Xue H, Geng Q, Miao Y, Xu M, ***et al.*** Hydrogen sulfide ameliorates senescence in vascular endothelial cells through ameliorating inflammation and activating PPAR delta/SGLT2/STAT3 signaling pathway. *Acta Biochim Biophys Sin* 2023, 55: 1358
20. Wang R, Yuan T, Wang J, Chen Y, Zhao J, Li M, Fang L, ***et al.*** Immunity and inflammation in pulmonary arterial hypertension: from pathophysiology mechanisms to treatment perspective. *Pharmacol Res* 2022, 180: 106238
21. Sun X, Moreno Caceres S, Yegambaram M, Lu Q, Pokharel MD, Boehme JT, Datar SA, ***et al.*** The mitochondrial redistribution of ENOS is regulated by AKT1 and dimer status. *Nitric Oxide* 2024, 152: 90–100
22. Ling WC, Liu J, Lau CW, Murugan DD, Mustafa MR, Huang Y. Treatment with salvianolic acid B restores endothelial function in angiotensin II-induced hypertensive mice. *Biochem Pharmacol* 2017, 136: 76–85
23. Guo J, Wang Z, Wu J, Liu M, Li M, Sun Y, Huang W, ***et al.*** Endothelial SIRT6 is vital to prevent hypertension and associated cardiorenal injury through targeting Nkx3.2-GATA5 signaling. *Circ Res* 2019, 124: 1448–1461
24. Li P, Guo Z, Feng R, Wu N, Zhong X, Fang Z, Hu Y, ***et al.*** Multi-omics analysis reveals the regulation of SIRT6 on protein processing of endoplasmic reticulum to alleviate oxidative stress in endothelial cells. *Clin Transl Med* 2022, 12: e1039
25. Khan RI, Nirzhor SSR, Akter R. A review of the recent advances made with SIRT6 and its implications on aging related processes, major human diseases, and possible therapeutic targets. *Biomolecules* 2018, 8: 44
26. He Y, Yang G, Sun L, Gao H, Yao F, Jin Z, Zheng Z, ***et al.*** SIRT6 inhibits inflammatory response through regulation of NRF2 in vascular endothelial cells. *Int Immunopharmacol* 2021, 99: 107926
27. Ji W, Liu S, Dai J, Yang T, Jiang X, Duan X, Wu Y. Hydrogen sulfide defends against the cardiovascular risk of Nw-nitro-L-argininemethyl ester-induced hypertension in rats via the nitric oxide/endothelial nitric oxide synthase pathway. *Chin Med J* 2014, 127: 3751–3757
28. Zhao W, Wang R. H_2S-induced vasorelaxation and underlying cellular and molecular mechanisms. *Am J Physiol Heart Circ Physiol* 2002, 283: H474–H480
29. Bibli SI, Hu J, Sigala F, Wittig I, Heidler J, Zukunft S, Tsilimigras DI, ***et al.*** Cystathionine γ lyase sulfhydrates the RNA binding protein human antigen R to preserve endothelial cell function and delay atherogenesis. *Circulation* 2019, 139: 101–114
30. Li Z, Xia H, Sharp III TE, LaPenna KB, Katsouda A, Elrod JW, Pfeilschifter J, ***et al.*** Hydrogen sulfide modulates endothelial-mesenchymal transition in heart failure. *Circ Res* 2023, 132: 154–166
31. Liu Z, Han Y, Li L, Lu H, Meng G, Li X, Shirhan M, ***et al.*** The hydrogen sulfide donor, GYY 4137, exhibits anti-atherosclerotic activity in high fat fed apolipoproteinE$^{-/-}$ mice. *Br J Pharmacol* 2013, 169: 1795–1809
32. Miao Y, Zhang S, Liang Z, Wang Y, Tian D, Jin S, Guo Q, ***et al.*** Hydrogen sulfide ameliorates endothelial dysfunction in aging arteries by regulating ferroptosis. *Nitric Oxide* 2023, 140-141: 77–90
33. Huang S, Shao T, Liu H, Wang Q, Li T, Zhao Q. SIRT6 mediates MRTF-A deacetylation in vascular endothelial cells to antagonize oxLDL-induced ICAM-1 transcription. *Cell Death Discov* 2022, 8: 96