Explorations of the therapeutic potential of influencing metabolism during critical illness

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A short course of infusion of a hydrogen sulfide–donor attenuates endotoxemia induced organ injury via stimulation of anti–inflammatory pathways, with no additional protection from prolonged infusion
Abstract

Organ failure is associated with increased mortality and morbidity in patients with systemic inflammatory response syndrome. Previously, we showed that a short course of infusion of a hydrogen sulfide (H$_2$S) donor reduced metabolism with concurrent reduction of lung injury. Here, we hypothesize that prolonged H$_2$S infusion is more protective than a short course in endotoxemia with organ failure. Also, as H$_2$S has both pro– and anti–inflammatory effects, we explored the effect of H$_2$S on interleukin production.

Endotoxemia was induced by an intravenous bolus injection of LPS (7.5 mg/kg) in mechanically ventilated rats. H$_2$S (2 mg/kg/hr) or vehicle (saline) was infused and organ injury was determined after either 4 or 8 hours. A short course of H$_2$S infusion was associated with reduction of lung and kidney injury. Prolonged infusion did not enhance protection. Systemically, infusion of H$_2$S increased both the pro–inflammatory response during endotoxemia, as demonstrated by increased TNF–α levels, as well as the anti–inflammatory response, as demonstrated by increased IL–10 levels. In LPS–stimulated whole blood of healthy volunteers, co–incubation with H$_2$S had solely anti–inflammatory effects, resulting in decreased TNF–α levels and increased IL–10 levels. Co–incubation with a neutralizing IL–10 antibody partly abrogated the decrease in TNF–α levels. In conclusion, a short course of H$_2$S infusion reduced organ injury during endotoxemia, at least in part via upregulation of IL–10.
Introduction

Systemic inflammatory response syndrome (SIRS) is associated with development of organ failure (1), presumably caused by excessively produced pro-inflammatory cytokines triggered by a non-infectious insult (2). Organs most commonly failing are the lungs due to development acute respiratory distress syndrome (ARDS) (3) and the kidneys due to development of acute kidney injury (AKI) (1). The development of ARDS or AKI contributes to adverse outcome of patients with SIRS (4–6). Treatment of SIRS is supportive, aimed at optimizing supportive care with early goal directed therapy.

Hydrogen sulfide (H\textsubscript{2}S) is considered to be a toxic gas, responsible for many deadly accidents (7). In the last two decades however, H\textsubscript{2}S has been embraced as a third gasotransmitter, like nitric oxide and carbon monoxide with biological effects on the cardiovascular, nervous and inflammatory system (8). Controversies exist on the role of H\textsubscript{2}S on the inflammatory response in SIRS (9;10). Whereas endogenously produced H\textsubscript{2}S aggravated inflammation and organ failure (9;10), exogenous administration of H\textsubscript{2}S donors offered protection in various models of SIRS (11–13). Also, H\textsubscript{2}S administered exogenously protected against organ failure resulting from ischemia reperfusion (14–16).

Previously, we showed that infusion of H\textsubscript{2}S for four hours induced a so-called suspended animation–like state, a state of reversible reduction of metabolism associated with reduced lung inflammation in mechanically ventilated rats (17). Here, we hypothesize that prolonged H\textsubscript{2}S infusion is more protective than a short course of H\textsubscript{2}S infusion in endotoxemia. As controversies about the pro- and anti-inflammatory nature of H\textsubscript{2}S pursue, we also investigated the effect of H\textsubscript{2}S on inflammation, both in rats with endotoxemia as well as in human blood in vivo stimulated with LPS.

Material and Methods

After approval by the animal care and use committee of the Academic Medical Center in Amsterdam, the Netherlands, rats (Sprague Dawley ± 350 gram) were purchased from Harlan laboratories (The Hague, The Netherlands) and were given access to food and water ad libidum in our animal facility until the time of experiments. All animal procedures were carried out in compliance with Institutional Standards for Human Care and Use of Animal Laboratory Animals.
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\textit{H}_2\textit{S} donor

Preparations of a \textit{H}_2\textit{S} donor were made by diluting NaHS (Sigma Aldrich, Steinheim, Germany) in sterile saline (0.9\%) to a stock solution of 5 mg/ml and infused at 2 mg/kg/hour as described before (17).

\textit{LPS, experimental protocol and fluid strategy}

Rats were anesthetized with intraperitoneal injection of a mix (0.15 ml/100g body weight) containing 90 mg/kg ketamine, 0.5 mg/kg medetomidine and 0.05 mg/kg atropine. Next, a catheter was inserted in the tail vein, after which a bolus of LPS (from \textit{E. coli}, L4131, 7.5 mg/kg, Sigma Aldrich, Steinheim, Germany) to induce endotoxemia or volume matched saline in the controls was injected. A tracheotomy was performed, after which the metal cannula was connected to a ventilator (Servo 300, Siemens, Sweden). Hemodynamic parameters were monitored by a carotid artery catheter connected to a monitor. Urine production was monitored. Anesthesia was continuously infused (50 mg/kg ketamine at 0.5 ml/100g/hr), as well as bicarbonate (0.84\% at 1 ml/100g/hr) and H\textsubscript{2}S. Controls received vehicle (saline 0.9\%). Fluid infusion was increased to 1 ml/hr to treat shock in the LPS groups. Norepinephrine (1.5 \textmu g/kg/min) was started when mean arterial pressure dropped below 65 mmHg and stopped when mean arterial pressure stabilized. In the animals infused with NaHS, heating pad was shut off after baseline measurements.

\textit{Mechanical ventilation and experimental groups}

Tidal volumes were measured using a pneumotachometer (HSE, Harvard apparatus, Manheim, Germany) specific for rats. The pneumotachometer was calibrated using a 1 mL syringe according to the manufacturer’s instruction. The animals were mechanically ventilated with 8 ml/kg, with positive end–expiratory pressure at 5 cm H\textsubscript{2}O and respiratory rates at 50 breaths/min. Respiratory mechanics were recorded using respiration software (HSE–BDAS basic data acquisition, Harvard apparatus, Manheim, Germany). The fraction of inspired oxygen was set at 50\% and inspiration to expiration ratio at 1:2. Recruitment maneuvers were applied every 60 minutes by increasing the tidal volume to 16 ml/kg during 5 breaths. Peak inspiratory pressure (PIP) was noted hourly. Exhaled CO\textsubscript{2} was measured by a side stream analyzer (CWE Inc., Ardmore, PA, USA). After baseline measurements, the following experimental groups were formed: animals challenged with saline (controls (C), n=4) or LPS and subsequently infused with placebo (saline) or H\textsubscript{2}S during 4 or 8 hours (n=8 per group). Immediately after discontinuation of H\textsubscript{2}S infusion (after 4 or 8 hours), animals were sacrificed and organs harvested.
**Exsanguination and assays**

The rats were bled and the lungs were removed en block. Bronchoalveolar lavage fluid (BALF) was obtained by flushing the left lung (3 x 2.5 ml saline) after ligation of the right lung. Cell counts were determined using a hemocytometer (Z2 Coulter Particle Counter, Beckman Coulter; Florida, USA) and cell differentiation were done on Giemsa–stained cytopsins in BALF. BALF and plasma were centrifuged at 260 x g or 850 x g respectively during 10 minutes at 4°C and supernatant stored at –80°C. The middle right lobe, left kidney and liver lobe were homogenized in 4 volumes of saline and diluted (1:1) in lysis buffer (150mM NaCl, 15mM Tris, 1mM MgCl₂ H₂O, 1mM CaCl₂, 1% Triton x–100, 100 µg/mL Pepstatin A, leupeptin and aprotinin, pH 7.4) and incubated at 4°C for 30 min. Cell free supernatants were obtained by centrifugation (1500 g, 4°C for 10 minute) and stored at –80°C.

Protein levels were measured according to instructions from the manufacturer (Bradford, Oz Biosciences, Marseille, France) in BALF supernatant and urine, as were cytokines and chemokine in BALF supernatant, plasma and organ tissue: interleukin (IL)–6, IL–1β, tumor necrosis factor (TNF)–α, IL–10 and the chemo–attractant CINC3 (R&D Systems; Abingdon, United Kingdom).

Plasma levels of aspartate amino–transaminase (AST) were measured using standard enzymatic methods at 37°C (Roche Diagnostics, the Netherlands), and creatinine by enzymatic PAP (Roche Diagnostics, the Netherlands). The right lung top and part of the liver were fixed in 10% formaldehyde and embedded in paraffin, after which 4–µm sections were cut and stained with hematoxylin–eosin. The slides were analyzed by a pathologist who was blinded for group identity. Edema, hemorrhage, infiltration, wall thickness and hyperinflation were scored on a scale of 0 – 4: 0 for normal lungs, 1 for <25% lung involvement, 2 for 25 – 50% involvement, 3 for 50 – 75% involvement and 4 for >75% lung involvement (17). Liver necrosis, parenchymal inflammation and portal inflammation were scored on a scale 0 – 3 as described (18). Total histopathology score is the sum score of all parameters in the lung or liver respectively. The remaining right lobes were weighted to determine wet to dry weight, as was the right kidney.

**Whole blood stimulation**

To study the role of IL–10 in H₂S–induced protection, blood was drawn from 6 healthy volunteers, diluted 1:1 in RPMI, stimulated with LPS (10 µg/mL, E. Coli, Sigma Aldrich, Germany) or saline and incubated with 40 or 80 mM H₂S at 37°C and 5% CO₂. In a separate set of experiments, IL–10 was neutralized with anti–IL10 neutralizing antibody (2 µg/mL, R&D Systems; Abingdon, United Kingdom) in co–incubation with 40mM H₂S. After 2, 4 and 8 hours of incubation, blood was centrifuged at 600 g for 10 minutes, at 4°C. Supernatant was stored at −20° until further analysis. When cytokines where above detection limits, samples including the samples below detection limit were further diluted.
Statistical analysis
Data are expressed as mean with SEM in the tables and in the figures. Intergroup differences were analyzed by analysis of variance (ANOVA) and Bonferroni’s post–hoc test, or by a Kruskal Wallis test with Mann–Whitney U test depending on data distribution. The effect of \( \text{H}_2\text{S} \) on cytokine levels were compared to the vehicle treated stimulated blood within the group by ANOVA with Bonferroni’s post hock test. A \( p \) value of < 0.05 was considered significant. Statistical analyses were done using Prism (Graphpad Prism 5, CA, USA).

Results
LPS caused organ injury
Injection of LPS caused a drop in arterial pressure within two hours, requiring norepinephrine infusion to maintain mean arterial pressure above 65 mmHg for a duration of 30 minutes in all experimental groups. There was no difference in cumulative norepinephrine and fluid infusion between the experimental groups. Heart rate remained stable (not shown). Endotoxemia was associated with lung injury, with an increase in pulmonary edema and increased BALF protein levels after 4 hours of LPS injection compared to saline controls, together with an increase in PIP (figure 1). Furthermore, BALF levels of IL–1\( \beta \), IL–6 and chemokine CINC3 were elevated compared to saline controls (figure 2), associated with increased lung injury score (histology sum score 4.5 ± 1 vs. 0 ± 0 in controls, \( p < 0.05 \), figure 3). LPS also resulted in kidney injury, characterized by increased plasma creatinine levels as well as increased kidney edema and IL–1\( \beta \) levels, but not TNF–\( \alpha \) levels in kidney homogenates (\( p < 0.05 \), figure 4). Plasma levels of AST were increased, indicating liver cell damage, with a concomitant increase in levels of IL–1\( \beta \), IL–6 and TNF–\( \alpha \) in liver homogenates (\( p < 0.05 \), figure 5) and liver histology score (histology sum score 4.6 ± 0.3 vs. 0.8 ± 0.3 in controls, \( p < 0.05 \)).

Organ injury persisted after 8 hours compared to saline controls, with high plasma and BALF cytokine levels, lung injury score (histology score 3.3 ± 0.7 vs. 0.5 ± 0.3 in control, \( p < 0.05 \), figure 3) and markers of kidney and liver injury (liver histology score 4.8 ± 0.4 vs. 0.5 ± 0.5 in controls, \( p < 0.05 \)). Systemically, LPS caused a pro–inflammatory response, with increased levels of IL–1\( \beta \), IL–6 and TNF–\( \alpha \) compared to saline controls (\( p < 0.05 \), figure 6). Eight hours after LPS injection, the level of anti–inflammatory cytokine IL–10 was decreased in organ homogenates.
**H₂S induced a suspended animation like state in endotoxemic rats**

In line with our previous report (17), infusion of H₂S induced physiologic changes consistent with a hypo metabolic state after 4 hours, including a reduction in body temperature (figure 7), exhaled CO₂ (figure 1) and heart rate (from 326 ± 16 to 196 ± 11, all p<0.05). Parameters did not further decrease during prolonged infusion of H₂S. Mean arterial pressure was not affected.

**Table:** Arterial blood gas analysis during short and prolonged course of hydrogen sulfide (H₂S) or vehicle (saline) infusion in rats with endotoxemia.

<table>
<thead>
<tr>
<th>Time (hour)</th>
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<th>8 hours</th>
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<tr>
<td></td>
<td>control</td>
<td>endotoxemia</td>
</tr>
<tr>
<td>pH</td>
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</tr>
<tr>
<td></td>
<td>end</td>
<td>7.38±0.02</td>
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<tr>
<td>PaCO₂ (kPa)</td>
<td>baseline</td>
<td>4.6±0.3</td>
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<tr>
<td></td>
<td>end</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>PaO₂ (kPa)</td>
<td>baseline</td>
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<td></td>
<td>end</td>
<td>30±2</td>
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<td>HCO₃⁻ (mmol/L)</td>
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<td></td>
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Data are means ± SEM. N=8 per group. * Vehicle vs. H₂S p < 0.05.
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Figure 1: Lung mechanics (A, C and E) and lung injury markers (B, D and F) in animals with endotoxemia (LPS) during a short (T=4 hours) and prolonged (T=8 hours) course of hydrogen sulfide (H₂S) or vehicle (saline) infusion. Peak inspiratory pressure (PIP) and end tidal (et) CO₂ were calculated as relative change (%) compared to baseline. Straight line represents vehicle, gray straight line endotoxemia and dotted line endotoxemia with H₂S. Data shown as mean with SEM (n=8 per group), * p< 0.05. MVV: minute volume ventilation, BALF: bronchoalveolar lavage fluid.

The effect of H₂S on organ failure in endotoxemic rats
A short course of H₂S infusion reduced lung edema, protein leak and cell influx compared to saline controls (figure 1 p < 0.05), together with a reduction in BALF levels of IL–6 and CINC3 (figure 2, p < 0.05 for all). Lung histopathology score was not reduced in these animals (4.3 ± 0.4 sum score, p = ns, figure 3). Improvement in inflammatory markers was accompanied by a reduction in peak inspiratory pressures needed to maintain a tidal volume of 8 ml/kg. A prolonged course of H₂S infusion reduced BALF protein levels and lung histology score (1.4 ± 0.4 sum score, p < 0.05, figure 3) but failed to reduce cell influx, or BALF levels of pro-inflammatory cytokines, whereas pulmonary edema was even aggravated by prolonged H₂S infusion.
A short course of H$_2$S infusion reduces organ injury in endotoxemia

A short course of H$_2$S infusion reduced plasma creatinine levels and IL–1β levels in kidney homogenate compared to saline controls. A prolonged course of H$_2$S infusion also resulted in reduction of creatinine levels, with a concomitant reduction in kidney edema and urine protein leakage (p < 0.05, figure 4). Surprisingly, kidney IL–6 levels increased after H$_2$S. H$_2$S did not reduce plasma levels of AST and liver histology score (4.4 ± 0.5 during short and 4.1 ± 0.5 sum score during prolonged course of H$_2$S infusion) and increased liver TNF–α levels (figure 5). A prolonged course of H$_2$S treatment significantly reduced IL–6 levels in liver homogenate (figure 5).

The effect of H$_2$S on systemic host response in endotoxemia

The systemic pro–inflammatory response caused by LPS injection was significantly augmented after infusion of H$_2$S, exemplified by a marked increase in plasma levels of IL–1β and TNF–α (p < 0.05, figure 6). IL–6 remained at the same level compared to saline controls. Besides augmenting the pro–inflammatory response, H$_2$S also increased an anti–inflammatory response, demonstrated by a marked increase in plasma IL–10 levels. Given this differential effect of H$_2$S on the systemic inflammatory response, additional in vitro experiments were performed.
Figure 3: Representative microphotographs of lung tissue slides of animals with endotoxemia (C – F) or controls (A and B) during a short (T=4 hours) (A, C, E) and prolonged (T=8 hours) (B, D, F) course of hydrogen sulfide (H₂S) (E and F) or vehicle (saline) (C and D) infusion. Magnification 100x.
A short course of H$_2$S infusion reduces organ injury in endotoxemia.

**Figure 4:** Kidney injury markers (A, C and E) and interleukin (IL) levels in kidney homogenates (hmg) (B, D, F and G) in animals with endotoxemia (LPS) during a short (T=4 hours) and prolonged (T=8 hours) course of hydrogen sulfide (H$_2$S) or vehicle (saline) infusion. Data shown as mean with SEM, (n=8 per group), * p<0.05.
Figure 5: Aspartate transaminase (AST) (A) and cytokine levels in liver homogenates (hmg) (B–E) of animals with endotoxemia (LPS) during a short (T=4 hours) and prolonged (T=8 hours) course of hydrogen sulfide (H₂S) or vehicle (saline) infusion. Data shown as mean with SEM, (n=8 per group), * p< 0.05.
A short course of H$_2$S infusion reduces organ injury in endotoxemia

The effect of H$_2$S on cytokine production in whole blood of healthy human volunteers stimulated with LPS. The role of IL–10.

We further explored the effects of H$_2$S on cell response in LPS–stimulated whole blood of healthy volunteers. H$_2$S decreased the pro–inflammatory response in vitro, as the gradual rise in TNF–α levels after stimulation with LPS was inhibited by co–incubation with H$_2$S compared to saline controls (p<0.05, figure 8). There was no apparent dose–response relationship. Also, co–incubation of LPS–stimulated whole blood with H$_2$S significantly increased IL–10 levels (p<0.05, figure 8). Given the increase in IL–10 levels after H$_2$S treatment, we explored the role of IL–10 in the protective effect of H$_2$S found in the SIRS model. Co–incubation with a neutralizing anti–IL10 antibody partly abrogated the H$_2$S–induced decrease in TNF–α levels (figure 9).

Figure 6: Plasma cytokine levels (A–D) in animals with endotoxemia (LPS) during a short (T=4 hours) and prolonged (T=8 hours) course of hydrogen sulfide (H$_2$S) or vehicle (saline) infusion. Data shown as mean with SEM, (n=8 per group), * p < 0.05.
Figure 7: Body temperature in animals with endotoxemia (LPS) during a short (T=4 hours, A) and prolonged (T=8 hours, B) course of hydrogen sulfide (H₂S) or vehicle (saline) infusion. Straight line represents vehicle, gray straight line endotoxemia and dotted line endotoxemia with H₂S (n=8 per group). * LPS vs. LPS with H₂S p < 0.05.

Figure 8: Levels of TNF-α (A) and IL-10 (B) in whole blood stimulation with LPS or vehicle and co incubation with 40 and 80 mM hydrogen sulfide. The experiment was performed in 3 independent experiment with 2 donors each experiment. Data shown as mean with SEM, (n=8 per group), * p < 0.05.
A short course of H₂S infusion reduces organ injury in endotoxemia

Figure 9: Levels of TNF–α in whole blood stimulation with LPS or vehicle (V) and co incubation with 40mM hydrogen sulfide (H₂S) and a neutralizing IL–10 antibody. The experiment was performed in 3 independent experiment with 2 donors each experiment. Data shown as mean with SEM, (n=8 per group), * p< 0.05.

Discussion

In endotoxemia, a short course of infusion of H₂S protected against the development of lung and kidney injury. Prolonged H₂S infusion did not further enhance protection. The protective effect of H₂S during endotoxemia was associated with increased IL–10 levels systemically.

In whole blood stimulations, co–incubation with H₂S yielded an anti–inflammatory effect, which was abolished when IL–10 was neutralized. Of note, H₂S infusion resulted in a systemic pro–inflammatory response.

The reduction in organ injury after H₂S infusion has been shown before in various models of SIRS (11–13;17). Lung injury was clearly reduced, exemplified by reduced lung edema and protein leakage, as found before in a model of ventilator–induced lung injury (17). Importantly, peak pressures needed to generate the predefined tidal volume in endotoxemia could be reduced after H₂S infusion, suggesting that H₂S prevented lung injury. H₂S clearly reduced kidney injury. Presumably, the vasodilatory effect of the compound may have contributed to the observed protection (19;20). Of note, IL–6 levels in kidney homogenates were increased after H₂S treatment. This inconsistent response between organs may indicate a tissue dependent response to H₂S (21). The effect of H₂S on liver damage was less clear. Levels of IL–6 were reduced but TNF–α levels were clearly increased. As IL–10 levels were also increased in the liver, this suggests both a pro–inflammatory as well as an anti–inflammatory response. As the TNF–α response was also present systemically, differences between kidney and liver may be related to perfusion rate, as renal blood flow decreases during shock,
rendering the kidney the first organ to fail in the multiple organ failure syndrome. Prolonged \( H_2S \) infusion offered no additional protection in endotoxemia compared to a short course. Presumably, accumulating \( H_2S \) may have caused toxic effects, as a single dose of 10 mg/kg can already aggravate organ injury (22). In line, pre-treatment with \( H_2S \) was not associated with organ protection, while low concentration of \( H_2S \) infusion offered protection in piglets with hemorrhagic shock (23). Although protection against kidney injury was more apparent after prolonged \( H_2S \) infusion, lung injury worsened with prolonged infusion, probably due to increased need for fluid administration to maintain adequate blood pressure during prolonged mechanical ventilation. Our prolonged ventilation model has limitations. After 8 hours, ventilated control group showed high levels of pulmonary protein leakage and cell influx. Also, IL–1 levels in the kidney and plasma AST of controls were similar to LPS infected animals. These findings probably reflect detrimental effects of mechanical ventilation on the lung (24) and distant organ injury (25). Thereby, to determine whether prolonged \( H_2S \) or mechanical ventilation contributed most to outcome is not clear. However, we believe that this model is a reflection of the clinical situation of patients with SIRS, who can be ventilated for prolonged periods of time. Taken together, we conclude that prolonged infusion of \( H_2S \) is not recommended in SIRS.

Surprisingly, \( H_2S \)-mediated protection at the organ level was associated with an aggravation of the systemic pro-inflammatory response in endotoxemia. \( H_2S \) does not have a specific target receptor and a wide range of biological effects have been noted, including both pro- and anti-inflammatory activities (26). Inflammatory effects of \( H_2S \) may occur at the leukocyte-endothelium interface, as inhibition of endogenous \( H_2S \) synthesis reduced leukocyte rolling and adherence to blood vessels (27). As LPS causes mobilization of immune cells from the bone marrow (28) and \( H_2S \) prevents adhesion and transmigration of immune cells (27), it may be hypothesized that increased amounts of circulating immune cells contribute to the pro-inflammatory response. Thereby, LPS and \( H_2S \) may act synergistically. A direct toxic effect of \( H_2S \) on vascular endothelium can not be excluded. We found previously that infusion of high doses of \( H_2S \) did not generate an inflammatory response, but did result in statistically non-significant increases in pulmonary edema and cytokine levels (17). However, in isolated endothelial cells stimulated with TNF–α, \( H_2S \) attenuated the inflammatory response (29). In line with this, we found that \( H_2S \) reduced the production of TNF–α in whole blood stimulated with LPS. Contrasting results have been attributed to differences in dose of \( H_2S \). Thereby, an explanation for the systemic inflammatory response may be the high dose used in our study. In line with this, prolonged \( H_2S \) infusion was associated with increased systemic inflammation and can not be recommended.

In SIRS, a compensatory anti-inflammatory response is simultaneously generated to counterbalance the pro-inflammatory response (30), which is mainly mediated by production of IL–10 (31). Interestingly, in our study, the protective effect of \( H_2S \) was associated with
enhanced systemic IL–10 levels, as well as elevated IL–10 levels in the liver. This accords with findings in a lung injury model, in which exogenous H$_2$S reduced injury with a concomitant increase in IL–10 levels (12). Given that anti–IL10 abrogated the H$_2$S–induced decrease in TNF–α production in vitro, we suggest that IL–10 is an important mediator in H$_2$S–mediated organ protection during endotoxemia. A word of caution is that whole blood stimulations are limited since cytokine release does not include all sources such as endothelium and locally activated immune cells.

**Conclusions**

During endotoxemia, infusion of H$_2$S was associated with a reduction in lung and kidney injury, possibly via upregulation of IL–10. Given that lungs and kidneys are the first failing organs in SIRS and that the number and severity of organ failure contributes to mortality in SIRS [4–6], the possible therapeutic effect of a short course of H$_2$S infusion in SIRS should be further explored. The aggravated systemic inflammatory response however, limits its clinical applicability.
References


A short course of H₂S infusion reduces organ injury in endotoxemia


