

Review

Bench-to-bedside review: Hydrogen sulfide – the third gaseous transmitter: applications for critical careFlorian Wagner¹, Pierre Asfar^{2,3}, Enrico Calzia¹, Peter Radermacher¹ and Csaba Szabó^{4,5}¹Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Klinik für Anästhesiologie, Universitätsklinikum, Parkstrasse 11, 89073 Ulm, Germany²Laboratoire HIFIH, UPRES EA 3859, IFR 132, Université d'Angers, 49933 Angers, France³Département de Réanimation Médicale et de Médecine Hyperbare, Centre Hospitalo-Universitaire, 49933 Angers, France⁴Ikaria, Seattle, WA 98102, USA⁵Department of Anesthesiology, The University of Texas Medical Branch, 610 Texas Avenue, Galveston, TX 77555-0833, USACorresponding author: Peter Radermacher, peter.radermacher@uni-ulm.de

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Critical Care 2009, **13**:213 (doi:10.1186/cc7700)**Abstract**

Hydrogen sulfide (H₂S), a gas with the characteristic odor of rotten eggs, is known for its toxicity and as an environmental hazard, inhibition of mitochondrial respiration resulting from blockade of cytochrome c oxidase being the main toxic mechanism. Recently, however, H₂S has been recognized as a signaling molecule of the cardiovascular, inflammatory and nervous systems, and therefore, alongside nitric oxide and carbon monoxide, is referred to as the third endogenous gaseous transmitter. Inhalation of gaseous H₂S as well as administration of inhibitors of its endogenous production and compounds that donate H₂S have been studied in various models of shock. Based on the concept that multiorgan failure secondary to shock, inflammation and sepsis may represent an adaptive hypometabolic response to preserve ATP homeostasis, particular interest has focused on the induction of a hibernation-like suspended animation with H₂S. It must be underscored that currently only a limited number of data are available from clinically relevant large animal models. Moreover, several crucial issues warrant further investigation before the clinical application of this concept. First, the impact of hypothermia for any H₂S-related organ protection remains a matter of debate. Second, similar to the friend and foe character of nitric oxide, no definitive conclusions can be made as to whether H₂S exerts proinflammatory or anti-inflammatory properties. Finally, in addition to the question of dosing and timing (for example, bolus administration versus continuous intravenous infusion), the preferred route of H₂S administration remains to be settled – that is, inhaling gaseous H₂S versus intravenous administration of injectable H₂S preparations or H₂S donors. To date, therefore, while H₂S-induced suspended animation in humans may still be referred to as science fiction, there is ample promising preclinical data that this approach is a fascinating new therapeutic perspective for the management of shock states that merits further investigation.

Introduction

Hydrogen sulfide (H₂S), a colorless, flammable and water-soluble gas with the characteristic odor of rotten eggs, has been known for decades because of its toxicity and as an environmental hazard [1,2]. Inhibition of mitochondrial respiration – more potent than that of cyanide [3] – resulting from blockade of cytochrome c oxidase is the main mechanism of H₂S toxicity [4,5]. During recent years, however, H₂S has been recognized as an important signaling molecule of the cardiovascular system, the inflammatory system and the nervous system. Alongside nitric oxide (NO) and carbon monoxide, therefore, H₂S is now known as the third endogenous gaseotransmitter [1,6].

Since H₂S is a small ubiquitous gaseous diffusible molecule, its putative interest for intensive care research is obvious. Consequently, inhibitors of its endogenous production as well as compounds that donate H₂S have been studied in various models of shock resulting from hemorrhage [7-9], ischemia/reperfusion [10-18], endotoxemia [19-21], bacterial sepsis [22-25] and nonmicrobial inflammation [26-29] – which, however, yielded rather controversial data with respect to the proinflammatory or anti-inflammatory properties of H₂S. The present article reviews the current literature on the therapeutic potential of H₂S, with a special focus on clinically relevant studies in – if available – large animal models.

Biological chemistry

In mammals, H₂S is synthesized from the sulfur-containing amino acid L-cysteine by either cystathionine-β-synthase or

H₂S = hydrogen sulfide; IFN = interferon; IL = interleukin; LPS = lipopolysaccharide; Na₂S = sodium disulfide; NaHS = sodium hydrogen sulfide; NF = nuclear factor; NO = nitric oxide; PAG = D,L-propargylglycine; TNF = tumor necrosis factor; TUNEL = terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling.

Table 1

Physicochemistry and biology of hydrogen sulfide

Environmental toxicology	Toxic gas originating from sewers, swamps, and putrefaction
Endogenous sources	Synthesized in various tissues from L-cysteine by cystathionine- β -synthase or cystathionine- γ -lyase
Pharmacological inhibitors	D,L-propargylglycine and β -cyanoalanine (limited selectivity, unspecific side-effects)
Elimination kinetics	Half-life within minutes; metabolites comprise thiosulfate, sulfite, and sulfate
Receptors and targets	Potassium-dependent ATP channels (others?); cytochrome c oxidase
Vascular effects	Vasodilatation or vasoconstriction (depending on local oxygen concentration)
Biological effects	Radical scavenging, upregulation of heme oxygenase-1. Toxicology: pulmonary irritant, mitochondrial poison
Inflammatory effects	Dose-dependently proinflammatory or anti-inflammatory and anti-apoptotic effects

Table adapted from [1].

cystathionine- γ -lyase, both using pyridoxal 5'-phosphate (vitamin B₆) as a cofactor [30-32]. This synthesis results in low micromolar H₂S levels in the extracellular space, which can be rapidly consumed and degraded by various tissues. Similarly to NO and carbon monoxide, H₂S is a lipophilic compound that easily permeates cell membranes without using specific transporters. Via direct inhibition, NO as well as carbon monoxide are involved in the regulation of cystathionine- β -synthase, but not cystathionine- γ -lyase, which can be activated by lipopolysaccharide (LPS) [1,6].

There are three known pathways of H₂S degradation: mitochondrial oxidation to thiosulfate, which is further converted to sulfite and sulfate; cytosolic methylation to dimethylsulfide; and sulfhemoglobin formation after binding to hemoglobin [6]. Similar to NO and carbon monoxide, H₂S can also bind to hemoglobin – which was therefore termed the common sink for the three gaseous transmitters [33]. Consequently, saturation with one of these gases might lead to enhanced plasma concentrations and, subsequently, to biological effects of the other gases [1]. Table 1 summarizes the physicochemistry of H₂S in mammalian tissues.

Mechanisms of H₂S

H₂S exerts its effects in biological systems through a variety of interrelated mechanisms (for a review see [1]). Our current knowledge of the biology of H₂S predominantly stems from *in vitro* studies in various cell and isolated organ systems, either using cystathionine- γ -lyase inhibitors such as D,L-propargylglycine (PAG) and β -cyanoalanine, or administration of H₂S gas or H₂S donors such as sodium disulfide (Na₂S) and sodium hydrogen sulfide (NaHS). While high (high micromolar to millimolar) levels are invariably accompanied with cytotoxic effects [34] – which result from free radical generation, glutathione depletion, intracellular iron release and proapoptotic action through both the death receptor and mitochondrial pathways [35] – lower (low micromolar) levels have been shown to exert either cytoprotective (antinecrotic or antiapoptotic) effects [10-13,36] or proapoptotic proper-

ties [37-39], depending on the cell type and on the experimental conditions.

Cytochrome c oxidase, a component of the oxidative phosphorylation machinery within the mitochondrion, is one intracellular target of H₂S [4,5]. Both the toxic effects of H₂S as well as the induction of a so-called “suspended animation” [40,41] are referred to in this inhibition of mitochondrial respiration [42,43], and thus may represent a possible mechanism for the regulation of cellular oxygen consumption [44].

Activation of potassium-dependent ATP channels is another major mechanism of H₂S, which in turn causes vasodilation, preconditioning against ischemia/reperfusion injury and myocardial protection [45]. Various findings support this concept [1,6,46]: potassium-dependent ATP channel blockers (sulfonylurea derivatives – for example, glibenclamide) attenuated the H₂S-induced vasodilation both *in vivo* and *in vitro* [47,48], and stimulation of potassium-dependent ATP channels was demonstrated in the myocardium, pancreatic β cells, neurons and the carotid sinus [6]. Moreover, glibenclamide reversed the otherwise marked Na₂S-related increase of the hepatic arterial buffer response capacity that counteracts reduction of portal venous flow, whereas PAG decreased this compensatory mechanism [49].

An endothelium-dependent effect seems to contribute to these vasodilatory properties: in human endothelial cells, H₂S caused direct inhibition of the angiotensin-converting enzyme [50], and, finally, H₂S can enhance the vasorelaxation induced by NO [51,52]. The interaction between H₂S and NO with respect to vascular actions is, however, fairly complex: low H₂S concentrations may cause vasoconstriction as a result of an attenuated vasorelaxant effect of NO due to scavenging of endothelial NO and formation of an inactive nitrosothiol [52-54]. The local oxygen concentration apparently assumes importance for the vasomotor properties of H₂S as well [55]: while H₂S had vasodilator properties at 40 M oxygen concentration (that is, an oxygen partial

pressure of approximately 30 mmHg), it exerted vasoconstrictor effects at a 200 M oxygen concentration (that is, an oxygen partial pressure of approximately 150 mmHg) [56]. Finally, the H₂S-related inhibition of oxidative phosphorylation also contributes to the vasodilatation [57].

Owing to its SH group that allows reduction of disulfide bonds and radical scavenging, H₂S also exerts biological effects as an antioxidant [9], in particular as an endogenous peroxynitrite scavenger [58], which is consistent with its cytoprotective effects in various cell-based experiments [59,60]. In this context the effect of H₂S on intracellular signal pathways assumes particular importance: in LPS-stimulated macrophages, pretreatment with physically dissolved gaseous H₂S or the H₂S-donor NaHS was affiliated with diminished activation of the nuclear transcription factor NF- κ B and inhibition of the inducible isoform of the NO synthase. This effect coincided with increased expression of heme oxygenase-1, and co-incubation with carbon monoxide mimicked the cytoprotection exerted by H₂S [61].

Conflicting data are available on the effects of H₂S on other intracellular signal transduction pathways; for example, the mitogen-activated protein kinase pathway and the phosphatidylinositol-3-kinase/Akt pathway [20,61-65]. Depending on the cell lines used, both inhibitory [20] and activating [36,61,64] effects on p38 mitogen-activated protein kinase were reported, whereas H₂S seems not to affect the stress-activated protein kinase c-Jun N-terminal kinase [61,65]. In contrast, activation of the extracellular signal-regulated kinase 1/2 pathway has been implicated in the H₂S-related ischemic preconditioning [48], both its proinflammatory [63,65] and anti-inflammatory [20,61] effects, as well as in the induction of apoptosis [62]. While the influence of H₂S on extracellular signal-regulated kinase seems to be rather comprehensible [25], studies exploring the effect on downstream pathways result in conflictive statements.

Jeong and colleagues reported that H₂S enhances NO production and inducible NO synthase expression by potentiating IL-1 β -induced NF- κ B in vascular smooth muscle cells [63], which is consistent with the H₂S-induced NF- κ B activation and subsequent proinflammatory cytokine production in IFN γ -primed monocytes [65]. Nevertheless, any H₂S effect on NF- κ B and its transcription-regulated mediators (for example, inducible NO synthase, cytokines and apoptotic factors) may be cell-type dependent and stimulus dependent. In fact, in addition to the above-mentioned decreased NF- κ B activation and inducible NO synthase expression in LPS-stimulated macrophages [61], H₂S administration also attenuated inducible NO synthase expression, NO production, as well as TNF α secretion in microglia exposed to LPS [20].

In the context of these contradictory findings, the doses of the H₂S donors administered may assume particular importance. Even the physiologically relevant concentrations

[36,64] might have to be reconsidered due to overestimation of basal H₂S levels: murine plasma sulfide levels are reported between 10 and 34 M [21,22], and are increased up to 20 to 65 M after endotoxin injection [21] or cecal ligation and puncture [22]. A reduction of plasma sulfide concentration from 50 M to ~25 M, finally, was reported in patients with coronary heart disease [1], whereas plasma sulfide levels increased from 44 to 150 M in patients with sepsis [21]. It should be noted, however, that the distinct techniques used by various groups to determine sulfide levels may account for the marked variability in the baseline values reported. The various derivatization methods, which are inherent to the analytic procedures, are likely to liberate sulfide from its bound forms so that the exact amount of free and bioavailable sulfide may be lower than frequently reported [66]. In fact, Mitsuhashi and colleagues reported that the blood sulfite concentrations (that is, the product of mitochondrial sulfide oxidation) were 3.75 ± 0.88 M only in patients with pneumonia (versus 1.23 ± 0.48 M in healthy control individuals) [67]. Infusing 2.4 and 4.8 mg/kg/hour in anesthetized and mechanically ventilated pigs over 8 hours resulted in maximum blood sulfide levels of 2.0 and 3.5 M, respectively (baseline levels 0.5 to 1.2 M) in our experiments [16].

Metabolic effects of H₂S: induction of suspended animation

Suspended animation is a hibernation-like metabolic status characterized by a marked yet reversible reduction of energy expenditure, which allows nonhibernating species to sustain environmental stress, such as extreme changes in temperature or oxygen deprivation [41,68].

In landmark work, the Roth's group provided evidence that inhaled H₂S can induce such a suspended animation [40,41]: in awake mice, breathing 80 ppm H₂S caused a dose-dependent reduction of both the respiratory rate and the heart rate as well as of oxygen uptake and carbon dioxide production, which was ultimately associated with a drop in body core temperature to levels ~2°C above ambient temperature [40]. All these effects were completely reversible after H₂S washout, and thereafter animals presented with a totally normal behavior. A follow-up study confirmed these observations, and the authors demonstrated using telemetry and echocardiography that the bradycardia-related fall in cardiac output coincided with an unchanged stroke volume and blood pressure. These physiologic effects of inhaled H₂S were present regardless of the body core temperature investigated (27°C and 35°C) [69].

It is noteworthy that anesthesia may at least partially blunt the myocardial effect of inhaled H₂S. In mechanically ventilated mice instrumented with left ventricular pressure volume conductance catheters and assigned to 100 ppm inhaled H₂S, we found that hypothermia alone (27°C) but not normothermic H₂S inhalation (38°C) decreased the cardiac output due to a fall in heart rate, whereas both the stroke volume as

Table 2**Cardiac effects of inhaled H₂S in anesthetized and mechanically ventilated mice during normothermia and hypothermia**

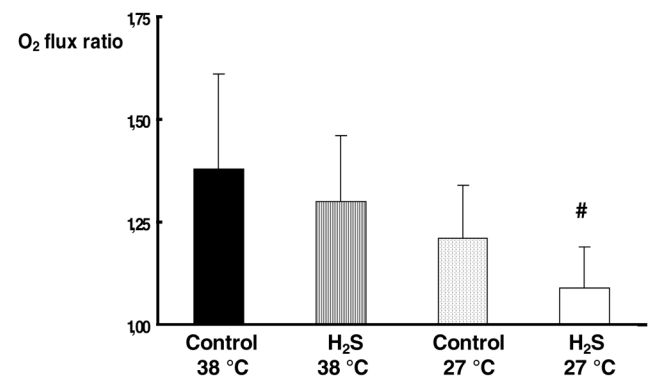
	Control, 38°C	H ₂ S, 38°C	Control, 27°C	H ₂ S, 27°C
Heart rate (beats/min)	350 (289 to 437)	324 (274 to 387)	112 (96 to 305)*	116 (96 to 327)*
Mean arterial pressure (mmHg)	62 (57 to 72)	60 (57 to 65)	45 (37 to 63)*	48 (41 to 59)*
Stroke volume (l)	33 (19 to 62)	29 (23 to 53)	27 (21 to 39)	25 (20 to 32)
Ejection fraction (%)	45 (38 to 55)	40 (35 to 48)	50 (37 to 57)	47 (35 to 54)
End-diastolic pressure (mmHg)	16 (12 to 18)	15 (12 to 16)	15 (11 to 22)	14 (11 to 18)

Cardiac effects of inhaled hydrogen sulfide (H₂S) (100 ppm over 5 hours) in anesthetized and mechanically ventilated mice instrumented with left ventricular pressure volume conductance catheters during normothermia (38°C) and hypothermia (27°C) [62]. Data presented as median (range), *n* = 8 in each group. **P* < 0.05 versus control, 38°C.

well as the parameters of systolic and diastolic function remained unaffected (Table 2) [70]. Interestingly, inhaled H₂S in combination with hypothermia, however, was concomitant with the least stimulation of oxygen flux induced by addition of cytochrome c during state 3 respiration with combined complex I and complex II substrates (Figure 1) [71]. Since stimulation by cytochrome c should not occur in intact mitochondria, this finding suggests better preservation of mitochondrial integrity under these conditions [72].

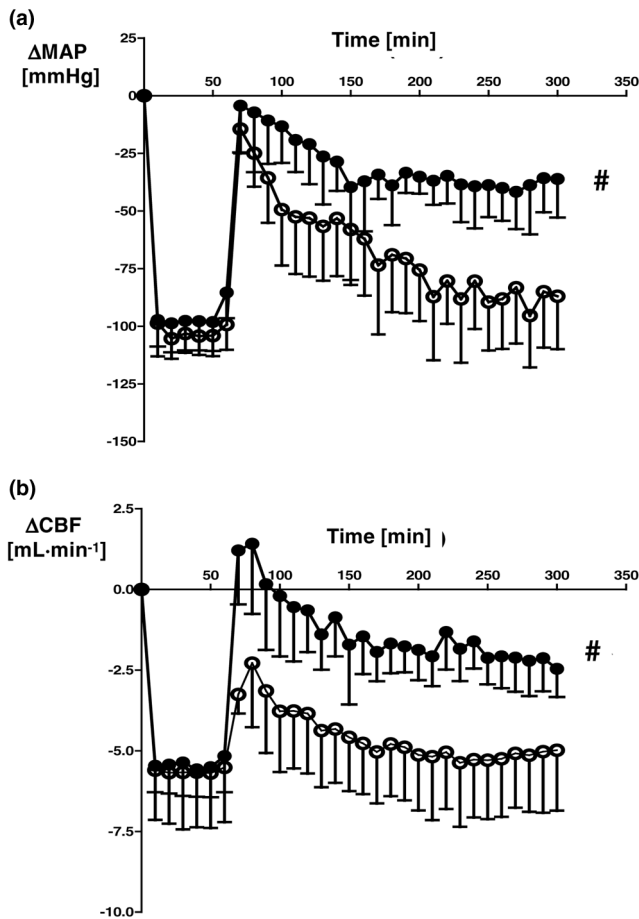
In good agreement with the concept that a controlled reduction in cellular energetic expenditure would allow maintenance of ATP homeostasis [41] and thus of improving outcome during shock states due to preserved mitochondrial function [73,74], the group of Roth and colleagues subsequently demonstrated that pretreatment with inhaled H₂S (150 ppm) for only 20 minutes markedly prolonged survival without any apparent detrimental effects for mice exposed to otherwise lethal hypoxia (5% oxygen) [75] and for rats undergoing lethal hemorrhage (60% of the calculated blood volume over 40 minutes) [8]. It is noteworthy that in the latter study the protective effect was comparable when using either inhaled H₂S or a single intravenous bolus of Na₂S [75]: parenteral sulfide administration has a number of practical advantages (ease of administration, no need for inhalation delivery systems, no risk of exposure to personnel, no issues related of the characteristic odor of H₂S gas) and, in particular, avoids the pulmonary irritant effects of inhaled H₂S, which can be apparent even at low inspiratory gaseous concentrations [76]. Finally, it is noteworthy that hypothermia is not a prerequisite of H₂S-related cytoprotection during hemorrhage: the H₂S donor NaHS improved hemodynamics, attenuated metabolic acidosis, and reduced oxidative and nitrosative stress in rats subjected to controlled hemorrhage at a mean blood pressure of 40 mmHg (Figure 2) [9].

The clinical relevance of murine models may be questioned because, due to their large surface area/mass ratio, rodents can rapidly drop their core temperature [77]. In fact, other authors failed to confirm the metabolic effect of inhaled H₂S

Figure 1

Cytochrome c-stimulated mitochondrial oxygen flux in livers from anesthetized and mechanically ventilated mice. Ratio of mitochondrial oxygen flux in homogenized livers from anesthetized and mechanically ventilated mice *after* addition in relation to *before* addition of cytochrome c. Since stimulation by cytochrome c should not occur in intact mitochondria, the smallest value (that is, a ratio close to 1.00) suggests preservation of mitochondrial integrity. Animals were subjected to inhaled hydrogen sulfide (H₂S) (100 ppm over 5 hours) or vehicle gas during normothermia (38°C) and hypothermia (27°C) [63]. Data presented as mean ± standard deviation, *n* = 8 in each group. #*P* < 0.05 versus control, 38°C.

in anesthetized and mechanically ventilated piglets (body weight ~6 kg) or in H₂S-sedated and spontaneously breathing sheep (body weight ~74 kg) exposed to up to 80 or 60 ppm H₂S, respectively [78,79]. These findings may be due to the dosing or timing of H₂S, and are in contrast to recent data from our own group: in anesthetized and mechanically ventilated swine (body weight ~45 kg) that underwent transient thoracic aortic balloon occlusion, infusing the intravenous H₂S donor Na₂S over 10 hours reduced the heart rate and cardiac output without affecting the stroke volume, thereby reducing oxygen uptake and carbon dioxide production and, ultimately, core temperature [16]. The metabolic effect of H₂S coincided with an attenua-

Figure 2

Hydrogen sulfide-related hemodynamic effects in rats subjected to hemorrhage and subsequent retransfusion. Time course of the difference in (a) mean blood pressure (Δ MAP) and (b) carotid blood flow (Δ CBF) in rats subjected to 60 minutes of hemorrhage (MAP 40 mmHg) and subsequent retransfusion of shed blood. Ten minutes prior to retransfusion, animals received vehicle ($n = 11$; open circles) or the hydrogen sulfide donor sodium hydrogen sulfide (bolus 0.2 mg/kg, $n = 11$; closed circles) [9]. Data presented as mean (standard deviation). # $P < 0.05$ versus controls.

tion of the early reperfusion-related hyperlactatemia – suggesting a reduced need for anaerobic ATP generation during the ischemia period – and an improved noradrenaline responsiveness, indicating both improved heart function and vasomotor response to catecholamine stimulation [16].

H₂S-induced cytoprotection during ischemia-reperfusion

Deliberate hypothermia is a cornerstone of the standard procedures to facilitate neurological recovery after cardiac arrest and to improve postoperative organ function after cardiac and transplant surgery. Consequently, several authors investigated the therapeutic potential of H₂S-induced suspended animation after ischemia-reperfusion injury – and

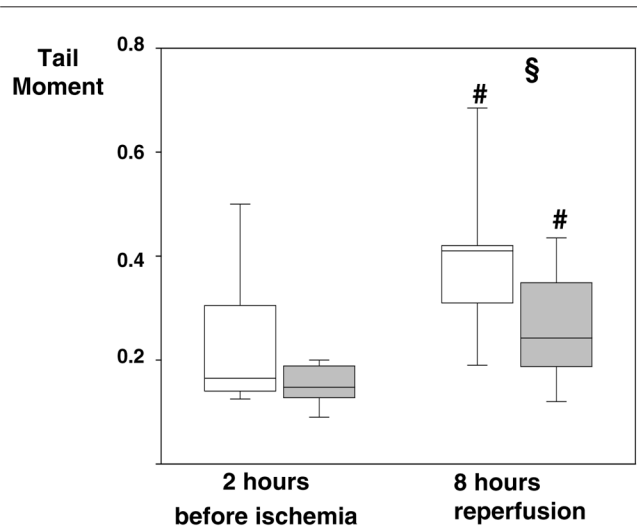
H₂S protected the lung [14], the liver [12], the kidney (Figure 3) [17,80], and, in particular, the heart [10,11,13,15, 18,62,81-83]. H₂S administered prior to reperfusion therefore limited the infarct size and preserved left ventricular function in mice [10] and in swine [11].

While these findings were obtained without induction of hypothermia, preserved mitochondrial function documented by an increased complex I and complex II efficiency assumed major importance for the H₂S-induced cytoprotection [10]. The important role of preserved mitochondrial integrity was further underscored by the fact that 5-hydroxydeconate, which is referred to as a mitochondrial potassium-dependent ATP-channel blocker, abolished the anti-apoptotic effects of H₂S [18]. Clearly, anti-inflammatory and anti-apoptotic effects also contributed to the improved postischemic myocardial function: treatment with H₂S was associated with reduced myocardial myeloperoxidase activity and an absence of the increase in the IL-1 β levels (that is, attenuated tissue inflammation [10,18]), as well as complete inhibition of thrombin-induced leukocyte rolling, a parameter for leukocyte-endothelium interaction [10]. Moreover, the ischemia-reperfusion-induced activation of p38 mitogen-activated protein kinase, of c-Jun N-terminal kinase and of NF- κ B was also attenuated by H₂S [18]. Finally, H₂S exerted anti-apoptotic effects as shown by reduced TUNEL staining [10,11] and by expression of cleaved caspase-9 [18], caspase-3 [10,11], poly-ADP-ribose-polymerase [11] and the cell death-inducing proto-oncogene c-fos [13].

Controversial role of H₂S in animal models of inflammation

Despite the promising data mentioned above, it is still a matter of debate whether H₂S is a metabolic mediator or a toxic gas [84] – particularly given the rather controversial findings on the immune function reported in various models of systemic inflammation. In fact, H₂S exerted both marked pro-inflammatory effects [19,21-25,27,85] and anti-inflammatory effects [9,10,18,20,28-30]. Studies using inhibitors of endogenous H₂S production such as PAG demonstrated pronounced proinflammatory effects of H₂S: PAG attenuated organ injury, blunted the increase of the proinflammatory cytokine and chemokine levels as well as the myeloperoxidase activity in the lung and liver, and abolished leukocyte activation and trafficking in LPS-induced endotoxemia [19,21] or cecal ligation and puncture-induced sepsis [22-25,86]. In good agreement with these findings, the H₂S donor NaHS significantly aggravated this systemic inflammation [21-25,86]. Although similar results were found during caerulein-induced pancreatitis [27,87], the role of H₂S during systemic inflammatory diseases is still a matter of debate. Zanardo and colleagues reported reduced leukocyte infiltration and edema formation using the air pouch and carrageenan-induced hindpaw edema model in rats injected with the H₂S donors NaHS and Na₂S [30]. Moreover, in mice with acute lung injury induced by combined burn and smoke

Figure 3



Hydrogen sulfide attenuation of oxidative DNA damage in the kidney after organ ischemia–reperfusion. Oxidative DNA damage (tail moment in the alkaline version of the comet assay [89]) in kidney tissue biopsies prior to (left panel) and after 2 hours of organ ischemia and 8 hours of reperfusion (right panel) in control swine ($n = 7$; open box plots) and in animals treated with the hydrogen sulfide donor sodium disulfide (Na_2S) ($n = 8$; grey box plots). Renal ischemia was induced by inflating the balloon of an intra-aortic catheter positioned at the renal artery orifices. Na_2S infusion was infused before kidney ischemia (2 mg/kg/hour over 2 hours) as well as during the first 4 hours of reperfusion (1 mg/kg/hour) [72]. Data presented as median (quartiles, range). $\#P < 0.05$ versus before ischemia, $\$P < 0.05$ versus control.

inhalation, a single Na_2S bolus decreased tissue IL-1 β levels, increased IL-10 levels, and attenuated protein oxidation in the lung, which ultimately resulted in markedly prolonged survival [28].

Variable dosing and timing make it difficult to definitely conclude on the proinflammatory and/or anti-inflammatory effects of H_2S : while the median sulfide lethal dose in rats has been described to be approximately 3 mg/kg intravenously [1], studies in the literature report on doses ranging

from 0.05 to 5 mg/kg. In addition, there are only a small number of reports on continuous intravenous infusion rather than bolus administration. Finally, the role of the suspended animation-related hypothermia *per se* remains a matter of debate. While some studies report that spontaneous hypothermia and/or control of fever may worsen the outcome [88], other authors describe decreased inflammation [89] and improved survival after inducing hypothermia in sepsis [90].

We found in anesthetized and mechanically ventilated mice undergoing sham operation for surgical instrumentation that normothermic H_2S (100 ppm) inhalation (38°C) over 5 hours and hypothermia (27°C) alone comparably attenuated the inflammatory chemokine release (monocyte chemotactic protein-1, macrophage inflammatory protein-2 and growth-related oncogen/keratinocyte-derived chemokine) in the lung tissue. While H_2S did not affect the tissue concentrations of TNF α , combining hypothermia and inhaled H_2S significantly decreased tissue IL-6 expression (Table 3) [91].

Conclusions

Based on the concept that multiorgan failure secondary to shock, inflammation and sepsis may actually be an adaptive hypometabolic response to preserve ATP homeostasis [92] – such as has been demonstrated for the septic heart [93] – and thus represent one of the organism's strategies to survive under stress conditions, the interest of inducing a hibernation-like suspended animation with H_2S is obvious. Investigations have currently progressed most for the treatment of myocardial ischemia [94]. It must be underscored, however, that only a relatively small proportion of the published studies was conducted in clinically relevant large animal models [11,16,95], and, furthermore, that the findings reported are controversial [16,78,79].

Moreover, several crucial issues warrant further investigation before the clinical application of this concept. First, the role of hypothermia for any suspended animation-related organ protection is well established [96], but its impact remains a matter of debate for H_2S -related organ protection. Clearly, in the rodent studies [10,12,18,28], any cytoprotective effect

Table 3

Lung tissue concentrations of inflammatory chemokines after inhaling H_2S during normothermia or hypothermia				
	Control, 38°C	H_2S , 38°C	Control, 27°C	H_2S , 27°C
TNF α (pg/mg protein)	67 (52 to 90)	75 (60 to 88)	76 (54 to 88)	71 (60 to 81)
IL-6 (pg/mg protein)	449 (264 to 713)	366 (252 to 483)	338 (140 to 500)	260 (192 to 339)*
MCP-1 (pg/mg protein)	194 (102 to 280)	114 (77 to 138)*	99 (68 to 168)*	106 (48 to 150)*
MIP-2 (pg/mg protein)	613 (278 to 1049)	284 (214 to 357)*	306 (231 to 376)*	283 (248 to 373)*
KC (pg/mg protein)	435 (268 to 602)	296 (255 to 332)*	309 (217 to 401)*	329 (301 to 366)*

Lung tissue concentrations of monocyte chemotactic protein-1 (MCP-1), macrophage-inflammatory protein-2 (MIP-2), growth-related oncogen/keratinocyte-derived chemokine (KC), TNF α , and IL-6 after inhaling hydrogen sulfide (H_2S) (100 ppm over 5 hours) during normothermia (38°C) or hypothermia (27°C) [83]. Data presented as median (range), $n = 5$ in each group. * $P < 0.05$ versus control, 38°C.

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was apparent without a change in core body temperature, but localized metabolic effects cannot be excluded [10]. In addition, the role of any H₂S-related hypothermia remains controversial in the context of systemic inflammation [88]. Second, similar to the friend and foe character of NO, no definitive conclusions can be made as to whether H₂S exerts proinflammatory or anti-inflammatory properties [1,6,85]. Finally, in addition to the question of dosing and timing (for example, bolus administration versus continuous intravenous infusion), the preferred route of H₂S administration remains to be settled: while inhaling gaseous H₂S probably allows easily titrating target blood concentrations, it is well established that this method can also directly cause airway irritation [76].

While H₂S-induced suspended animation in humans to date may still be referred to as science fiction, there are ample promising preclinical data that this approach is a fascinating new therapeutic perspective for the management of shock states that merits further investigation.

Competing interests

CS is an officer and stockholder of Ikaria (Seattle, WA, USA), a company involved in the commercial development of hydrogen sulfide. PR received research grants from Ikaria. FW, PA and EC declare that they have no competing interests.

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