Hydrogen sulfide ameliorates acute lung injury induced by infrarenal aortic cross-clamping by inhibiting inflammation and angiopoietin 2 release

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Objective: **Infrarenal aortic cross-clamping (IAC) is a common procedure during infrarenal vascular operations. It often causes ischemia-reperfusion injury to lower limbs, resulting in systemic inflammation response and damage to remote organs (particularly lungs). Hydrogen sulfide (H_2S) is a gaseous mediator that has been shown to have a protective effect against lung injury.**

Methods: **Wistar rats underwent IAC for 2 hours, followed by 4 hours of reperfusion. GYY4137 (a slow-releasing H_2S donor) and DL-propargylglycine (PAG, an inhibitor of cystathionine γ-lyase) were preadministered to rats 1 hour before IAC, and their effects on severity of lung injury and related mechanisms were investigated.**

Results: **IAC induced a significant increase in plasma levels of H_2S, H_2S-synthesizing activity, and cystathionine γ-lyase expression in lung tissues compared with sham operation. Administration of GYY4137 significantly increased the levels of H_2S but had little effect on H_2S-synthesizing activity, whereas PAG reduced H_2S levels and H_2S-synthesizing activity. Preadministration of GYY4137 significantly attenuated acute lung injury induced by IAC, evidenced by reduced histologic scores and wet lung contents; improved blood gas parameters; reduced cell counts and protein amounts in bronchoalveolar lavage fluids; and reduced myeloperoxidase activity in lung tissues and plasma levels of tumor necrosis factor α, interleukin 6, and interleukin 1β. However, PAG further aggravated the severity of lung injury and displayed opposite effects to GYY4137. In exploration of the mechanisms, we found that IAC increased the release of angiopoietin 2 (Ang2) and its expression in lung tissues. GYY4137 attenuated the increase of Ang2 release and expression and increased the phosphorylation of Akt and the activation of its downstream factors, glycogen synthase kinase 3β and ribosomal protein S6 kinase; PAG showed opposite effects.**

Conclusions: **The study indicates that H_2S may play a protective role in IAC-induced acute lung injury in rats by inhibiting inflammation and Ang2 release. (J Vasc Surg 2015;■:1-8.)**

Clinical Relevance: **The incidences of peripheral arterial diseases and aortic abdominal aneurysms are steadily increasing because of arteriosclerosis in the elderly. The postoperative morbidity of infrarenal vascular operations is closely linked to or accompanied by ischemia-reperfusion-induced injury and inflammation. This study provides a novel approach for alleviating acute lung injury induced by infrarenal aortic cross-clamping, which is a common procedure during infrarenal vascular operations. Given that hydrogen sulfide could protect multiple organs against several risk events, the results warrant further investigation by using GYY4137 in preventing acute lung injury during infrarenal vascular surgical interventions.**

Infrarenal aortic cross-clamping (IAC) is a common procedure used in infrarenal vascular operations, during which local tissues suffer from ischemia-reperfusion (IR) injury, especially in surgical interventions involving prolonged ischemic periods.[1] IAC-induced IR injury can lead to systemic appearance of proinflammatory cytokines, provoke systemic inflammatory syndrome, or even cause a life-threatening disorder called multiple organ dysfunction.[2] Among the affected organs, lungs bear the first round of cytokine attack; thus, acute lung injury caused by prolonged IAC is of primary importance.[1] It is essential to seek potential agents to attenuate IAC-induced acute lung injury.

Hydrogen sulfide (H_2S) is the third gaseous mediator, following nitric oxide and carbon monoxide, and has been recognized as a crucial signaling molecule with a wide range of physiologic functions affecting most organs in animals and humans.[3] H_2S is endogenously produced in mammalian cells mainly by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) from L-cysteine and homocysteine, respectively.[3] The biologic importance of H_2S has also shed light on the pathogenesis of various human diseases and paved the way for therapeutic interventions.[3] H_2S has demonstrated a potential role in preserving the function and limiting injury of multiple organs

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particularly induced by IR.[4] We have previously reported that H_2S showed a protective role in myocardial IR-induced injury in diabetics,[5] and administration of sodium hydrosulfide, a donor of H_2S, attenuated the severity of liver injury and inhibited the production of inflammatory factors induced by hepatic IR.[6] Recent studies reveal that exogenous H_2S protects lungs from injury induced by hyperoxia,[7,8] oleic acid,[9] lipopolysaccharide,[10] ozone,[11] limb IR,[12] and burn and smoke inhalation[13] and protects alveolar growth in the experimental O_2-induced neonatal lung injury.[14]

However, it is unknown whether H_2S could also protect against IAC-induced acute lung injury. Therefore, we designed this study to examine the possible effects of H_2S in a rat model of IAC. In this study, we applied GYY4137, a water-soluble and slow-releasing H_2S donor,[15] and DL-propargylglycine (PAG), an irreversible inhibitor of CSE,[6] to investigate their potential effects on severity of IAC-induced acute lung injury and underlying mechanisms.

METHODS

Animals and experiment design. Male Wistar rats (200-250 g) were supplied by the Animal Research Center of the First Affiliated Hospital, Harbin Medical University, China. This study was approved by the Animal Ethics Committee of Harbin Medical University, in compliance with Experimental Animal Regulations by the National Science and Technology Commission. Animals maintained under standard conditions were fed rodent chow and water. Forty-eight rats were randomly assigned into one group of sham operation and three IAC groups. Each group had 12 rats. Sham-operated rats and one group of IAC rats received an injection of 1.0 mL of normal saline. Rats in the other two IAC groups received an injection of GYY4137 (133 μmol/kg, body weight, dissolved in 1.0 mL of normal saline) or PAG (50 mg/kg, body weight, dissolved in 0.2 mL of normal saline; Sigma-Aldrich, Shanghai, China), respectively. The doses were selected on the basis of previous reports.[5,6,15] A group of six healthy untreated rats and two groups of six sham-operated rats receiving an injection of GYY4137 or PAG were also included in this study. Saline, GYY4137, and PAG were intraperitoneally administered 1 hour before the commencement of surgery.

Surgical procedures, gas analysis, and sampling. All surgical procedures were performed under aseptic conditions. Rats were anesthetized by an intraperitoneal injection of the mixture of ketamine (90 mg/kg body weight) and xylazine (20 mg/kg body weight). Anesthesia was maintained by intravenous injections of 25 mg/kg/h of ketamine and 2.5 mg/kg/h of xylazine through the right jugular vein. Heparin (60 IU/kg, body weight) was administered 5 minutes before IAC. Rats were placed on a heating pad to maintain a body temperature of 37°C ± 1.0°C. A midline laparotomy was performed after the skin was shaved and sterilized with 10% povidone-iodine solution. In the IAC groups, the infrarenal part of the aorta was isolated and occluded with an atraumatic clip above the bifurcation of the abdominal aorta. The abdominal cavity was temporarily closed with metal clips, and the wound was covered with a plastic wrap to minimize the loss of heat and fluid. The clip was removed 2 hours later, and reperfusion was initiated. The abdominal wall was closed with a continuous suture, and reperfusion was allowed for 4 hours. Aortic occlusion and reperfusion were corroborated by the loss and resurrection of the pulsation on the distal aorta. Rats in the sham groups underwent the same surgical procedures except for clamping of the infrarenal aorta.

Blood gas analysis was performed at 0, 2, and 4 hours after the commencement of reperfusion from the left carotid artery through a polyethylene catheter (PolyE polyethylene tubing; Harvard Apparatus, Holliston, Mass). At the end of reperfusion, a median sternotomy was performed, and blood samples were collected from the right ventricle. Blood samples were centrifuged at 1000*g* for 10 minutes, and the plasma was decanted and stored at −80°C. The left lung lobe was ligated, and a bronchoalveolar lavage (BAL) was performed using 5 mL of normal saline through a 16-gauge tracheal catheter in the right lung lobe.[16] The BAL fluid was immediately collected and centrifuged at 1000*g* for 10 minutes at 4°C. The supernatant was analyzed for protein contents (Pierce BCA Protein Assay; Thermo Scientific, Rockford, Ill). The cell pellet was resuspended, and total cell number was counted using a hemocytometer. The upper lobe of the right lung was fixed in 10% buffered formalin. The remaining part of the right lung was snap-frozen in liquid nitrogen and stored at −80°C. The wet/dry ratio was calculated from the left lung to evaluate tissue wet content.

Histologic analysis. Formalin-fixed lung specimens were embedded in paraffin, sectioned (3 μm), stained with hematoxylin-eosin, and examined by an independent pathologist in a blinded manner. Evaluation of histologic damage was performed with a histologic score as described previously.[11] Briefly, the severity of lung injury was expressed as the sum of the individual score grades of 0 (no symptoms), 1 (mild), 2 (moderate), and 3 (severe) for each of the following five symptoms: (1) alveolar edema, (2) atelectasis, (3) hemorrhage, (4) infiltration of polymorphonuclear leukocytes, and (5) vascular congestion. In addition, the thickness of alveolar walls was measured in 20 high-power fields (magnification ×1000) randomly selected from each sample.

Enzyme-linked immunosorbent assay (ELISA). The concentrations of tumor necrosis factor α (TNF-α), interleukin (IL) 6, IL-1β, and angiopoietin 2 (Ang2) in plasmas were measured with ELISA kits (R&D Systems, Shanghai, China) according to the manufacturer's instruction. Absorbance was read at a wavelength of 450 nm using an ELISA reader.

Measurement of plasma H_2S, H_2S-synthesizing activity, and myeloperoxidase (MPO) activity in lung tissues and immunoblotting analysis. These methods have been described previously.[5,6] Further details are provided in the Supplementary Methods (online only).

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