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# $H_2S$ in acute lung injury: a therapeutic dead end(?)

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## Abstract

This review addresses the plausibility of hydrogen sulfide ($H_2S$) therapy for acute lung injury (ALI) and circulatory shock, by contrasting the promising preclinical results to the present clinical reality. The review discusses how the narrow therapeutic window and width, and potentially toxic effects, the route, dosing, and timing of administration all have to be balanced out very carefully. The development of standardized methods to determine in vitro and in vivo $H_2S$ concentrations, and the pharmacokinetics and pharmacodynamics of $H_2S$-releasing compounds is a necessity to facilitate the safety of $H_2S$-based therapies. We suggest the potential of exploiting already clinically approved compounds, which are known or unknown $H_2S$ donors, as a surrogate strategy.

**Keywords:** Suspended animation, Gaseous mediator, Hypometabolism, Inflammation, Oxidative stress, Translational medicine

## Background

This review explores the plausibility of hydrogen sulfide ($H_2S$) therapy for acute lung injury (ALI) and circulatory shock. $H_2S$ is a toxic gas with a characteristic smell of rotten eggs, and is also produced endogenously by three different enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate-sulfurtransferase (MST) [1]. In 1996 and 1997, physiological roles of $H_2S$ in the brain and vascular smooth muscle, respectively [2, 3], were discovered, which led to its classification as the third "endogenous gaso-transmitter" [4], besides nitric oxide and carbon monoxide.

In 2005, in a hallmark study, Blackstone et al. demonstrated that inhaled $H_2S$ (80 ppm, ambient temperature 13 °C) can induce a "suspended-animation" like state by reduction of the metabolic rate in spontaneously breathing mice. This was accompanied by a fall in body temperature down to 15 °C [5]. The metabolic rate dropped by 90% after 6 h of $H_2S$ exposure. The effect was fully reversible upon transferring the mice into room air and room temperature [5]. These findings led to high hopes and a

frenzy of speculation regarding the ability of $H_2S$ to induce a hypometabolic state which could be exploited in patient care [6]. However, the fact that this effect was first shown in experimental conditions (low ambient temperature, no maintenance of body temperature, and no anesthesia) that are contrary to the current clinical practice, some drawbacks have to be anticipated in translating this effect to critical care medicine. Interestingly, $H_2S$-induced hypometabolism and hypothermia could be reproduced in mice at room temperature, but could not be confirmed in anesthetized sheep [7]. In anesthetized pigs, Simon et al. did report a sulfide-induced drop in metabolism in a model of aortic occlusion with intravenous sulfide administration [8]. However, in large animals, the effect seems to take longer to manifest and is not as pronounced as in rodents. Thus, the concept of $H_2S$-induced "suspended animation" or hypometabolism should remain in the realm of science fiction (as suggested by Drabek et al. [9]), but it is also true that potentially therapeutic effects of $H_2S$ independent of hypometabolism [10–12]: anti-inflammatory, antioxidant, organ-specific benefits, regulation of blood pressure, and glucose metabolism [13–17], are encouraging for the clinical development of $H_2S$ donors and have not yet been fully explored [18]. After a brief introduction into the role of $H_2S$ in the lung, its role in chronic lung diseases and modes of exogenous $H_2S$ administration, we will review the current literature of exogenous $H_2S$ administration in preclinical models of acute lung injury (ALI, mostly rodents), translationally more relevant models of lung injury and circulatory shock (resuscitated large animal models), and finally conclude with the current status of clinical trials of $H_2S$ therapies and an outlook on future clinical development.

## The role of $H_2S$ in the lung

High levels of $H_2S$ gas have been shown to be an environmental hazard, entering the body through the lung and being further distributed via the bloodstream [17]. $H_2S$ as a byproduct of various industries and pollutant arising from sewers can cause a "knock-down" effect upon inhalation of > 500 ppm: pulmonary injury, loss of consciousness, cardiopulmonary arrest, and death [19]. Generally, 10–20 ppm of $H_2S$ are considered to be safe to inhale acutely [17]. The effects of a chronic low-level exposure to $H_2S$ on lung toxicity have not been well characterized, and epidemiological studies are controversial, either reporting no relevant effect [20], or reduced lung function [21]. Bates et al. investigated the effects of naturally occurring $H_2S$ in geothermal areas on pulmonary health and found no detrimental effect and surprisingly even suggest a potential benefit on lung function [22].

$H_2S$ reportedly plays a role in lung development [23], and a deficiency in the endogenous $H_2S$ enzymes impairs alveolarization [24]. In the adult lung, the expression of the endogenous enzymes has been identified in a variety of pulmonary compartments in different species: rodents [25–27], bovine [28], and humans [29–32]. An upregulation of the endogenous $H_2S$ enzymes has been reported to play a role in the adaptive response to injury [27, 33]. However, the role of endogenous $H_2S$ in the adult lung is not well established.

## $H_2S$ in chronic lung diseases

Chronic pulmonary diseases have been found to be associated with reduced $H_2S$ serum levels in patients [34] and suppressed pulmonary CSE expression [31]. Even though a

few preclinical studies report pro-inflammatory effects of $H_2S$ in general (e.g., [35, 36]), it seems well established that the predominant $H_2S$ effect in the pathophysiology of chronic pulmonary diseases is anti-inflammatory [25, 31, 32, 37, 38]. Interestingly, low expression of the $H_2S$-producing enzymes was shown to compromise the anti-inflammatory effects of glucocorticoid therapy in asthma [31, 39]. Low levels of CSE expression and $H_2S$ production in early development have been correlated to a higher susceptibility to allergic asthma in young mice [40]. The protective role of $H_2S$ in chronic inflammatory lung diseases has been thoroughly reviewed by Chen and Wang ([41]: animal models [25, 37, 39, 42] and human studies [34, 43]) and reported more recently (animal models: [38, 44] human: [31], human in vitro: [32]). There are numerous studies reporting a potential benefit of exogenous $H_2S$ administration in chronic lung diseases [25, 32, 38, 44, 45].

### Possible strategies for exogenous administration of $H_2S$

The possible strategies for exogenous administration of $H_2S$ have been reviewed recently by Szabo and Papapetropoulos [17] and comprise the following: inhalation of gaseous $H_2S$ and intraperitoneal (i.p.) or intravenous (i.v.) administration of various $H_2S$-releasing compounds: $H_2S$-releasing salts (e.g., $Na_2S$, NaHS) and slow $H_2S$-releasing donors (GYY4137, AP39, diallyl-trisulfide (DATS)). Regarding the effects of exogenous $H_2S$ on inflammation reveals that short-term free sulfide levels as a consequence of the administration of $H_2S$-releasing salts can have detrimental effects, whereas a slow continuous $H_2S$ release from slow-releasing donors attenuated inflammation (demonstrated in vitro by [46] and thoroughly reviewed by [13]). An overview of currently available $H_2S$-releasing compounds is given in Table 1.

## Therapeutic potential of $H_2S$ during acute lung injury

In the following subsections, 70 articles investigating the effects of exogenous $H_2S$ administration in various models of acute lung injury are reviewed. These articles were identified in a literature search on PubMed in August 2019 with the search term "hydrogen sulfide" in combination with either "acute lung injury" or "ventilator-induced lung injury" or "shock" and "lung." Articles that were not available in English or did not deal with exogenous $H_2S$ administration were excluded.

### Ventilator-induced lung injury (VILI)

The effects of exogenous $H_2S$ in murine models of VILI are mostly reported to be anti-inflammatory. Only one study reports an acceleration of VILI with 60 ppm of $H_2S$ gas

**Table 1** Overview of various sulfide donors and their sulfide release

| Donor category | Compounds | Sulfide release |
|---|---|---|
| **Inhalation** | Gaseous $H_2S$ | Rapid, high risk of toxic peak concentrations |
| **Sulfide-releasing salts** | $Na_2S$, NaHS, IK-1001 | Rapid, high risk of toxic peak concentrations |
| **Slow-releasing donors** | GYY4137, AP39, DATS, SG-1002 | Slow, toxicity ultimately not clear |
| **Clinically available compounds** | Sodium thiosulfate (STS), Ammonium tetrathiomolybdate (ATTM), Zofenopril | Slow, good safety profile |

administration as an inhaled gas [47]. However, in the same study, pre-treatment with an intra-arterial bolus of $Na_2S$ (0.55 mg/kg) before starting harmful ventilation could attenuate lung inflammation and oxidative stress [47]. The latter is well in accordance with the protective effects of $H_2S$ in VILI reported by Aslami et al. and Wang et al., who observed reduced inflammation and improved lung function in animals with VILI, treated with a continuous infusion of 2 mg/kg/h NaHS or DATS, respectively [48, 49]. In contrast to the harmful effects of gaseous $H_2S$ administration (60 ppm) [47], four separate reports from a different group all indicate a beneficial effect of 80 ppm of $H_2S$: anti-inflammatory and anti-apoptotic effects [11], attenuated lung damage [50], antioxidant effects [51], and prevention of edema formation, even with a reduced $H_2S$ administration time [52]. These contrasting results might be due to the fact that the latter group used a milder VILI protocol with a tidal volume of 12 ml/kg over a longer time (6 h) [11, 50–52] rather than 40 ml/kg for 4 h as [47]. In conclusion, these results suggest an overall beneficial effect of $H_2S$ in VILI.

### Pancreatitis-induced acute lung injury (ALI)

Up to 1/3 of all pancreatitis patients develop ALI or acute respiratory distress syndrome (ARDS), which accounts for 60% of pancreatitis-related deaths [53]. Inhibition of cystathionine-γ-lyase (CSE) had anti-inflammatory effects in a murine model of pancreatitis-induced lung injury [54]. In a follow-up experiment, Bhatia et al. 2006 reported an induction of lung inflammation and histological damage in response to i.p. injection of 10 mg/kg NaHS in mice [55]. The effects were only present 1 h post-injection and by 3 and 6 h, the inflammatory state had returned to baseline [55], suggesting that the toxic effects were a transitory consequence of NaHS-induced high peak sulfide concentrations, which were quickly cleared. Besides Bhatia et al. 2005 [54], three more studies report a benefit of the inhibition of endogenous $H_2S$ production by CSE (either chemically or genetic deletion) on pancreatitis-induced ALI in murine models [56–58]. However, as mentioned previously, the effects of $H_2S$ on inflammation are controversial: in other studies, both the administration of ACS15 ($H_2S$-releasing diclofenac) and NaHS pre-treatment (10–15 mg/kg) led to an attenuation of inflammation in pancreatitis-induced ALI [59, 60]. The context of $H_2S$ administration seems to be crucial: in a healthy animal, 10 mg/kg NaHS induces transient lung inflammation, whereas this kind of pre-treatment is anti-inflammatory in subsequent pancreatitis-induced ALI. Furthermore, the role of CBS in the CSE inhibition experiments is not clear—it could potentially be upregulated in response to CSE inhibition. Neither of the CSE inhibition experiments report pulmonary $H_2S$ levels; thus, no causal conclusions about the role of $H_2S$ itself in inflammation can be drawn from these studies.

### Burn and/or smoke-induced lung injury

Acute lung injury is common in burn injury patients and can also be aggravated by the inhalation of smoke. In a murine model of hot water-induced skin burn, Zhang et al. observed aggravated lung inflammation and histological damage in animals treated with NaHS (10 mg/kg) [61], which could be mediated by transient toxic peak sulfide release, which has to be anticipated with this dose of NaHS. In contrast, in a similar model, Ahmad et al. report attenuated pulmonary cell infiltration and oxidative stress with the

administration of AP39 [62]. However, confoundedly, another arm in this study was treated with AOAA, an inhibitor of endogenous $H_2S$ enzymes [63], which had the same effects as AP39, prompting their conclusion of a "complex pathogenic role of $H_2S$ in burns" [62]. However, the authors neither report $H_2S$ levels nor the expression levels of the endogenous enzymes, which makes it difficult to interpret their data. In the lung, the upregulation of the endogenous $H_2S$ enzymes can represent an adaptive response to stress [27]. Thus, it is tempting to speculate that their apparently ambivalent results may be attributed to AOAA and AP39 having a similar regulatory effect on the endogenous $H_2S$ enzymes, which has not been investigated or reported yet. In fact, Han et al. report attenuated lung injury and antioxidant effects of spontaneous breathing of 80 ppm $H_2S$ in a rat model of cotton smoke-induced ALI [64]. In a combined model of smoke- and flame burn-induced lung injury, Esechie et al. were able to demonstrate attenuated inflammation and improved 5 days survival due to subcutaneous $Na_2S$ treatment [65]. They were also able to confirm this protective effect of $Na_2S$ in a large animal (ovine) model of smoke and burn injury, where a 24-h primed continuous i.v. infusion of $Na_2S$ after injury ameliorated pulmonary pathophysiological changes [66]. Overall, $H_2S$ seems to mediate protective effects in burn- and/or smoke-induced ALI.

#### Endotoxin-induced ALI

All studies investigating the effects of exogenous $H_2S$ in LPS-induced lung inflammation were performed in rodents and reported beneficial effects, regardless of the mode of LPS (locally or systemically) and $H_2S$ (salt, slow-releasing donor, inhalation) administration. Inhalation of 80 ppm $H_2S$ after intranasal LPS attenuated lung histological damage and had anti-inflammatory and antioxidative effects [67, 68]. Pre-treatment with GYY4137 also attenuated lung injury and cell infiltration after LPS inhalation [69]. Both GYY4137 and NaHS pre-treatment also attenuated lung injury and inflammation after intratracheal LPS exposure [70, 71]. A therapeutic administration of $H_2S$, either sodium thiosulfate (STS) or GYY4137, after intratracheal LPS ameliorated pulmonary inflammation as well [72, 73]. GYY4137 also attenuated cell infiltration in the lung after i.v. injection with LPS. Pre-treatment with GYY4137 had antioxidant and anti-inflammatory effects in i.p. injection of LPS. NaHS administration 3 h after i.v. LPS attenuated inflammation and oxidative stress and protected the mitochondria in the lung [74].

#### Polymicrobial sepsis-induced ALI

In contrast to studies investigating endotoxin administration, the role of exogenous $H_2S$ in murine models of cecal ligation and puncture (CLP, abdominal sepsis) is controversial: both beneficial and detrimental effects have been reported. In a resuscitated murine model, 100 ppm of inhaled $H_2S$ had minor anti-inflammatory effects, though not mediating protective effects in CLP [75]. A variety of studies report aggravation of sepsis-induced lung injury by NaHS [76–82]. However, in all these models, NaHS was administered as an i.p. bolus and did not comprise any additional resuscitative measures. The route of administration might also be a confounding factor combined with the CLP. Furthermore, the dose of $H_2S$ that was used in these studies was much higher than the dose of the previously mentioned LPS experiments (i.e., 10 mg/kg during CLP

versus 0.78–3.12 mg/kg i.p. NaHS during LPS). In fact, 1 h i.v. administration of NaHS at a rate of 1 and 3 mg/(kg × h) after CLP attenuated oxidative stress and cell infiltration in the lung [83]. High peak sulfide levels achieved by the bolus administration of a high dose of $H_2S$ can exert toxic detrimental effects, whereas achieving a less pronounced elevation of sulfide levels over a longer period of time could exert a benefit [13]. In a model of enterocolitis, the slow-releasing $H_2S$ donor GYY4137 attenuated lung inflammation and edema, whereas $Na_2S$ (20 mg/kg 3 times daily) had no effect [84].

#### Oleic acid-induced ALI

ALI is most commonly modeled in mice by an intravenous injection of oleic acid (OA) [85]. Studies investigating exogenous $H_2S$ administration in this model consistently report beneficial effects: attenuated edema formation, reduced cell infiltration, and anti-inflammatory and antioxidant effects of NaHS pre-treatment [86–89].

#### Oxidative lung injury

In models of hyperoxia- or ozone-induced ALI, NaHS administration exerted anti-inflammatory and antioxidative effects [90–92]. However, hyperoxia cannot only induce lung damage, depending on the experimental protocol: hyperoxia, as an experimental therapy in combined fracture healing and blunt chest trauma, exerted lung-protective effects. Interestingly, these protective effects were associated with an amelioration of the stress-induced upregulation of endogenous $H_2S$ enzymes and thus restoring the naive state of protein expression [27].

#### Trauma-induced ALI

Blunt chest trauma induces mechanical and inflammatory injury to the lung [93]. In a resuscitated, murine model of thoracic trauma, a continuous i.v. infusion of $Na_2S$ (0.2 mg/(kg × h)) had no effect on lung mechanics and gas exchange, but reduced apoptosis and cytokine production [33]. These effects were even more pronounced in combination with hypothermia [33]. Inhaled $H_2S$ (100 ppm) attenuated inflammation and cell infiltration in the lung in a non-resuscitated rat model of thoracic trauma [94]. However, in both these studies, the effects of $H_2S$ were rather weak and a clear benefit could not have been determined [33, 94], in contrast to models of other types of injury. Interestingly, an upregulation of pulmonary CSE expression in response to combined acute on chronic lung disease, i.e., thoracic trauma after cigarette smoke exposure, was suggested to be an adaptive response to injury [27, 95], in that a genetic deletion of CSE in the same kind of acute on chronic trauma was associated with aggravated ALI [96].

#### ALI in various types of ischemia/reperfusion injury (I/R)

In a rat model of lung transplantation, NaHS (0.7 mg/kg i.p.) improved lung function and reduced cell infiltration and oxidative stress [97]. NaHS pre-treatment was beneficial in limb I/R-induced lung injury, due to anti-inflammatory effects and attenuated edema formation [98]. GYY4137 pre-treatment has been tested in infrarenal aortic cross clamping, as well as lung I/R, and beneficial effects have been reported in both

types of lung injury: anti-inflammatory and antioxidant activity, respectively [99, 100]. Results in models of hemorrhagic shock are controversial. One study found a beneficial effect of an i.p. bolus of NaHS in a rat model: attenuated edema formation, cell infiltration, and necrosis [101]. Another study of HS in mice determined pulmonary anti-inflammatory effects of AP39; however, the mortality rate in the treated arm of this study was very high due to profound vasodilation [102]. Using a lower dose of AP39 yielded no effects at all [102]. These opposite effects of exogenous $H_2S$ administration in these two experiments might be due to the different $H_2S$-releasing compounds used or resuscitative measures. Chai et al. [101] performed the re-transfusion/resuscitation only with fluid administration, whereas Wepler et al. [102] used re-transfusion of shed blood and a full-scale small animal intensive care unit (ICU) setup (see below), which certainly changes the pathophysiology. In general, the role of $H_2S$ in hemorrhagic shock is controversial, with either a beneficial [103–108], harmful [109, 110], or no impact [111, 112].

## Translational medicine—$H_2S$ in large animal models of shock

Animal models with the purpose to identify relevant novel therapeutic strategies for patient care should reflect the clinical situation as closely as possible. In the context of ALI and shock research, the clinical practice for patient care in the ICU has to be reflected in experimental models to facilitate the translation from preclinical research to the clinical reality, i.e., temperature management, frequent blood gas analysis, lung-protective mechanical ventilation, hemodynamic monitoring, fluid administration, and catecholamine support titrated to the mean arterial pressure (MAP) [113]. Metabolic and organ-specific differences between small and large animals need to be taken into account [114, 115], as well as the challenge of reproducing the patient's pathophysiology (e.g., comorbidities and premedication).

In particular for $H_2S$, in a translational scenario, the implementation of intensive care measures (e.g., maintenance of body temperature, anesthesia, fluid resuscitation) might interfere with its effects, thus contributing to the lack of a hypometabolic effect in resuscitated rodent intensive care models [10, 33, 75, 102]. In large animals, the effects of $H_2S$ administration, in general, have been less robust, not only due to the intensive care measures, but also due to their large body size and different metabolic and thermoregulatory phenotype [114]. Large resuscitated animal studies reflect (i) no or very limited effects [8, 103, 112, 116–118], (ii) organ-specific effects [66], or (iii) beneficial effects restricted to a narrow timing and dosing window [119, 120].

As aforementioned, the induction of suspended animation by $H_2S$ inhalation was successful in small animals [5]; however, the translation to larger animals and eventually humans has proven to be challenging. Small animals have a much higher metabolic rate in relation to their body weight than large animals [121]; thus, the induction of a hypometabolic state is much easier to perform in small animals [114]. To induce that same state in a larger animal, a much higher dose of $H_2S$ would be needed, harboring the risk of toxicity [114]. However, the challenges of measuring $H_2S$/sulfide in biological samples make it difficult to perform dose-finding studies.

Nonetheless, several studies in large animal models explored the therapeutic potential in various types of ALI. $Na_2S$ in an ovine model of burn reduced mortality and

improved gas exchange [66]. In porcine models, $Na_2S$ was further studied in hemorrhagic shock, where it attenuated lung damage when administered at the time of reperfusion, however largely unrelated to hypothermia [120]. Administration of STS in the acute phase of resuscitation (24 h) after hemorrhagic shock in a porcine comorbid atherosclerotic model showed only a limited effect by improved gas exchange and lung mechanics in comparison to vehicle-treated animals (Table 2, [122]). Nußbaum et al. investigated the effects of GYY4137 during long-term resuscitated septic shock in pigs with atherosclerosis: GYY4137 treatment led to a preferential utilization of carbohydrates; however, they did not observe any major benefit of the treatment, gas exchange was not affected, and they did not further investigate lung tissue [117]. Unfortunately, none of the other large animal studies report lung function or lung histopathology. Still, it seems that exogenous $H_2S$ can mediate lung-protective effects in translationally relevant large animal models, when carefully timed and titrated.

## Clinical trials of exogenous $H_2S$ administration in ALI

To be able to answer the question posted in the title of this review, the clinical development of $H_2S$-releasing compounds has to be taken into consideration as well. As we shift from large animal preclinical studies to clinical trials, a search on *clinicaltrials.gov* (August 2019) for the term "sulfide" revealed a total of 64 clinical trials (see Fig. 1). Only two trials were found, which focused on a lung pathology (i.e., asthma), falling into the category "observational" in Fig. 1, investigating the potential use of $H_2S$ as a biomarker. There are no interventional clinical trials addressing the therapeutic potential of exogenous $H_2S$ in lung injury or lung disease. Of the 50 interventional trials identified, only 20 were evaluating $H_2S$ donors, 8 evaluated their intervention based on $H_2S$ as a biomarker, and 5 suggested $H_2S$ as a part of the mechanism of their intervention (see Fig. 1). The category "other" in Fig. 1 includes contrast agents, chemotherapeutics, and dietary supplements with a sulfide moiety. Only 6 of the 20 interventional

**Table 2** Lung function in a resuscitated comorbid porcine model of hemorrhagic shock [122]

| Timepoint | Group assignment | Horowitz index (mmHg) | PEEP ($cmH_2O$) |
|---|---|---|---|
| Baseline | Control | 400 (338, 448) | 0 |
| | Thiosulfate | 351 (328, 427) | 0 |
| After shock (start of STS infusion) | Control | 376 (322, 431) | 0 |
| | Thiosulfate | 352 (283, 405) | 0 |
| 24 h after shock (end of STS infusion) | Control | 387 (326, 418) | 10 (10, 10) |
| | Thiosulfate | 385 (355, 417) | 10 (10, 10) |
| 48 h after shock | Control | 230 (195, 270)# | 12.5 (12.5, 15) |
| | Thiosulfate | 299 (263, 339)* | 11.3 (10, 12.5) |
| 72 h after shock | Control | 289 (106, 323)# | 15 (12.5, 15) |
| | Thiosulfate | 337 (300, 387) | 10 (10, 12.5)* |

Atherosclerotic pigs were surgically instrumented and, after a short recovery period, underwent 3 h of hemorrhagic shock (target mean arterial pressure 40 ± 5 mmHg). Seventy-two hours of resuscitation comprised re-transfusion of the shed blood and fluid and catecholamine administration targeted to the pre-shock mean arterial pressure. Further details about the experimental protocol can be found in [122]. STS was administered during the first 24 h of resuscitation after hemorrhagic shock. Effects on lung function were most pronounced at 48 h after hemorrhagic shock. Data shown are median (lower quartile, upper quartile)

*Significant to control group

#Significant to baseline ($p < 0.05$ in two-way ANOVA)

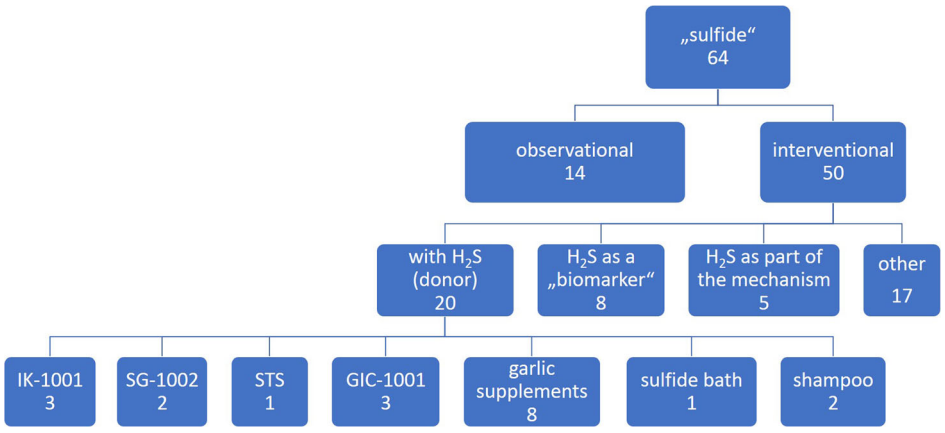


**Fig. 1** Overview of clinical trials found with the search term "sulfide" listed on clinicaltrials.gov

trials with $H_2S$ donors are relevant to intensive care (see Fig. 2), excluding skin diseases, colonoscopy, and arthritis.

IK-1001, a solution generated by bubbling $H_2S$ gas into an aqueous solution, was the first compound, designated to administer $H_2S$, under investigation in clinical trials in 2009. The first trial of IK-1001 targeted "renal impairment" (NCT00879645) and was terminated prematurely (actual recruitment of 28 participants) because investigators were unable to determine sulfide levels. The issue of not being able to reliably measure sulfide is of course critical for clinical approval of a compound: how would one ever be able to determine the safety of a compound that cannot be measured? One complexity is the fact that exogenous sulfide is highly volatile and rapidly bound and/or metabolized in vivo [27]. Various sulfide pools are available in biological systems and sulfide engages in many different chemical reactions [123], suggesting that these endogenous pools are highly dynamic. Exogenous administration of $H_2S$ might change the balance

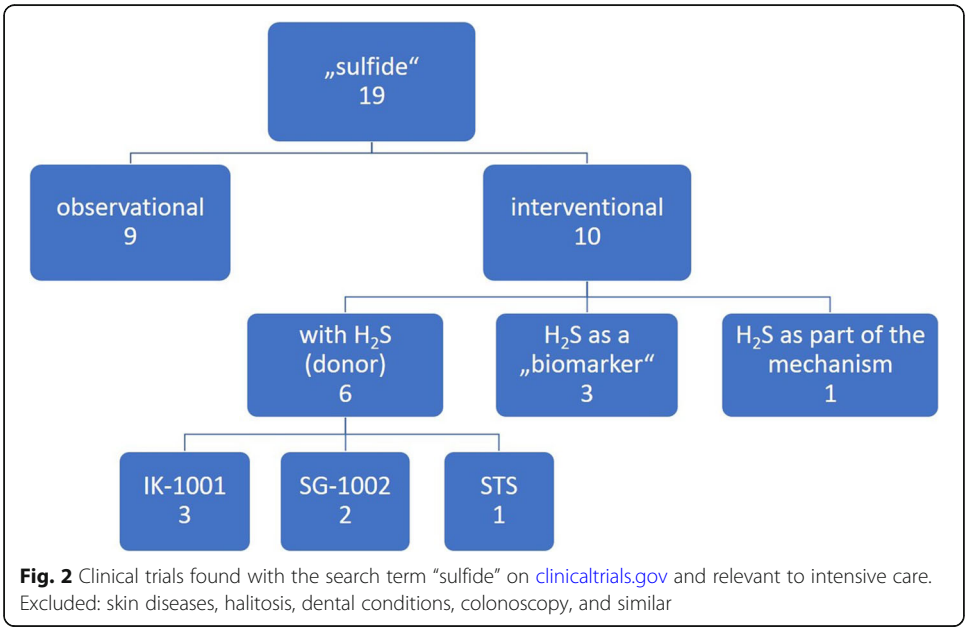


**Fig. 2** Clinical trials found with the search term "sulfide" on clinicaltrials.gov and relevant to intensive care. Excluded: skin diseases, halitosis, dental conditions, colonoscopy, and similar

of this whole system in ways that we do not fully understand yet. The second trial with IK-1001 in coronary artery bypass (NCT00858936) was terminated after recruiting 6 participants with reasons not reported. The third trial in ST-elevation myocardial infarct (STEMI, NCT01007461) was withdrawn by company decision—non-safety related.

As mentioned above, IK-1001 is an aqueous solution of physically dissolved $H_2S$, thus resembling the characteristics of the administration of $H_2S$-releasing salts or inhaled $H_2S$ (see also Table 1). Neither administration of $H_2S$ via inhalation nor injection of $H_2S$-releasing salts will likely be ever used in clinical practice, due to airway mucosal damage and the potential of toxic peak sulfide concentrations, respectively [27]. In fact, inhalation of 300 ppm $H_2S$, though sub-lethal, is used as a model to study lung injury [124, 125]. Efforts to avoid the airway irritation of gaseous $H_2S$ using extracorporeal membrane lung ventilation in a preclinical study were successful, but there was no improvement on the outcome from cardiopulmonary bypass [126].

SG-1002, a mixture of organic sulfide-releasing compounds and salts, has been under investigation in heart failure. A phase I trial revealed the compound to be safe and well tolerated (NCT01989208); a follow-up phase II trial is still in progress with no results posted yet (NCT02278276).

An interesting perspective for $H_2S$-based therapeutics is the reconsideration of compounds that are already clinically approved and have only recently been identified to be able to release $H_2S$: (i) sodium thiosulfate (STS) [17, 127], approved for cyanide detoxification and cisplatin overdosage; (ii) ammonium tetrathiomolybdate (ATTM) [128, 129], approved for Wilson's disease, a copper metabolism disorder; and (iii) zofenopril [130], an inhibitor of angiotensin converting enzyme approved for hypertension. These compounds all have been tested extensively and are known to have good safety profiles (see also Table 1).

For example, Dyson et al. showed ATTM led to a 50% reduction of infarct size in rat models of myocardial and cerebral I/R as well as improved survival after hemorrhagic shock [129]. The good safety profile of STS [131] in particular might be related to the fact that thiosulfate itself is an endogenous intermediate of oxidative $H_2S$ metabolism [127] and is suggested to be "a circulating 'carrier' molecule of beneficial effects of $H_2S$" [132], in particular under hypoxic conditions [127]. The clinical trial of IK-1001 in renal impairment even used thiosulfate as an indirect measure of $H_2S$ release from their compound (NCT00879645), although ultimately not successful. STS is currently under investigation in a phase 2 clinical trial to preserve cardiac function in STEMI (NCT02899364). With regard to the lung, as mentioned previously, STS was beneficial in murine models of intratracheal LPS and CLP [72]. Our own group's findings support these results from Sakaguchi et al.: we determined a beneficial effect of STS to the lung, i.e., improved gas exchange and lung mechanics in a translationally relevant large animal model of hemorrhagic shock (Table 2). Thus, STS is a very promising compound for the development of therapeutic $H_2S$ administration in ALI in a clinical setting.

## Conclusions

Exogenous $H_2S$ administration has been demonstrated to be beneficial in various preclinical models of lung injury. However, due to the narrow therapeutic window and width, and potentially toxic effects, the route, dosing, and timing of administration all have to be balanced out very carefully. The development of methods to determine $H_2S$

levels and/or the pharmacokinetics and pharmacodynamics of $H_2S$-releasing compounds is absolutely necessary to facilitate the safety of $H_2S$-based therapies. Awaiting the results of currently ongoing clinical trials and the re-evaluation of already approved $H_2S$-releasing compounds for novel indications could likely help to prove that $H_2S$ is in fact not a therapeutic dead end [6].

#### Abbreviations

ALI: Acute lung injury; ARDS: Acute respiratory distress syndrome; ATTM: Ammonium tetrathiomolybdate; CBS: Cystathionine-β-synthase; CLP: Cecal ligation and puncture; CSE: Cystathionine-γ-lyase; DATS: Diallyl-trisulfide; $H_2S$: Hydrogen sulfide; ICU: Intensive care unit; I/R: Ischemia reperfusion; i.p.: Intraperitoneal; i.v.: Intravenous; LPS: Lipopolysaccharide; MAP: Mean arterial pressure; MST: 3-mercaptopyruvate-sulfurtransferase; OA: Oleic acid; ppm: parts per million; STS: Sodium thiosulfate; VILI: Ventilator-induced lung injury

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#### Authors' contributions

TM drafted the manuscript. OM and PR critically reviewed and edited the manuscript. TM, ND, MW, HG, DACM, CH, TD, and OM were involved in the acquisition and interpretation of data. All authors read and approved the final version.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Ethics approval and consent to participate

Not applicable

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#### Competing interests

The authors declare that they have no competing interests.

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