Role of Hydrogen Sulfide in Severe Burn Injury–Induced Inflammation in Mice

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Endogenous hydrogen sulfide (H_2S) is naturally synthesized in many types of mammalian cells from L-cysteine in the reactions catalyzed by cystathionine-β-synthase and cystathionine-γ-lyase (CSE). H_2S has been demonstrated to play a proinflammatory role in various animal models of hindpaw edema, acute pancreatitis, lipopolysaccharide-induced endotoxemia and cecal ligation, and puncture–induced sepsis. Full-thickness burns that exceed 25% of the total body surface area (TBSA) produce a profound systemic inflammatory reaction characterized by leukocyte activation and plasma leakage in the microvasculature of tissues and organs remote from the wound. The aim of this study was to investigate the effect of local burn injury on induced distant organ endogenous H_2S release and expression of CSE. Male BALB/c mice were subjected to 30% TBSA full-thickness burn and treated with saline (administered intraperitoneally (i.p.)); DL-propargylglycine (PAG, 50 mg/kg i.p.), which is a CSE inhibitor; or sodium hydrosulfide (NaHS, 10 mg/kg i.p.), which is an H_2S donor. PAG was administered either 1 h before or 1 h after the burn injury, whereas NaHS was given at the same time as the burn injury. Measurements of liver myeloperoxidase (MPO) activities, liver H_2S-synthesizing activity, plasma H_2S level and liver and lung CSE mRNA expression and histological examination of tissues were performed after burn injury. Burn injury significantly increased the plasma H_2S level and liver H_2S synthesis 8 h after burn compared with the sham group. Burn injury also resulted in a significant upregulation of CSE mRNA in liver and lung. Prophylactic as well as therapeutic administration of PAG significantly reduced burn-associated systemic inflammation, as evidenced by MPO activity and histological changes in liver and lung. Injection of NaHS significantly aggravated burn-associated systemic inflammation. Therefore, our findings show for the first time the role of H_2S in contributing to inflammatory damage after burn injury.

INTRODUCTION

Patients with severe burns become susceptible to sepsis and may develop systemic inflammation and multiple organ failure, especially acute lung injury, which are common causes of morbidity and mortality (1). A profound systemic inflammatory response characterized by leukocyte activation and plasma leakage in the microvasculature of tissues or organs remote from the wound was produced in response to full-thickness burns that exceed 25% of the total body surface area (TBSA) (2). The degree of inflammatory response correlated directly with the percentage of TBSA burn (3). Systemic inflammatory-mediator release results in a massive inflammatory reaction. The development of methods for identifying and blocking specific inflammatory mediators has been a major focus of investigation (4,5).

Endogenous hydrogen sulfide (H_2S) is synthesized through degradation of L-cysteine by cystathionine-γ-lyase (CSE, EC4.4.1.1) or cystathionine-β-synthase (CBS, EC4.2.1.22). Both enzymes were found to be expressed in many mammalian tissues (6). CBS converts homocysteine to cystathionine and hydrolyses L-cysteine to equimolar amounts of serine and H_2S, whereas CSE converts cystathionine to L-cysteine, yielding pyruvate, ammonia and H_2S (7). H_2S is becoming recognized as a regulator of many physiological and pathological processes (8). For example, H_2S exhibits potent vasodilator activity both *in vitro* and *in vivo*, most likely by opening vascular smooth muscle K (ATP) channels (9,10). In addition, H_2S also functions as a neuromodulator in the brain. Physiological H_2S can facilitate the induction of hippocampal long-term potentiation and selectively enhance excitatory postsynaptic currents mediated by the *N*-methyl-D-aspartate receptor (11). In addition to its functions as a neuromodulator and a vasodilator, H_2S may contribute to inflammatory disease. In our laboratory, we have ob-

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served that H_2S is associated with inflammation, such as hindpaw edema (12), acute pancreatitis (13), lipopolysaccharide (LPS)-induced endotoxemia (14) and cecal ligation and puncture (CLP)-induced sepsis (15).

The present study was designed to investigate the role of endogenous H_2S in systemic inflammation and organ injury caused by burn injury in mice. Plasma H_2S concentration, liver H_2S synthesizing activity and CSE gene expression in tissues 8 hours after burn injury were analyzed to determine whether H_2S has proinflammatory effects on systemic inflammation in mice subjected to burn injury. Then DL-propargylglycine (PAG) was used to further confirm the role of H_2S in mice subjected to burn injury.

MATERIALS AND METHODS

Mouse Burn Injury Model

All experiments were approved by Institutional Animal Care and Use Committee of DSO National Laboratories and were conducted in accordance with their established guidelines. Groups of male BALB/c mice, 6–8 wks old, were anesthetized with ketamine (160 mg/kg) plus xylazine (4 mg/kg) and the dorsal hair was clipped. Mice were placed in an insulating mold device with an opening calculated to expose 30% TBSA. The exposed skin was immersed in 95°C water for 8 s. This procedure has been shown to produce an anesthetic full-thickness burn (16). Sham mice, which served as controls, were anesthetized, shaved, and exposed to 24°C water. After sham or burn injury, mice were resuscitated with 1 mL of 0.9% sterile normal saline solution by intraperitoneal (i.p.) injection and were individually housed. PAG (50 mg/kg, i.p.; Sigma-Aldrich, St Louis, MO, USA), an irreversible inhibitor of CSE (17), or saline was given i.p. to mice either 1 h before ("prophylactic") or 1 h after ("therapeutic") the burn injury, respectively. In the sodium hydrosulfide (NaHS) intervention experiment, mice subjected to burn

injury were simultaneously given NaHS (10 mg/kg i.p.; Sigma-Aldrich) or saline. Eight hours after sham or burn injury, animals were killed by an i.p. injection of a lethal dose of pentobarbitone (90 mg/kg). Blood samples were drawn from the right ventricles by using heparinized syringes and were then centrifuged (1000g for 10 min, 0°C–4°C). Thereafter, plasma was aspirated and stored at –80°C for H_2S measurement. Samples of lung and liver were stored at –80°C for subsequent measurement of tissue myeloperoxidase (MPO) activity, H_2S-synthesizing activity and CSE mRNA expression.

Measurement of Plasma H_2S

Aliquots (120 µL) of plasma were mixed with distilled water (100 µL), trichloroacetic acid (10% wt/vol, 120 µL), zinc acetate (1% wt/vol, 60 µL) and N,N-dimethyl-p-phenylenediamine sulfate (20 µmol/L; 40 µL) in 7.2 mol/L HCl and FeCl$_3$ (30 µmol/L; 40 µL) in 1.2 mol/L HCl. The absorbance of the resulting solution (670 nm) was measured 10 min thereafter by spectrophotometry (Tecan Systems, San Jose, CA, USA) (18). H_2S was calculated against a calibration curve of NaHS (3.125–100 µmol/L). Results showed plasma H_2S concentrations in the micromolar range.

Assay of Liver H_2S-Synthesizing Activity

H_2S-synthesizing activity in liver homogenates was measured similarly as described elsewhere (14). Briefly, the assay mixture contained 100 mmol/L potassium phosphate buffer (pH 7.4), L-cysteine (20 µL, 20 mmol/L), pyridoxal 5′-phosphate (20 µL, 2 mmol/L), 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 H_2S, followed by 10% wt/vol trichloroacetic acid (250 µL) to denature the protein and

stop the reaction. Subsequently, N,N-dimethyl-p-phenylenediamine sulfate (20 µmol/L; 133 µL) in 7.2 mol/L HCl was added, immediately followed by FeCl$_3$ (30 µmol/L; 133 µL) in 1.2 mol/L HCl. The absorbance of the resulting solution at 670 nm was measured by spectrophotometry (Tecan Systems). The H_2S concentration was calculated against a calibration curve of NaHS. Results were then corrected for the DNA content of the tissue sample (19) and expressed as micromoles of H_2S formed per microgram of DNA.

MPO Estimation

Tissue samples were thawed, homogenized in 20 mmol/L phosphate buffer (pH 7.4) and centrifuged (13,000g, 10 min, 4°C), and the resulting pellets were resuspended in 50 mmol/L phosphate buffer (pH 6.0) containing 0.5% wt/vol hexadecyltrimethylammonium bromide (Sigma-Aldrich). The suspension was subjected to four cycles of freezing and thawing and further disrupted by sonication (40 s). The samples were then centrifuged (13,000g, 5 min, 4°C), and the supernatants were used for the MPO assay. The reaction mixture consisted of the supernatant (50 µL), 1.6 mmol/L tetramethylbenzidine (Sigma-Aldrich), 80 mmol/L sodium phosphate buffer (pH 5.4) and 0.3 mmol/L 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 mol/L H_2SO4, and the absorbance was measured at 405 nm. This absorbance was then corrected for the DNA content of the tissue sample (19), and the results were expressed as fold increase over normal control.

Morphological Examination

Samples of lung and liver were fixed in 10% vol/vol neutral phosphate-buffered formalin and subsequently dehydrated through a graded ethanol series as described previously (13,20). After impregnation in paraffin wax, tissue samples were sectioned. Liver and lung

sections (4–5 μm) were stained with hematoxylin-eosin and examined with light microscopy by using a Carl Zeiss microscope (objective lens magnification 40×; eyepiece magnification 10×).

Reverse Transcription–Polymerase Chain Reaction Analysis of Liver and Lung CSE mRNA

Total RNA from liver and lung was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol (13). The concentration of isolated nucleic acids was determined spectrophotometrically by measuring the absorbance at 260 nm, and the integrity was verified by GelRed™ nucleic acid gel staining (Biotium, Hayward, CA, USA) of 18S and 28S rRNA bands on a denaturing agarose gel. All samples were thereafter stored at –80°C until required for analysis. RNA (1 μg) was reverse transcribed by using an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) 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 polymerase chain reaction (PCR) amplification by iQ Supermix (Bio-Rad). The primer sequences for detection of CSE and 18S were as follows: CSE (forward) 5'-GACCTCAATAGTCGG CTTCGTTTC-3', CSE (reverse) 5'-CAGTTCTGCGTATGCTCCGTAATG-3'; 18S (forward) 5'-GTAACCCGTTGAACC CCATT-3', 18S (reverse) 5'-CCATC CAATCGGTAGTAGCG-3'. The PCR product sizes for CSE and 18S were 618 and 150 bp, respectively. PCR amplification was conducted in a MyCycler (Bio-Rad). The reaction mixture was first subjected to 95°C for 3 min, followed by an optimal cycle (CSE 30 cycles, 18S 20 cycles) of amplifications, consisting of 95°C for 50 s, optimal annealing temperature (CSE 63°C, 18S 59°C) for 60 s, and 72°C for 60 s. Final extension was at 72°C for 7 min. PCR products were analyzed on 1.5% (wt/vol) agarose gels containing GelRed™ nucleic acid gel stain (Biotium).
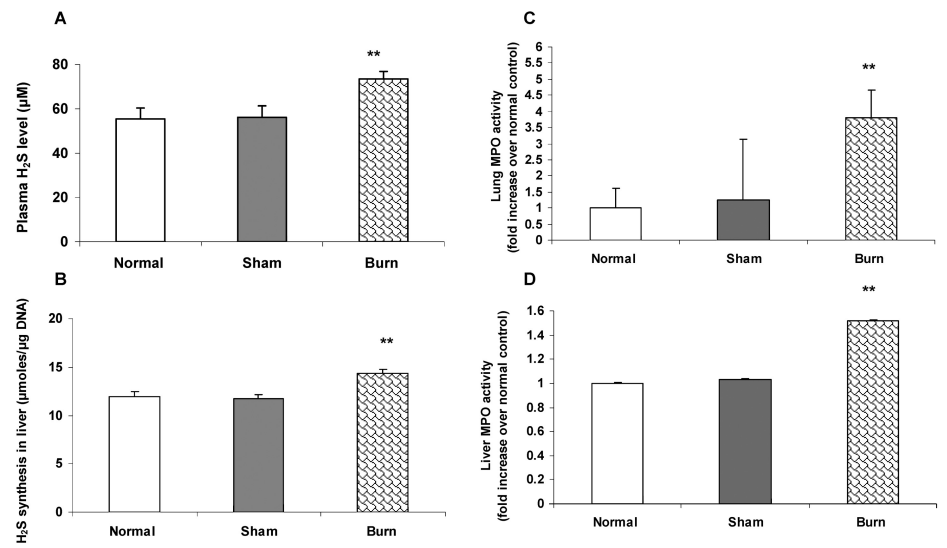
Figure 1. Plasma hydrogen sulfide (H$_2$S) concentration (A), liver H$_2$S-synthesizing activity (B) and lung (C) and liver (D) MPO activity 8 h after burn injury. Results shown are mean ± SEM (n = 9 animals/group). *P < 0.05 and **P < 0.01 when mice subjected to burn injury were compared with sham group.

Statistical Analysis

Data were expressed as mean ± the standard error of mean (SEM). The significance of differences among groups was evaluated by ANOVA with *post hoc* Tukey test when comparing three or more groups. A P value < 0.05 was considered to indicate a statistically significant difference.

RESULTS

### Burn Injury Is Associated with Increase in Plasma H$_2$S Level and H$_2$S-Synthesizing Activity in Liver

Burn injury resulted in a significant increase by 1.31-fold in the plasma H$_2$S level (P < 0.01; Figure 1A) compared with the sham group. The amounts of the formation of H$_2$S in lung were undetectable. Therefore only liver H$_2$S-synthesizing activity was measured. As expected, the amounts of H$_2$S formed in liver after burn injury were significantly enhanced by 1.23-fold compared with the sham group (P < 0.01; Figure 1B). In addition, those mice subjected to burn injury showed a significant systemic inflammation, which was indicated by the lung and liver MPO activity, a marker of

tissue neutrophil infiltration. The lung MPO activity (fold increase over normal control) was significantly increased by 3.06-fold 8 h after burn injury compared with that in the sham group (P < 0.01; Figure 1C). Similarly, in the liver, a significant elevation (1.48-fold burn versus sham) in MPO activity, but smaller than that in the lung, was observed 8 h after burn injury (P < 0.01; Figure 1D) compared with that in the sham group.

Liver and Lung CSE mRNA Expression Were Upregulated by Burn Injury

Burn injury resulted in a significant upregulation of CSE gene expression in both liver and lung. The expression level of CSE mRNA was significantly increased by 4.06-fold in the liver of mice subjected to burn injury compared with that in the sham group (P < 0.01; Figure 2A). In addition, a significant upregulation of CSE gene expression was found in the lungs of mice subjected to burn injury. Compared with the expression level of CSE mRNA in the lung from the sham group, CSE gene expression was significantly elevated by 1.83-fold in the lungs of mice subjected to burn injury (P < 0.01; Figure 2B).

A

B
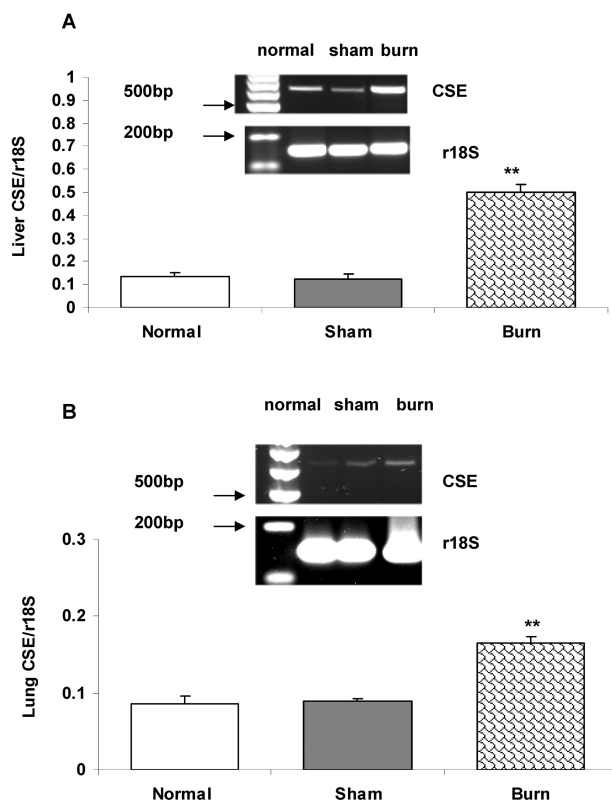
Figure 2. Liver (A) and lung (B) CSE mRNA expression in normal, sham and burn group (n = 5 animals/group). Mouse 18S RNA is shown as a control. The bar graph shows the quantification of band intensity. Data were normalized to controls and expressed as fold-change ratio to control levels. **$P < 0.01$ when mice subjected to burn injury were compared with sham group.

PAG Blocks H$_2$S Formation and Attenuates Systemic Inflammation Caused by Burn Injury

Administration of PAG either 1 h before (prophylactic) or 1 h after (therapeutic) burn injury almost completely abolished the H$_2$S-synthesizing activity in the liver ($P < 0.01$; Figure 3A). In addition, both prophylactic and therapeutic administration of PAG not only reduced the plasma H$_2$S level significantly 8 h after burn injury ($P < 0.05$; Figure 3B) compared with saline injection group, but also recovered it to a level comparable to that of mice in the sham group. Therefore, PAG prophylactic and therapeutic treatment abolished H$_2$S synthesizing activity in the liver and restored the plasma H$_2$S to a similar level of that in sham group and did not eliminate the circulatory H$_2$S. These results indicate that CSE

plays an important role in endogenous H$_2$S formation after burn injury.

Prophylactic and Therapeutic Administration of PAG Mitigated the Systemic Inflammation and Multiple Organ Damage

MPO activity 8 h after burn injury in the lungs from mice pretreated or posttreated with PAG was significantly reduced compared with MPO activity in the lungs of saline-injected mice (both $P < 0.01$; Figure 3C). In addition, with PAG pretreatment or posttreatment, a significant reduction in liver MPO activity was also seen consistently ($P < 0.05$; Figure 3D). It seems that CSE may be involved in the systemic inflammation induced by burn injury. Furthermore, histological examination of lung and liver tissues revealed significant alveolar

thickening, severe leukocyte infiltration in the interstitium and alveoli, hepatocyte swelling and marginated, pavemented and transmigrated neutrophils in mice subjected to burn injury compared with the sham group. These morphological changes in both lung and liver suggested the presence of systemic inflammation and multiple organ damage. Both prophylactic and therapeutic administration of PAG attenuated the histological damage in lung and liver tissue after burn injury (Figure 4A–J), providing evidence (in addition to MPO activity and H$_2$S measurement) that CSE may be involved in the systemic inflammation and multiple organ damage induced by burn injury.

CSE mRNA Expression Was Reduced in the Liver and Lung

Consistently, administration of prophylactic or therapeutic PAG significantly reduced the increased liver CSE gene expression induced by burn injury ($P < 0.05$; Figure 5A). In addition, the upregulated lung CSE gene expression induced by burn injury was reduced by both prophylactic and therapeutic administration of PAG ($P < 0.01$; Figure 5B). However, both CSE expression in liver and lung in mice subjected to burn injury treated with PAG were reduced only to a level comparable to that of mice in the sham group. It appears that PAG has an inhibitory effect on both liver and lung CSE gene expression induced by burn injury.

Administration of H$_2$S Donor Aggravates Systemic Inflammation Induced by Burn Injury

To directly investigate the potential proinflammatory role of H$_2$S in mice subjected to burn injury, the H$_2$S donor drug NaHS was applied. The injection of NaHS at the time of burn injury significantly aggravated the systemic inflammatory response and increased multiple organ damage. NaHS injection caused a further and pronounced increase in lung MPO activity in mice subjected to burn injury ($P < 0.01$; Figure 6A). However,
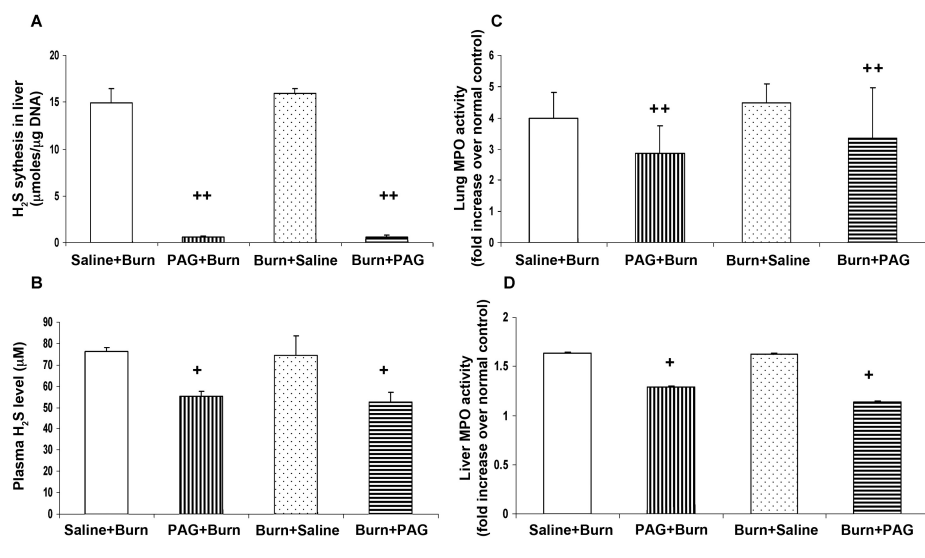
Figure 3. Effect of PAG administration on liver H_2S synthesizing activity (A), plasma H_2S concentration (B) and lung (C) and liver (D) MPO activity 8 h after burn injury. Mice with burn injury were randomly given PAG (50 mg/kg i.p.) or saline 1 h before (PAG + burn or saline + CLP) or 1 h after (burn + PAG or burn + saline) burn injury. Eight hours after burn injury, plasma H_2S concentration and H_2S-synthesizing activity in liver were measured as described in Materials and Methods. Results shown are mean ± SEM (n = 9 animals/group). [+]$P < 0.05$, [++]$P < 0.01$ when PAG treated animals were compared with saline treated animals.

ness could be easily detected in the lung from NaHS-injected mice compared with saline-treated mice (Figure 6C, D). However, histological images of liver sections from the mice subjected to burn injury and NaHS injection were similar to those of mice subjected to burn injury and saline injection (Figure 6E, F).

DISCUSSION

The gas H_2S is increasingly recognized to be a novel regulator of important physiologic functions and to be involved in the pathogenesis of various diseases. Most endogenous H_2S is generated during L-cysteine metabolism in many types of mammalian cells in reactions catalyzed by the pyridoxal phosphate–dependent enzymes CSE and CBS (21).

The present study investigated the role of H_2S in mice subjected to burn injury. As demonstrated in this study and previous studies (22,23), burn injury resulted in a significant systemic inflammatory response, and multiple organ damage characterized by results of both biochemical (MPO activity) and histological (hematoxylin-eosin–stained section) analysis were observed in lung and

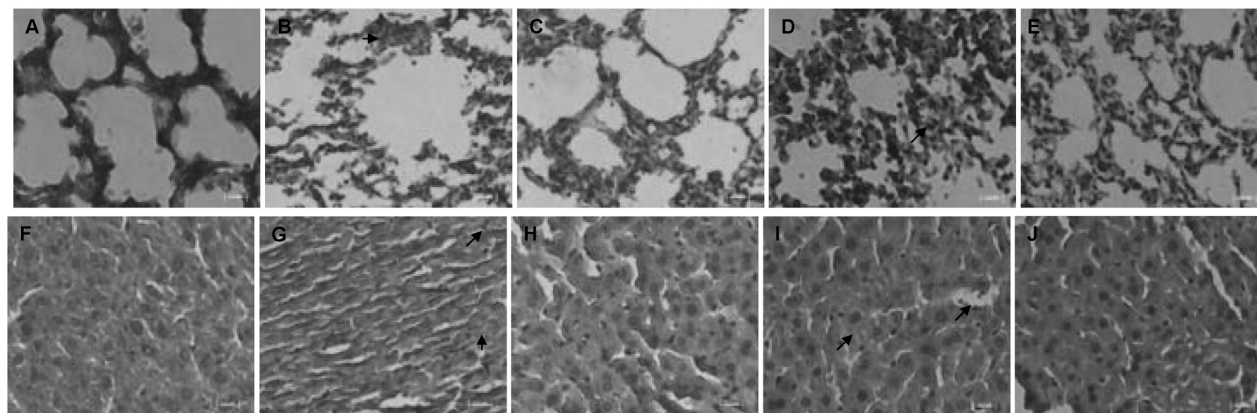NaHS injection only slightly increased the liver MPO activity in mice subjected to burn injury (Figure 6B). In addition, NaHS also resulted in histological evidence of more severe organ injury in the lung. Obvious epithelial disorganization, the presence of airway exudates, leukocyte infiltration and alveolar wall thick-

Figure 4. Morphological changes in lung and liver 8 h after induction of burn injury with or without PAG treatment in sections stained with hematoxylin and eosin. (A) Lung from sham group; (B) lung from mice subjected to burn injury with saline pretreatment; (C) lung from mice subjected to burn injury with PAG prophylactic treatment; (D) lung from mice subjected to burn injury with saline posttreatment; (E) lung from mice subjected to burn injury with PAG therapeutic treatment; (F) liver from sham group; (G) liver from mice subjected to burn injury with saline pretreatment; (H) liver from mice subjected to burn injury with PAG prophylactic treatment; (I) liver from mice subjected to burn injury with saline posttreatment; (J) liver from mice subjected to burn injury with PAG therapeutic treatment. The scale bar is 2 μm. Mice subjected to burn injury demonstrated alveolar thickening and severe leukocyte infiltration in the interstitium (arrowed) in the lung (B,D) and hepatocyte swelling and transmigrated neutrophils (arrow) in the liver (G,I).
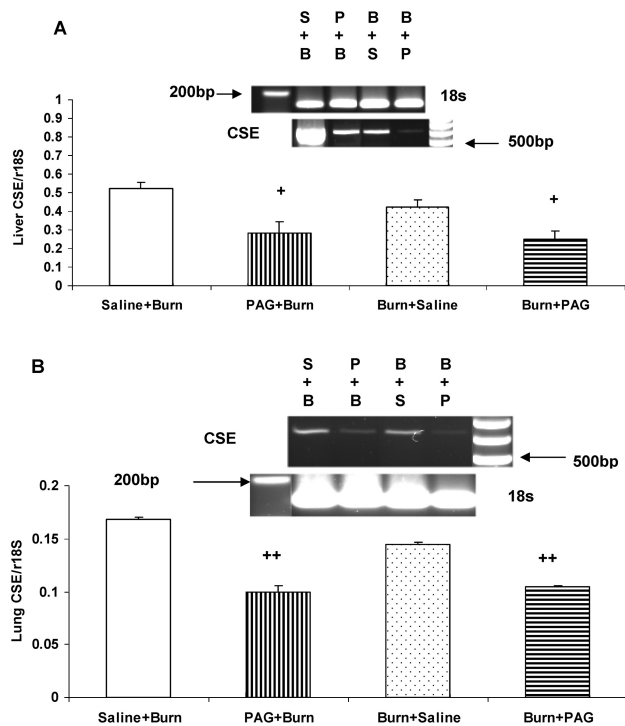
Figure 5. Effect of PAG administration on liver (A) and lung (B) CSE mRNA expression in mice with burn injury (n = 5 animals/group). Mice with burn injury were randomly given PAG (50 mg/kg i.p.) or saline 1 h before (PAG + burn or saline + CLP) or 1 h after (burn + PAG or burn + saline) burn injury. Mouse 18S RNA is shown as a control. The bar graph shows the quantification of band intensity. Data were normalized to controls and expressed as fold change ratio to control levels. $^{+}P < 0.05$, $^{++}P < 0.01$ when PAG-treated animals were compared with saline-treated animals.

liver from the mice subjected to burn injury. In addition, this study demonstrated that burn injury caused a significant increase in both plasma H_2S concentrations and liver H_2S synthesizing activity, and a significant upregulation of both liver and lung CSE mRNA expression 8 hours after burn injury. These findings are similar to those observed in our previous studies on other animal models. Our group previously reported that CLP-induced sepsis upregulates liver CSE mRNA expression and causes a significant increase in plasma H_2S concentration (15). A similar proinflammatory effect of H_2S has also been reported in other animal models of inflammatory diseases, such as hindpaw edema (12), acute pancreatitis (13) and LPS-induced endotoxemia (14). Altogether, with the well-established burn model, our findings indicated an important proinflammatory role of endogenous H_2S in the postburn inflammatory response.

Mice subjected to burn injury had significantly elevated plasma H_2S levels, CSE gene expression levels, and H_2S-synthesizing enzyme activity in liver. It is reasonable to conclude that burn injury may upregulate CSE expression and activity in liver and consequently raise the plasma levels of H_2S. However, the mechanism by which burn injury promotes the expression levels of CSE mRNA and CSE activity in liver is not clear. Results of our previous studies showed that blockage of H_2S formation by administration of PAG, an inhibitor of CSE enzyme activity, reduced the severity and mortality of sepsis, whereas exogenous H_2S resulted in augmented MPO activity as well as histological changes in lung and liver (15). Hence, to

further confirm the proinflammatory role of H_2S in the postburn inflammatory response and to investigate any role of H_2S in burn-injury–induced CSE mRNA expression, we added the CSE inhibitor PAG.

We demonstrated in mice subjected to burn injury that prophylactic as well as therapeutic administration of PAG significantly alleviated the systemic inflammation characterized by reduced lung and liver MPO activity and multiple organ damage compared with saline-treated mice subjected to burn injury. In addition, PAG administration significantly reversed the liver H_2S-synthesizing activity and consequently reduced the plasma H_2S concentration. Furthermore, both prophylactic and therapeutic PAG administration resulted in a significant downregulation of liver and lung CSE gene expression compared with saline-treated mice subjected to burn injury, but still higher than those in sham group. Inhibition of the gene expression and activity of CSE was found in WKY rats when the endogenous production of H_2S was deprived by administration of PAG (24). Therefore, inhibition of the activity and expression of CSE by PAG could significantly alleviate burn injury–associated inflammation and multiple organ damage. PAG reduced the hepatocellular, pancreatic and neuromuscular injury and abolished the increase in MPO activity and in the formation of H_2S in the liver caused by endotoxemia (25). Inhibition of H_2S biosynthesis by PAG also reduced hemorrhagic shock–induced inflammatory responses and organ injury (26). These findings combined with results from the present study indicate that PAG could partially reverse the proinflammatory function of H_2S. These data also indicate that H_2S-synthesis inhibitors may be useful in the treatment of the systemic inflammatory response and organ injury associated with burn injury, sepsis and shock, and suggest a new approach to the development of novel drugs for this condition.

To directly investigate the potential proinflammatory role of H_2S in mice sub-
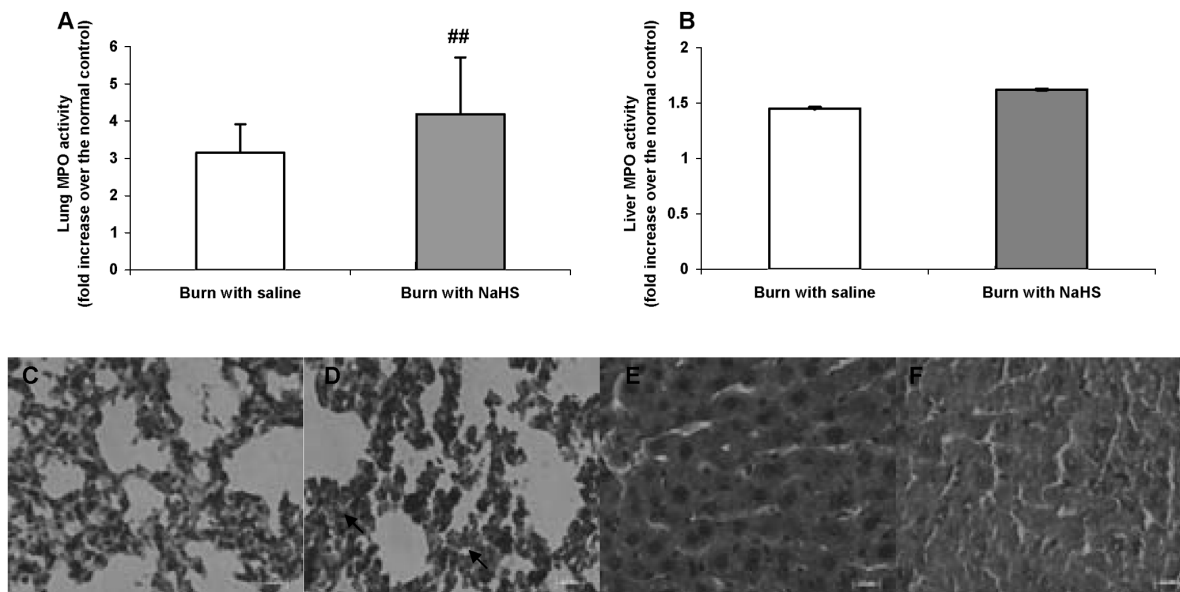
Figure 6. Effect of H₂S donor NaHS administration on MPO activity in lung (A) and liver (B) from mice subjected to burn injury. Mice subjected to burn injury were randomly given NaHS (10 mg/kg i.p.) or saline at the same time of burn injury. Eight hours after burn injury, MPO activity in lung and liver was measured as described in Materials and Methods. Results shown are mean ± SEM (n = 9 animals/group). $^{##}P < 0.01$ when NaHS-treated mice were compared with saline-treated mice. (C–F) Morphological changes in lung and liver from mice subjected to burn injury with NaHS administration in hematoxylin and eosin–stained sections. (C) Lung from mice subjected to burn injury with saline simultaneously; (D) lung from mice subjected to burn injury with NaHS simultaneously; leukocyte infiltration and alveolar wall thickness (arrow) can be easily detected. (E) Liver from mice subjected to burn injury with saline simultaneously; (F) liver from mice subjected to burn injury with NaHS simultaneously. The scale bar is 2 μm.

jected to burn injury, H₂S donor NaHS was also applied. Our previous studies in other animal models showed that exogenous H₂S from injected NaHS aggravated the systemic inflammation characterized by elevated MPO activity in lung and liver, and the tissue damage characterized by increased neutrophil infiltration in liver and lung tissue samples (14,15). This study also showed that NaHS injection produces an elevated inflammatory response in mice subjected to burn injury treated with NaHS, a response characterized by a significant increase in lung MPO activity and tissue damage to the lung. However, exogenous H₂S from injected NaHS only slightly, but not significantly, increased the liver MPO activity and tissue damage to the liver. The increase in liver MPO activity was not as high as that in lung tissue, a finding that is consistent with the previous data from the literature and attributable to a tissue-specific difference (27).

Consequently, based on these results, we conclude that H₂S is one of the pivotal factors that determines the severity of inflammation and organ injury induced by burns. However, the precise mechanism by which H₂S modulates the systemic inflammation remains to be elucidated. Significant increases in dermal interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and IL-1β expression induced by burn injury have been reported (28). In addition, H₂S caused a significant increase in expression of IL-6, TNF-α and IL-1β *in vitro* and *in vivo* (29,30). Therefore, it is necessary to further investigate the possibility that H₂S modulates the development of the inflammatory response to burn injury via association with chemokines and cytokines. Furthermore, the interaction between H₂S and other gaseous mediators, such as nitric oxide (NO), suggests another possible way that H₂S promotes inflammation. A significant increase of NO and its metabolites was discovered in noninjured skin and skin wounds in

burned sheep (31). In addition, inhibition of NO production resulted in decreased damage to the postburn intestinal mucosal barrier function (32). H₂S can inhibit NO production in LPS-stimulated macrophages through a mechanism that involves the action of hemeoxygenase-1 (33). Anuar *et al.* reported that the NO donor nitroflurbiprofen can downregulate the biosynthesis of proinflammatory H₂S in LPS-treated rats (34). Thus, the possibility of interaction between H₂S and NO in the inflammatory response to burn injury warrants further study. Moreover, our group demonstrated that substance P contributed to the exaggerated pulmonary inflammatory damage after burn injury via activation of neurokinin-1–receptor signaling (35). The acute lung injury and inflammatory response were significantly attenuated in mice deficient in the *preprotachykinin-A* gene, which encodes for the classical bioactive neuropeptide substance P, whereas the exogenous administration of substance P to

preprotachykinin-A gene–deficient mice restored the inflammatory response and lung injury (36). Our group also found that H$_2$S upregulated the generation of substance P in a CLP-induced sepsis model (37). Thus there is much evidence to indicate that multifactorial and numerous mediators other than H$_2$S are involved in the inflammatory response to burn injury. The interaction between H$_2$S and other mediators of the proinflammatory effect requires further study.

In summary, burn injury upregulates CSE gene expression and leads to enhanced H$_2$S biosynthesis, which promotes inflammation, whereas administration of an inhibitor of H$_2$S synthesis substantially attenuates the inflammation and multiple organ injury caused by burn injury. In future studies we will investigate the molecular mechanism linked to H$_2$S and burn injury and burn-injury–associated organ injury.

ACKNOWLEDGMENTS

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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