Role of hydrogen sulfide in cecal ligation and puncture-induced sepsis in the mouse

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Submitted 18 November 2005; accepted in final form 16 January 2006

Zhang, Huili, Liang Zhi, Philip K. Moore, and Madhav Bhatia. Role of hydrogen sulfide in cecal ligation and puncture-induced sepsis in the mouse. Am J Physiol Lung Cell Mol Physiol 290: L1193–L1201, 2006.—Endogenous hydrogen sulfide (H2S) is naturally synthesized in various types of mammalian cells from L-cysteine in a reaction catalyzed by two enzymes, cystathionine-γ-lyase (CSE) and/or cystathionine-β-synthase. The latest studies have implied that H2S functions as a vasodilator and neurotransmitter. However, so far there is little information about the role played by H2S in systemic inflammation such as sepsis. Thus the aim of this study was to investigate the potential role of endogenous H2S in cecal ligation and puncture (CLP)-induced sepsis. Male Swiss mice were subjected to CLP-induced sepsis and treated with saline (ip), δL-propargylglycine (PAG, 50 mg/kg ip), a CSE inhibitor, or sodium hydrosulfide (NaHS, 10 mg/kg ip). PAG was administered either 1 h before or 1 h after the induction of sepsis, whereas NaHS was given at the same time of CLP. CLP-induced sepsis significantly increased the plasma H2S level and the liver H2S synthesis 8 h after CLP compared with sham operation. Induction of sepsis also resulted in a significant upregulation of CSE mRNA in liver. On the other hand, prophylactic as well as therapeutic administration of PAG significantly reduced sepsis-associated systemic inflammation, as evidenced by myeloperoxidase activity and histological changes in lung and liver, and attenuated the mortality of CLP-induced sepsis. Injection of NaHS significantly aggravated sepsis-associated systemic inflammation. Therefore, the effect of inhibition of H2S formation and administration of NaHS suggests that H2S plays a proinflammatory role in regulating the severity of sepsis and associated organ injury.

δL-propargylglycine; cystathionine-γ-lyase; myeloperoxidase

Sepsis is a common and serious medical condition caused by a severe systemic infection leading to a systemic inflammatory response, which frequently occurs after hemorrhage, trauma, burn, or abdominal surgery. It is a leading cause of morbidity and mortality in severely ill patients (2). However, the role of H2S in sepsis still remains unknown. Although some information has been generated from LPS injection studies, LPS injection is an adequate model of endotoxemia and cannot precisely mimic the changes observed during sepsis. On the other hand, the cecal ligation and puncture (CLP) model seems to resemble qualitatively as well as quantitatively the clinical observations of vascular reactivity and inflammation during polymicrobial peritonitis, bacteraemia, and systemic sepsis (23). Therefore, the aim of this study is to evaluate the possible role of H2S in CLP-induced sepsis.

MATERIALS AND METHODS

Induction of sepsis. All experiments were approved by the animal ethics committee of National University of Singapore and were carried out in accordance with established guiding principles for animal research. The previously described model of CLP-induced sepsis was used with minor modifications (3). Male Swiss albino mice (25–30 g) were lightly anesthetized with a mixture of ketamine and medetomidine [0.75 ml ketamine (100 mg/ml) and 1 ml medetomidine (1 mg/ml) dissolved in 8.25 ml of distilled water, 7.5ml/kg] under aseptic conditions. After the abdominal fur was shaved and a topical disinfectant was applied, a small midline incision was made through the skin and peritoneum of the abdomen to expose the cecum. The cecal appendage was ligated with Silkam 4-0 thread at 3–5 mm below the ileocecal valve without occluding the bowel passage and then perforated in two locations with a 22-gauge needle distal to the point of ligation. After this, a small amount of stool was squeezed out through both holes. Finally, the bowel was repositioned, and the abdomen was stitched up with sterile Permilene 5-0 thread. Animals with sham operation underwent the same procedure without CLP.

δL-propargylglycine (PAG; 50 mg/kg ip, Sigma), an irreversible inhibitor of CSE (29), or saline was administered either 1 h before (“prophylactic”) or 1 h after (“therapeutic”) the CLP or sham operation. In the sodium hydrosulfide (NaHS) intervention experiment, mice underwent CLP operation and were simultaneously given NaHS (10 mg/kg ip, Sigma) or saline. Eight hours after the operation, animals were killed by an ip injection of a lethal dose of pentobarbital (90 mg/kg). Blood samples were drawn from the right ventricles using heparinized syringes and centrifuged (4,000 rpm for 10 min, 0–4°C). Thereafter, plasma was aspirated and stored at −80°C for H2S measurement. Samples of lung and liver were stored at −80°C for subsequent measurement of tissue myeloperoxidase (MPO) activity and H2S synthesizing activity.

Measurement of plasma H2S. Aliquots (120 μl) of plasma were mixed with distilled water (100 μl), trichloroacetic acid (10% w/v),
120 μl), zinc acetate (1% wt/vol, 60 μl), and N,N-dimethyl-p-phenylene diamine sulfate (20 μM; 40 μl) in 7.2 M HCl and FeCl3 (30 μM; 40 μl) in 1.2 M HCl. The absorbance of the resulting solution (670 nm) was measured 10 min thereafter by spectrophotometry (Tecan Systems) (10). H2S was calculated against a calibration curve of NaHS (3.125–100 μM). Results showed plasma H2S concentration in the micromolar range.

Assay of liver H2S synthesizing activity. H2S synthesizing activity in liver homogenates was measured essentially as described elsewhere (17). Briefly, the assay mixture contained 100 mM potassium phosphate buffer (pH 7.4), L-cysteine (20 μl, 20 mM), pyridoxal 5-phosphate (20 μl, 2 mM), saline (30 μl), and 4.5% wt/vol tissue homogenate (430 μl). The reaction was performed in tightly sealed microcentrifuge tubes and initiated by transferring the tubes from ice to a water bath at 37°C. After incubation for 30 min, 1% wt/vol zinc acetate (250 μl) was added to trap evolved H2S followed by 10% wt/vol trichloroacetic acid (250 μl) to denature the protein and stop the reaction. Subsequently, N,N-dimethyl-p-phenylene diamine sulfate (20 μM; 133 μl) in 7.2 M HCl was added, immediately followed by FeCl3 (30 μM; 133 μl) in 1.2 M HCl. The absorbance of the resulting solution at 670 nm was measured by spectrophotometry (Tecan Systems). The H2S concentration was calculated against a calibration curve of NaHS. Results were then corrected for the DNA content of the tissue sample (16) and expressed as nanomoles of H2S formed per milligram of DNA.

MPO estimation. Tissue samples were thawed, homogenized in 20 mM phosphate buffer (pH 7.4), and centrifuged (13,000 g, 10 min, 4°C), and the resulting pellets were resuspended in 50 mM phosphate buffer (pH 6.0) containing 0.5% wt/vol hexadecyltrimethylammonium bromide (Sigma). The suspension was subjected to four cycles of freezing and thawing and further disrupted by sonication (40 s). The samples were then centrifuged (13,000 g, 5 min, 4°C), and the supernatants were used for the MPO assay. The reaction mixture consisted of the supernatant (50 μl), 1.6 mM tetramethylbenzidine (Sigma), 80 mM sodium phosphate buffer (pH 5.4), and 0.3 mM hydrogen peroxide (reagent volume: 50 μl). This mixture was incubated at 37°C for 110 s. The reaction was terminated with 50 μl of 0.18 M H2SO4, and the absorbance was measured at 405 nm. This

Fig. 1. Myeloperoxidase (MPO) activity in lung (A) and liver (B) 8 h after induction of sepsis by cecal ligation and puncture (CLP). Results shown are means ± SE (n = 12 animals/group). *Statistically significant difference (P < 0.01) between mice subjected to sham operation and normal mice. **P < 0.01 when mice subjected to CLP were compared with those with sham operation.

Fig. 2. Morphological changes in lung and liver 8 h after CLP-induced sepsis in hematoxylin and eosin-stained section. A: lung from mice subjected to sham operation. B: lung from mice subjected to CLP. C: liver from mice subjected to sham operation. D: liver from mice subjected to CLP.
absorbance was then corrected for the DNA content of the tissue sample (16), and the results were expressed as enzyme activity.

Morphological examination. Samples of lung and liver were fixed in 4% vol/vol neutral phosphate-buffered formalin and subsequently dehydrated through a graded ethanol series as described before (5, 6). After impregnation in paraffin wax, tissue samples were sectioned. Liver and lung sections (4–5 μm) were stained with hematoxylin–eosin and examined by light microscopy using a Carl Zeiss microscope (objective lens magnification ×40; eyepiece magnification ×10).

**RESULTS**

Induction of sepsis by CLP is associated with increase in plasma H$_2$S level and H$_2$S synthesizing activity in liver. CLP-induced systemic inflammation was indicated by the lung and liver MPO activity, a marker of tissue neutrophil infiltration, and histological changes of the two organs. As expected, lung MPO activity (fold increase over normal control) was significantly increased 8 h after CLP compared with that after sham operation ($P < 0.01$; Fig. 1A). Similarly in liver, a significant but smaller elevation in MPO activity was observed 8 h after CLP ($P < 0.01$; Fig. 1B).

Furthermore, morphological changes in both the lung and liver also suggested systemic inflammation and multiple organ damage. The lung sections from mice with CLP operation exhibited characteristic signs of lung injury, which included interstitial edema, alveolar thickening, and severe leukocyte infiltration in the interstitium and alveoli (Fig. 2B). In liver sections, hepatocyte swelling, slight hepatocyte necrosis, and

RT-PCR analysis of liver CSE mRNA. Total RNA from liver was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol (8). The concentration of isolated nucleic acids was determined spectrophotometrically by measuring the absorbance at 260 nm, and the integrity was verified by ethidium bromide staining of 18S and 28S rRNA bands on a denaturing agarose gel. All samples were thereafter stored at −80°C until required. RNA (1 μg) was reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) at 25°C for 5 min and 42°C for 30 min, followed by 85°C for 5 min. The cDNA was used as a template for PCR amplification by iQ Supermix (Bio-Rad). The primer sequences of β-actin and CSE were as follows: β-actin (forward) 5'-GGG CTG TAT TCC CCT CCA TC-3', β-actin (reverse) 5'-GTC ACG CAC GAT TTC CCT CTC-3'; CSE (forward) 5'-GAC CTC AAT AGT CGG CTT CGT TTC-3'; CSE (reverse) 5'-CAG TGC GTA TGC TCC GTA ATG-3'. The RT-PCR product sizes for β-actin and CSE were 552 and 618 bp, respectively. PCR amplification was carried out in a MyCycler (Bio-Rad) under the optimized conditions of 30 (CSE) or 21 cycles (β-actin) at 95°C for 50 s, 63°C for 1 min, and 72°C for 1 min. PCR products were analyzed on 1.5% wt/vol agarose gels containing 0.5 μg/ml ethidium bromide.

Statistics. Data were expressed as means ± SE. The significance of differences among groups was evaluated by ANOVA with post hoc Tukey’s test when comparing three or more groups. A $P$ value of 0.05 was considered to indicate a statistically significant difference.

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Furthermore, morphological changes in both the lung and liver also suggested systemic inflammation and multiple organ damage. The lung sections from mice with CLP operation exhibited characteristic signs of lung injury, which included interstitial edema, alveolar thickening, and severe leukocyte infiltration in the interstitium and alveoli (Fig. 2B). In liver sections, hepatocyte swelling, slight hepatocyte necrosis, and
Fig. 5. Effect of DL-propargylglycine (PAG) administration on liver H$_2$S synthesizing activity (A) and plasma H$_2$S concentration (B) in CLP-induced sepsis. Mice with CLP-induced sepsis were randomly given PAG (50 mg/kg ip) or saline 1 h before (PAG+CLP or saline+CLP) or 1 h after (CLP+PAG or CLP+saline) CLP operation. Eight hours after CLP or sham operation, plasma H$_2$S concentration and H$_2$S synthesizing activity in liver were measured as described in MATERIALS AND METHODS. Results shown are means ± SE (n = 12 animals/group). *P < 0.01 when mice subjected to CLP with saline injection were compared with mice subjected to sham operation. **P < 0.01 when PAG-treated animals were compared with saline-treated animals.

Fig. 6. Effect of PAG administration on MPO activity in lung (A) and liver (B) from mice with CLP-induced sepsis. Mice with CLP-induced sepsis were randomly given PAG (50 mg/kg ip) or saline 1 h before (PAG+CLP or saline+CLP) or 1 h after (CLP+PAG or CLP+saline) CLP operation. Eight hours after CLP or sham operation, MPO activity in lung and liver was measured as described in MATERIALS AND METHODS. Results shown are means ± SE (n = 12 animals/group). †Statistically significant difference (P < 0.01) between mice subjected to CLP with saline injection and those with sham operation. ‡P < 0.05 when mice subjected to CLP with saline injection were compared with animals with sham operation. *P < 0.05 when PAG-treated animals were compared with saline-treated animals. **P < 0.01 when PAG-treated animals were compared with saline-treated animals.
marginated, pavemented, and transmigrated neutrophil could be easily observed (Fig. 2D).

CLP-induced systemic inflammation significantly enhanced the plasma H2S level ($P < 0.05$; Fig. 3A) as well as H2S synthesizing activity in liver (Fig. 3B; $P < 0.01$) compared with sham operation.

Induction of sepsis by CLP upregulates liver CSE mRNA expression. CLP-induced sepsis resulted in a significant upregulation of CSE gene expression in liver. The expression level of CSE mRNA was significantly elevated in liver from mice with CLP operation compared with sham operation ($P < 0.01$; Fig. 4, A and B).

Prophylactic as well as therapeutic treatment with PAG blocks H2S formation and attenuates systemic inflammation caused by sepsis. Administration of PAG (50 mg/kg) either 1 h before (prophylactic) or 1 h after (therapeutic) CLP operation almost completely abolished the H2S synthesizing activity in the liver (Fig. 5A); therefore, it caused a significant reduction in systemic inflammation.
in plasma H2S level (Fig. 5B). PAG prophylactic as well as therapeutic intervention not only reduced the plasma H2S level significantly 8 h after CLP (both \( P < 0.01 \), compared with saline injection) but also recovered it to a comparable level of mice with sham operation. Because two pyridoxal-5'-phosphate-dependent enzymes, CSE and CBS, are responsible for the majority of the endogenous production of H2S in mammalian tissues, PAG prophylactic and therapeutic treatment, a CSE inhibitor (29), only restored the plasma H2S to a similar level of sham operation and did not eliminate the circulatory H2S.

Prophylactic and therapeutic administration of PAG mitigated the systemic inflammation and multiple organ damage caused by CLP-induced sepsis. Compared with saline-injected mice, MPO activity 8 h after CLP in the lung from mice pretreated or posttreated with PAG was significantly reduced (both \( P < 0.05 \); Fig. 6A). With PAG pretreatment or posttreatment, a significant reduction in liver MPO activity was also seen consistently (\( P < 0.05 \) and \( P < 0.01 \), respectively; Fig. 6B). In addition, prophylactic as well as therapeutic administration of PAG attenuated the histological damages in lung and liver after the induction of sepsis (Figs. 7 and 8).

Because prophylactic and therapeutic intervention of PAG attenuated systemic inflammation and multiple organ damage, PAG was tested for the ability to protect or potentially reverse the progression of lethal CLP-induced sepsis. We found that injection of PAG 1 h before or 1 h after CLP significantly decreased sepsis-induced mortality (both \( P < 0.01 \); Fig. 9, A and B).

Administration of H2S donor aggravates systemic inflammation induced by sepsis. To directly investigate the potential proinflammatory role of H2S in sepsis, the H2S donor drug NaHS was applied. The injection of NaHS at the time of induction of sepsis by CLP significantly aggravated systemic inflammatory response as well as multiple organ damage.

Exogenous H2S from the injected NaHS caused a further and pronounced rise in both lung and liver MPO activity in CLP-induced sepsis (both \( P < 0.01 \) compared with saline injection; Fig. 10, A and B). Moreover, NaHS also resulted in histological

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**Fig. 9.** Effect of prophylactic as well as therapeutic administration of PAG on survival rate of mice subjected to CLP. A: 1 h before CLP, mice were randomly given PAG (50 mg/kg ip) or saline. Their survival rates were monitored for 12 days. B: 1 h after CLP, mice were randomly given PAG (50 mg/kg ip) or saline. Their survival rates were monitored for 12 days.

**Fig. 10.** Effect of H2S donor sodium hydrosulfide (NaHS) administration on MPO activity in lung (A) and liver (B) from mice with CLP-induced sepsis. Mice with CLP-induced sepsis were randomly given NaHS (10 mg/kg ip) or saline at the same time of CLP operation. Eight hours after CLP or sham operation, MPO activity in lung and liver was measured as described in MATERIALS AND METHODS. Results shown are means ± SE (\( n = 12 \) animals/group). *\( P < 0.01 \) when mice subjected to CLP with saline injection were compared with mice with sham operation. †\( P < 0.05 \) when mice subjected to CLP with saline injection were compared with mice with sham operation. **\( P < 0.01 \) when NaHS-treated animals were compared with saline-treated mice.
evidence of more severe organ injury in lung and liver. In addition to obvious leukocyte infiltration and alveolar wall thickness, marked pulmonary congestion and the presence of numerous red blood cells could be easily detected (Fig. 11B). Consistently, the liver sections from the mice subjected to CLP with NaHS injection had more neutrophils infiltrating in the extravascular space (Fig. 11D).

DISCUSSION

Nearly 300 years have passed since the first description of the toxicity of H$_2$S (28). Although most research has focused on its toxic effect, more and more attention has been given to understanding its physiological roles in biological cells and pathological parts in various diseases. Most endogenous H$_2$S is produced by pyridoxal phosphate-dependent enzymatic pathway. CBS appears to be the main H$_2$S-forming enzyme in the central nervous system, whereas CSE is the main H$_2$S-forming enzyme in the cardiovascular system (21, 28). H$_2$S dilates blood vessels and gastrointestinal smooth muscles by opening muscle K$_{ATP}$ channels and promotes hippocampal long-term potentiation by enhancing the sensitivity of NMDA receptors to glutamate (21, 27). However, as a biological mediator, its role in inflammation needs to be clearly understood.

Recent studies have demonstrated that endogenous H$_2$S plays a potential proinflammatory role in several inflammatory conditions, such as hindpaw edema, acute pancreatitis, and LPS-induced endotoxemia (7, 8, 17). To the best of our knowledge, there is little information about the potential role of H$_2$S in sepsis, which is a common systemic inflammation in a clinical situation. Although one study has revealed the correlation between the endogenous level of H$_2$S produced by vascular tissues and hemodynamic parameters in CLP-induced sepsis (31), the relationship between H$_2$S and systemic inflammation in sepsis is unclear. The studies on inflammation induced by endotoxin injection showed an increase in H$_2$S synthesis in liver and kidney and its association with inflammation and multiple organ damage in endotoxic shock. However, LPS-induced endotoxemia cannot accurately reflect the cytokine profiles and reproduce the hemodynamic changes in sepsis (22, 23). In addition, during the acute phase of sepsis induced by intravenous injection of live Escherichia coli, cysteine metabolism, glutathione synthesis, and methionine transsulfuration in rats were significantly increased, suggesting an enhanced requirement in cysteine during sepsis (18, 19, 20). Unfortunately H$_2$S level and synthesis in these cases have not been tested.

CLP-induced sepsis caused a significant systemic inflammatory response and multiple organ damage characterized both biochemically (MPO activity in lung and liver) and histologically (hematoxylin-eosin-stained section). However, a slight increase in lung MPO activity 8 h after sham operation was obtained in our experiment. A similar phenomenon was also seen in a time-course study of lung MPO activity in CLP-treated mice (15). Iwamura et al. (15) found not only the time-dependent increase in the lung MPO activity after CLP but also the modest elevation after sham operation. Although lung MPO activity in the sham-operated group was significantly higher than that in normal mice, the survival rate after sham operation was sustained at 100% during the 12 days thereafter. It seemed logical to assume that the elevation was correlated with the nonspecific inflammation caused by sham operation. In addition, due to the tissue-specific difference, the increase in liver MPO activity was not as high as that in lung, which is consistent with the previous data from the literature (24).

With the use of CLP-induced sepsis, a well-established and clinically relevant animal model for sepsis, our findings indicated an important role of H$_2$S in sepsis-associated inflamma-

![Fig. 11. Morphological changes in lung and liver from mice subjected to CLP with or without NaHS administration in hematoxylin and eosin-stained section. A: lung from mice with saline injection at the same time of CLP. B: lung from mice with NaHS injection at the same time of CLP. C: liver from mice with saline injection at the same time of CLP. D: liver from mice with NaHS injection at the same time of CLP.](http://ajplung.physiology.org/)
tion. Mice with sepsis had significantly elevated plasma H2S levels, CSE gene expression levels, and H2S synthesizing enzyme activity in liver. It is reasonable to conclude that sepsis may upregulate both the CSE expression and the CSE activity in liver and consequently raise the plasma levels of H2S. However, the mechanism in which it indicates that sepsis promotes the expression levels of CSE mRNA and the CSE activity in liver is not clear. A recent study demonstrated that myeloid zinc finger protein-I or specificity protein-I consensus, which could enhance the CSE promoter activity and the repressive elements (acute myeloid leukemia-1, upstream stimulatory factor-1, or N-Myc consensus), were involved in the regulation of CSE basal transcriptional activity (14). Whether sepsis may modify these elements in CSE promoter and therefore raise the expression of CSE is to be investigated.

It has been shown that exogenous H2S by itself produces an inflammatory response in normal mice characterized by an increase in lung and liver MPO activity and tissue damage to the lung (17). To directly investigate the potential proinflammatory role of H2S in sepsis, H2S donor was also applied. Exogenous H2S from injected NaHS aggravated the systemic inflammation characterized by elevated MPO activity in lung and liver. Multiple organ damage caused by sepsis was also exacerbated by the injection of NaHS. Severe pulmonary congestion and increased neutrophil infiltration were noted in lung and liver sections. On the other hand, evidence of prophylactic as well as therapeutic administration of PAG in sepsis indirectly demonstrated the proinflammatory function of H2S. Inhibition of the activity of CSE by PAG could significantly alleviate inflammation, sepsis-associated organ damage, and mortality.

CLP-induced sepsis is, of course, multifactorial and numerous mediators other than H2S are involved in it. Blockage of the synthesis of H2S only partly reversed the pathological progression of sepsis. Consequently, based on these results, we conclude that H2S is one of the pivotal factors to determine the severity of inflammation and organ injury induced by sepsis. However, the precise mechanism by which H2S modulates the systemic inflammation remains to be elucidated.

According to the studies of H2S in the vascular system (21, 31), it seems reasonable to extrapolate that H2S may primarily increase vascular permeability and promote tissue edema by vasorelaxation, leading to the hemodynamic changes in septic shock. In addition to its vasodilator activity, H2S may contribute to inflammation in sepsis in several ways. In CLP-induced sepsis, the level of chemokines (KC chemokine; macrophage inflammatory protein-2) and cytokines (TNF, IL-1, IL-6) substantially increase and remain higher at 8 h after CLP operation (22). The profiles of these chemokines and cytokines also correlate with the severity of sepsis (11). Thus it is necessary to further investigate the possibility that H2S modulates the development of inflammation and affects the severity of sepsis via association with chemokines and cytokines. In addition, interaction between H2S and other gaseous mediators, such as nitric oxide (NO), raises another possible way that H2S promotes the inflammation. In vascular tissue, NO and H2S can affect each other in different ways: 1) H2S may decrease the sensitivity of the cGMP pathway to NO and the expression level of NO synthase, 2) NO may increase the expression of CSE and the cellular uptake of cysteine, and 3) H2S may modify KCa channels to decrease their sensitivity to NO (28).

In sepsis, upregulated inducible NO synthase and NO not only activate the microvascular system and decrease vasopressor responsiveness leading to hypotension but also inhibit leukocyte rolling and adhesion and impair neutrophil migration, worsening the severity of sepsis (9, 12, 26). Thus NO may function together with H2S to promote the inflammation and at the same time contribute to the elevation of H2S synthesis in sepsis. The possibility of interaction between H2S and NO in sepsis warrants further study.

In summary, the results in this article show that an increase in the H2S synthesis promotes the inflammation, whereas administration of an inhibitor of H2S synthesis substantially attenuates the inflammation and multiple organ injury caused by CLP-induced sepsis. The mechanism by which H2S may play a proinflammatory role in sepsis and sepsis-associated organ injury shall be the subject of future studies.

ACKNOWLEDGMENTS

We thank Mei Leng Shoon for help with the animal experiments.

GRANTS

This work was supported by Biomedical Research Council Grant R-184-000-094-305 and Office of Life Sciences Cardiovascular Biology Program Grant R-184-000-074-712, National University of Singapore.

REFERENCES


