

Review

H₂S in Critical Illness—A New Horizon for Sodium Thiosulfate?

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Abstract: Ever since the discovery of endogenous H₂S and the identification of its cytoprotective properties, efforts have been made to develop strategies to use H₂S as a therapeutic agent. The ability of H₂S to regulate vascular tone, inflammation, oxidative stress, and apoptosis might be particularly useful in the therapeutic management of critical illness. However, neither the inhalation of gaseous H₂S, nor the administration of inorganic H₂S-releasing salts or slow-releasing H₂S-donors are feasible for clinical use. Na₂S₂O₃ is a clinically approved compound with a good safety profile and is able to release H₂S, in particular under hypoxic conditions. Pre-clinical studies show promise for Na₂S₂O₃ in the acute management of critical illness. A current clinical trial is investigating the therapeutic potential for Na₂S₂O₃ in myocardial infarct. Pre-eclampsia and COVID-19 pneumonia might be relevant targets for future clinical trials.

Keywords: ischemia/reperfusion injury; inflammation; oxidative stress

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1. Introduction

This review summarizes the current evidence for the therapeutic potential of sodium thiosulfate (Na₂S₂O₃), a clinically approved H₂S donor with minimal side effects, in critical illness. H₂S has a variety of biological roles, such as the regulation of vascular tone, as well as anti-oxidant and anti-inflammatory properties, which could exert a potential therapeutic benefit in intensive care. However, to date, the narrow therapeutic window and potential for toxic adverse effects have prevented the application of gaseous inhaled H₂S in the clinical setting [1–5]. Other strategies for H₂S administration, such as H₂S-releasing salts (NaHS and Na₂S) and specifically developed H₂S slow-releasing donors (such as GYY4137 and AP39) with a better pharmacokinetic profile, have similar limitations for clinical use [5–8]. Na₂S₂O₃ is a detoxifying agent, which is clinically approved as an antidote for cyanide poisoning as well as chronic renal failure-induced calciphylaxis [9] and cisplatin overdose [10]. Na₂S₂O₃ can induce metabolic acidosis, but otherwise no major side effects have been reported [11–13], thus Na₂S₂O₃ is a promising H₂S donor for clinical use. The thiosulfate anion (S₂O₃²⁻) is an endogenous oxidation product of H₂S degradation, and, in turn, can serve as a source of H₂S [14]. In particular, under hypoxic conditions, 3-mercaptopyruvate sulfotransferase (3-MST) and rhodanese have been reported to be able to facilitate sulfide release from thiosulfate [14].

2. Biology of H₂S and S₂O₃²⁻

Originally, H₂S was known for its toxicity to humans, due to its reversible inhibition of cytochrome-c-oxidase, Complex IV of the mitochondrial electron transfer system (ETS), blocking its O₂ binding site. About 30 years ago, endogenous H₂S was first detected [15] and described as a gaseous neurotransmitter [16], and since then three different enzymes have been identified to be able to release H₂S endogenously: cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-MST. 3-MST produces H₂S from 3-mercaptopyruvate, which is generated from cysteine (see Figure 1). The sulfur atom is transported from 3-mercaptopyruvate by MST in the form of sulfane sulfur (MST-SSH). It was shown that H₂S could be released in the presence of dithiols (dihydrolipoic acid (DHLA), DTT, thioredoxin (Trx)); however, in the presence of monothiols (GSH, cysteine), no release of H₂S was observed [17]. H₂S oxidation represents another level of the regulation of endogenous H₂S availability, besides its endogenous production. In the mitochondria, H₂S can be oxidized by sulfide quinone oxidoreductase (SQR), which can contribute to the pool of electrons processed in the ETS [14,18,19]. Thus, small amounts of H₂S (nanomolar up to low micromolar concentrations [20]) can actually stimulate mitochondrial respiration. A physiological acceptor of sulfane sulfur from SQR-SSH has not been identified unequivocally, yet. Some authors have postulated that human SQR utilizes sulfite as persulfide acceptor, yielding thiosulfate as a product. Other authors have demonstrated that in addition to sulfite, GSH functions as a persulfide acceptor for human SQR, leading to GSSH [21]. It is also not excluded that there are SQR persulfide acceptors other than sulfite and GSH (e.g., DHLA, Trx, cysteine), which, after accepting sulfane sulfur, can be reduced by GSH [22]. Hydropersulfides (-SSH) are further oxidized by sulfur dioxygenase and sulfur transferase to thiosulfate [14]. Hemoproteins might also contribute to thiosulfate production from H₂S [23]. Thiosulfate is further oxidized into sulfate. In turn, in particular under hypoxic and reducing conditions, H₂S can be regenerated from exogenously administered thiosulfate by rhodanese and, reportedly, 3-MST [14] (see Figure 1), which is not surprising when considering that both thiosulfate and 3-mercaptopyruvate are, chemically speaking, sulfane sulfurs.

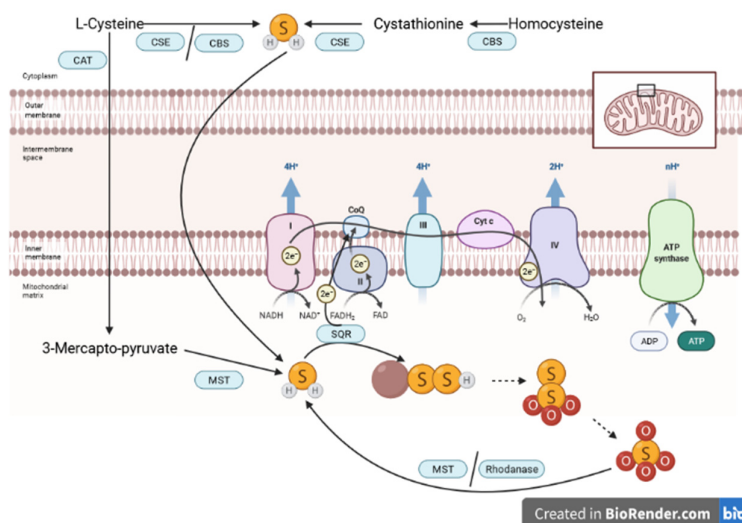


Figure 1. Sulfide generation and oxidation pathways. In the cytoplasm, cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS) facilitate H₂S release from L-Cysteine and homocysteine. Cysteine-aminotransferase uses L-cysteine to form 3-mercapto-pyruvate, which is then used by 3-mercaptopyruvate-sulfurtransferase (MST) for mitochondrial H₂S release. Sulfide quinone oxidoreductase (SQR) oxidizes H₂S to persulfides in the mitochondria, and persulfides are further oxidized, which ultimately results in the formation of thiosulfate and sulfate. MST and rhodanese can re-generate H₂S from thiosulfate, a process which can also happen non-enzymatically. Figure created in BioRender. Adapted from “Electron transport chain”, by BioRender.com (2022). Retrieved from <https://app.biorender.com/biorender-templates> (accessed on 15th March 2022).

Sulfide oxidation seems to be a critical process in cellular oxygen sensing [24]. Under normoxic conditions, sulfide is oxidized continuously into thiosulfate in the mitochondria [25], whereas under hypoxic conditions, sulfide oxidation is limited and cellular H₂S concentrations rise [19,25]. Hypoxic conditions additionally favor endogenous enzymatic H₂S production, which in turn seems to mediate intracellular signaling in response to hypoxia, such as hypoxic vasoconstriction and catecholamine release [25]. Furthermore, as mentioned above, thiosulfate itself can serve as a source of H₂S under hypoxic conditions, but not during normoxia [14].

We investigated the effects of Na₂S₂O₃ on mitochondrial respiration in an in vitro experiment in cultured rat primary cortical neurons. Cells were harvested after 4 h of incubation with either 4, 20, or 100 mM Na₂S₂O₃ for the assessment of mitochondrial oxygen consumption (JO₂) at the maximum respiratory activity of the electron transfer system in the uncoupled state. Indeed, we were able to identify a differential dose-dependent effect of Na₂S₂O₃ on mitochondrial respiration. It is likely that the cells were able to release H₂S from the administered Na₂S₂O₃, which (at a dose of 4 mM Na₂S₂O₃) either stimulated mitochondrial respiration as an electron donor via Complex II or (at a dose of 100 mM Na₂S₂O₃) inhibited Complex IV (see Figure 2).

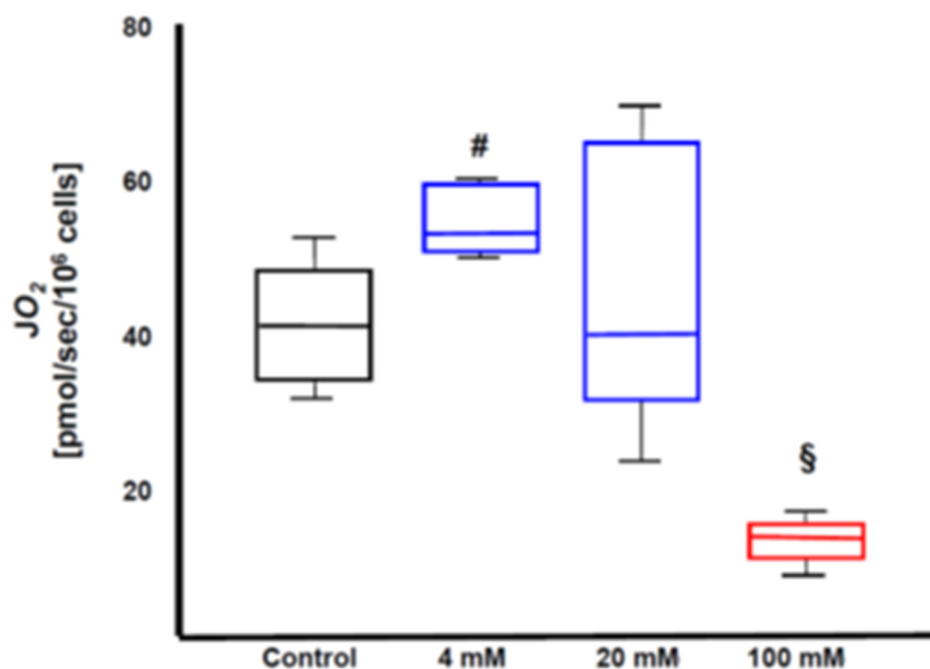


Figure 2. Effects of Na₂S₂O₃ on mitochondrial respiration (oxidative phosphorylation as assessed by routine O₂ consumption JO₂) in cultured cortical neurons from fetal rat brains. Primary neuron cultures from the fronto-temporal cortex were prepared from fetal rat brains (embryonic day 18) and seeded on poly-L-lysine-coated culture flasks. The cells were grown in neurobasal medium, and complemented with B27 supplement, L-glutamine, and penicillin/streptomycin. At day 22–24 of culturing, cells were incubated with PBS (control) or 4, 20, or 100 mM Na₂S₂O₃ for 4h and harvested in respiration medium (MIR05: 0.5 mM EGTA, 3 mM MgCl₂, 60 mM Lactobionic acid, 20 mM Taurine, 10 mM KH₂PO₄, 20 mM HEPES, 110 mM sucrose, 1 g/l bovine serum albumin). Mitochondrial oxygen consumption was determined after the addition of 10 mM pyruvate, 10 mM glutamate, 5 mM malate, 5 mM ADP, 10 μM cytochrome c, 10mM succinate, and uncoupling with 1.5 mM Carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazine (FCCP). N = 6 measurements in each group. Data are median (IQR), # *p* < 0.01 and § *p* < 0.0001 vs. control. A low Na₂S₂O₃ concentration (4 mM) increased, while a high concentration (100 mM) inhibited mitochondrial respiratory activity. Intermediate concentrations (20 mM) had variable effects.

Given the tight interplay of O_2 and H_2S availability, it is not surprising that H_2S plays a role in mediating many consequences of hypoxia: e.g., regulation of vascular tone, inflammation, oxidative stress, and apoptosis, all of which are affected by critical illness. For example, hemorrhagic shock-induced tissue ischemia and hypoxemia can trigger systemic hyper-inflammation [26], and reperfusion can cause ischemia/reperfusion injury (I/R), which contributes to systemic inflammation, oxidative stress, and multiple organ failure [27]. Even though the potential vaso-dilatory effects of H_2S donors could aggravate shock-induced hypotension [7], other beneficial H_2S effects might outweigh that risk. Still, the optimal timing and dosing window of H_2S donors in these conditions must be evaluated carefully. Exogenous H_2S administration can be facilitated by using inorganic H_2S -releasing salts ($NaSH$ and Na_2S), slow-releasing H_2S donors (GYY4137, AP39), and pre-existing clinically approved compounds, which have been recently identified to be able to release H_2S (ammonium tetrathiomolybdate (ATTM), $Na_2S_2O_3$). H_2S -releasing salts cause rapidly increasing, potentially toxic, peak H_2S concentrations, which dissipate quickly [28], can have pro-inflammatory effects [29], and damage the mitochondria [20,30]. Even when these peak concentrations are prevented, the therapeutic window for H_2S -releasing salts is very narrow, which is why they are unsuitable for clinical use [4–6,31]. In contrast, slow H_2S -releasing donors seem to have different effects, in that they rather seem to ameliorate inflammation [29]. However, translationally relevant *in vivo* results for slow-releasing H_2S donors also render them unlikely for clinical development, since they have failed to exert organ protection and/or even have adverse effects in these models so far [7,8], in spite of many promising pre-clinical studies in non-resuscitated rodents. ATTM showed organ protection in *in vivo* models of I/R [32,33]. However, it is not clear if ATTM is superior to standard treatment, because these studies did not include standard ICU measures. $Na_2S_2O_3$ is a clinically available compound with a good safety profile [12] and is reportedly able to release H_2S , in particular, under hypoxic conditions [14]. Further biochemical effects of STS are summarized in Figure 3. Its potential to reduce oxidized glutathione and its effects on vascular tone might be of particular importance for therapeutic approaches in critical care. Thus, $Na_2S_2O_3$ is a promising candidate to bring H_2S therapy in critical illness to the clinic. The following section will explore available literature reports of exogenous $Na_2S_2O_3$ administration in experimental models of critical illness.

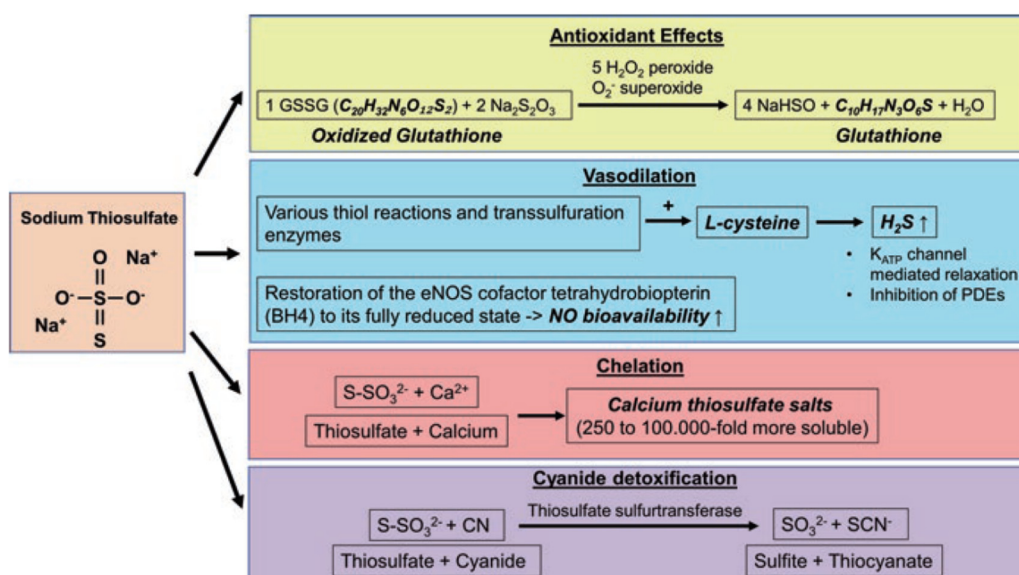


Figure 3. Biochemical effects of sodium thiosulfate. $Na_2S_2O_3$ can reduce oxidized glutathione [34], mediate vaso-dilation [35], work as a calcium chelator [36], and work as an antidote for cyanide [37]. Figure taken from [38]. Copyright 2019 Springer Nature Switzerland AG. Reprinted with permission.

3. Experimental Evidence for Beneficial Effects of Na₂S₂O₃ in Animal Models of Critical Illness

Animal studies investigating the effects of Na₂S₂O₃ in models of critical illness are summarized in Table 1.

3.1. Langendorff Heart Model and In Vivo I/R Experiments

In seven different *ex vivo* studies using the Langendorff rat heart model (i.e., isolated heart with retrograde perfusion with a nutrient-rich oxygenated buffer), with 30 min ischemia followed by 60 min reperfusion, a variety of beneficial effects of different modes of Na₂S₂O₃ administration have been reported. Both pre- [39–42] and post-conditioning [43] or Na₂S₂O₃ administration starting with reperfusion [41,42,44] were reported to reduce cardiac injury, apoptosis [39,43], inflammatory markers [39], and oxidative stress, [39,42–44] and to protect cardiac mitochondria [39,40,42–44], resulting in improved cardiac contractility [40,44] in comparison to non-Na₂S₂O₃-treated hearts. The same group of scientists was also able to confirm the beneficial pre-conditioning effects of Na₂S₂O₃ in *in vivo* rat model of myocardial I/R, i.e., left anterior descending artery ligation [39] and isoproterenol-induced myocardial infarction (MI) [45]. The beneficial effects of Na₂S₂O₃ in MI were also demonstrated in dogs [46]. In one of the *in vivo* rat MI studies, an additional beneficial effect on the brain—reduced apoptosis and oxidative stress associated with improved mitochondrial function—was determined [45]. Marutani et al. also reported a benefit of repeated i.p. Na₂S₂O₃ administration on the murine brain after cerebral I/R by bilateral common carotid artery occlusion: improved 20-day survival and better neuronal function in the treated animals [47].

Table 1. Summary of *in vivo/ex vivo* studies investigating the effects of Na₂S₂O₃ in models of critical illness.

First Author	Animal Model	Condition	Na ₂ S ₂ O ₃ Administration	Effects of Na ₂ S ₂ O ₃ (Compared to Vehicle)
Mouse models				
Tokuda 2012 [48]	Male C57BL/6J mice, aged 8–10 weeks	Endotoxemia: LPS (10 mg/kg i.p.) with 1 mL of saline (i.p.) at 0, 6 and 24 h after LPS	1 or 2 g/kg (i.p.) immediately after LPS	improved survival
Shirozu 2014 [49]	Male C57BL/6J mice, aged 8–12 weeks	Acute liver failure and endotoxemia: D-Galactosamine (300 mg/kg) and 1 mg/kg LPS (i.p.) with 1 mL of saline (i.p.) at 0, 6 and 24 h after LPS	2 g/kg (i.p.) at 30 min before and 3 h after LPS	liver injury ↓, antioxidant and anti-apoptotic effects, preserved mitochondrial membrane potential
Sakaguchi 2014 [50]	Male C57BL/6J mice, aged 8–10 weeks	Endotoxemia: intratracheal LPS (2 mg/kg) Polymicrobial septic shock: CLP with fluid resuscitation	2 g/kg (i.p.) at 0 and 12 h after LPS 0.5 g/kg (i.v.) at 10 min after CLP	cell accumulation and MPO in BALF ↓, lung edema ↓, lung IL-6, TNFα, NOS2, MMP9 ↓, lung NFκB signaling ↓
Marutani 2015 [47]	Male C57BL/6J mice, aged 8–9 weeks	Global cerebral I/R: bilateral common carotid artery occlusion, reperfusion after 40 min	10 mg/kg (i.p.) in 1 mL of 5% dextrose-enriched lactated Ringer's solution at 10 min after reperfusion and daily for 1 week	improved 20-day survival, neuronal function score ↑ at 24 h after reperfusion
Acero 2017 [51]	Female C57BL/6J mice, aged 2 months	Endotoxemia: LPS (3 mg/kg i.p.)	100, 350, 500, and 750 mg/kg (i.p.) immediately	brain IL1-β, COX-2, Iba-1, TSPO ↓ (at 500 mg/kg Na ₂ S ₂ O ₃) neuroinflammation ↓

			after LPS and at 8, 24, and 32 h	
Renieris 2021 [52]	Male and female mice, WT and CSE ^{-/-} (C57BL/6) aged 7–8 weeks	Sepsis: <i>P. aeruginosa</i> (i.p.) infection	2 g/kg (s.c.) daily for four days after infection	survival ↑ in WT mice
Gröger 2022a [53]	Male CSE ^{-/-} mice (C57BL/6J.129SvEv), aged 20–25 weeks	Polytrauma: blast wave-induced blunt chest trauma + hemorrhagic shock (1 h), retransfusion and intensive care management (6 h)	i.v. bolus (0.45 mg/g) at start of resuscitation	norepinephrine requirements, lactate ↓, Horowitz index and urine output ↑, lung IL-6 and MCP1 ↓, lung GR and NOS2 ↑, kidney IκBα and HO-1 ↑
Rat models				
Ravindran 2017 [43]	Male Wistar rats Langendorff heart model	30 min ischemia with 60 min reperfusion	1 mM (15 min) post-conditioning	apoptosis ↓, anti-oxidant defense ↑, preserved mitochondrial enzyme activity
Ravindran 2017 b [39]	Langendorff heart model	30 min ischemia with 60 min reperfusion	0.1 mM and 1 mM (10 min) pre-conditioning	myocardial injury, inflammatory cell infiltration, and interstitial oedema ↓
Ravindran 2017 b [39]	Rats	Left Anterior Descending Artery Ligation (30 min ischemia with 2h reperfusion)	1 mM (i.v., 15 min prior to ischemia)	oxidative stress ↓, mitochondrial protection
Ravindran 2018 [54]	Male Wistar rats (aged 6 weeks) with adenine-induced vascular calcification, Langendorff heart model	30 min ischemia with 60 min reperfusion	400 mg/kg orally for 28 days	calcification ↓, I/R-induced cardiac injury ↓ in non-calcified hearts, oxidative stress ↓ in non-calcified hearts, no effect in calcified hearts after I/R
Ravindran 2018 b [44]	Langendorff heart model	30 min ischemia with 60 min reperfusion	1 mM at start of reperfusion	contractility ↑, myocardial injury ↓, mitochondrial enzyme activity ↑, oxidative stress ↓, LV contractility ↑, cardiac injury ↓, recovered ATP production, PGC1α and mitochondrial copy number ↑, mitochondrial proteins ↑, better mitochondrial ultrastructure
Ravindran 2019 [40]	Male Wistar rats, 200–250 g Langendorff heart model	30 min ischemia with 60 min reperfusion	1 mM 15 min before ischemia	
Kannan 2019 [41]	Male Wistar rats Langendorff heart model	30 min ischemia with 60 min reperfusion	1 mM at 15 min before I/R 1 mM at reperfusion with(out) additional PAG (CSE/CBS inhibitor)	Infarct size ↓, less pronounced with PAG co-treatment
Ravindran 2020 [45]	Male Wistar rats (250–300 g)	Isoproterenol-induced MI	100 mg/kg (i.p.), 1 h before isoproterenol	cardiac injury ↓, ROS and caspase-3 and 9 ↓ (heart and brain), mitochondrial function ↑ (heart and brain)
Boovarahan 2021 [42]	Male Wistar rats (8–12 weeks, 250–300 g) Langendorff heart model	30 min ischemia with reperfusion	1 mM before ischemia or at reperfusion	Preconditioning protective through ↓oxidative stress, mitochondrial protection Postconditioning protective (different mechanism)
Schulz 2021 [55]	Male Wistar rats (320–380 g)	Polymicrobial sepsis: CASP	1 g/kg (i.p.) immediately after sepsis induction and at 24 h	24 h after sepsis induction: colonic and hepatic microcirculation ↑, mitochondria
large animal models				

Oksman 1982 [46]	Male and female dogs (15–25 kg)	Tourniquet shock experimental MI (descending coronary artery ligation)	500 µg/kg (i.v., 30 min after removal) 500 mg/kg (i.v., 15 min after ligation) 660 mg/kg (i.v. bolus in combination with other antioxidants, then continuous infusion of 190 mg/kg/h)	heart function ↑, blood pressure ↑ heart function ↑, minimal effects on blood pressure
Broner 1989 [56]	New Zealand white rabbits (approx. 3 kg)	<i>E. coli</i> septicemia with fluid resuscitation		well tolerated, no benefit
Datzmann 2020 [13]	Male and female Familial Hypercholesterolemia Bretoncelles Meishan pigs (reduced CSE protein levels), median age 24 months	Hemorrhagic shock (3 h), retransfusion and intensive care management	continuous i.v. infusion at start of retransfusion for 24 h (0.1 g/(kg·h))	PEEP and Horowitz-Index ↑ (48 h after shock), lung GR expression ↑, pH and BE ↓

Abbreviations: lipopolysaccharide (LPS), intravenous (i.v.), intraperitoneal (i.p.), cecal ligation and puncture (CLP), myeloperoxidase (MPO), bronchoalveolar lavage fluid (BALF), interleukin 6 (IL-6), tumor necrosis factor alpha (TNFα), inducible nitric oxide synthase (NOS2), matrix metalloproteinase 9 (MMP9), monocyte chemoattractant protein 1 (MCP1), glucocorticoid receptor (GR), ischemia-reperfusion injury (I/R), nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFκB), NFκB inhibitor alpha (IκBα), heme-oxygenase 1 (HO-1), positive end-expiratory pressure (PEEP), base excess (BE), left-ventricular (LV), adenosine -tris-phosphate (ATP), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), myocardial infarction (MI), colon ascendens stent peritonitis (CASP), l-propargylglycine (PAG), cyclo-oxygenase 2 (COX2), microglial marker ionized calcium-binding adapter molecule 1 (Iba-1), 18 kDa translocator protein (TSPO), ↑ higher, ↓ reduced.

3.2. Chronic Conditions

In rats with 28 days of oral administration of Na₂S₂O₃ and adenine-induced vascular calcification, Na₂S₂O₃ treatment alleviated the calcification [54]. In this study, hearts from Na₂S₂O₃-treated animals without vascular calcification were also characterized by reduced cardiac injury and diminished oxidative stress after the Langendorff I/R experiments [54]. However, this benefit of Na₂S₂O₃ did not extend to calcified hearts after I/R challenge [54]. Mohan et al. reported a beneficial effect of both oral preventative and therapeutic Na₂S₂O₃ on adenine-induced renal failure, which also rendered the mitochondria of treated animals more resistant to in vitro I/R [57]. Angiotensin-II (Ang-II)-induced hypertension, cardiac hypertrophy, and cardiac fibrosis was also alleviated by regular i.p. injections of Na₂S₂O₃ in rats, which were also associated with a reduction of oxidative stress [58]. In rats with l-NNA-administration, an inhibitor of nitric oxide synthases which causes hypertension and chronic heart disease, two weeks of oral administration of Na₂S₂O₃ ameliorated the hypertension, improved cardiac function [35], and improved glomerular filtration [59]. Interestingly, the authors of these studies did not observe any metabolic acidosis associated with the oral administration of Na₂S₂O₃ in their animals, which is an advantage of oral administration over infusion [35]: patients with i.v. administration of Na₂S₂O₃ have been reported to develop metabolic acidosis [11,60]. This begs the question of if oral administration is the preferable route of administration, even though oral Na₂S₂O₃ bioavailability seems much more limited when compared to i.v. infusion [12]. However, the effects of oral Na₂S₂O₃ on an acute injury in combination with the hypertension were not investigated by these groups. Chronic hypertension as a comorbidity might worsen the patient's outcome after critical illness, but is, in itself, not a condition that is normally treated in the intensive care unit. These reported "long-term" benefits show promise for Na₂S₂O₃ as a therapeutic agent but are not feasible for the acute management of critical illness [35,59]. However, it is tempting to speculate that "long-term" effects of oral Na₂S₂O₃ might still be associated with a benefit for the patient after an acute injury.

3.3. Endotoxemia and Sepsis

In models of endotoxemia, i.p. $\text{Na}_2\text{S}_2\text{O}_3$ post-treatment in mice led to improved survival [48], attenuated lung inflammation [50], and ameliorated neuroinflammation [51]. In animals with concomitant acute liver failure and LPS administration, $\text{Na}_2\text{S}_2\text{O}_3$ treatment decreased liver injury associated with anti-oxidant effects and preserved mitochondrial function [49]. In models of polymicrobial sepsis, $\text{Na}_2\text{S}_2\text{O}_3$ also had beneficial effects on the murine lung [50] and colonic and hepatic microcirculation in rats [55]. In contrast, in rabbits with *E. coli* septicemia and fluid resuscitation, $\text{Na}_2\text{S}_2\text{O}_3$ in combination with other antioxidants did not have a benefit [56].

3.4. Role of Endogenous H_2S Enzymes

To date, the role of endogenous H_2S -producing enzymes for the beneficial $\text{Na}_2\text{S}_2\text{O}_3$ -mediated effects is not entirely clear. In a study with mice with arteriovenous fistula (AVF)-induced chronic heart failure, the authors suggest that $\text{Na}_2\text{S}_2\text{O}_3$ exerted a benefit by attenuating the AVF-induced loss of CSE expression and thus enhancing endogenous H_2S generation [61]. In the Langendorff heart model, a co-treatment with the CSE and CBS inhibitor l-propargylglycine (PAG) attenuated $\text{Na}_2\text{S}_2\text{O}_3$ effects [41]. In *P. aeruginosa*-induced sepsis, daily s.c. $\text{Na}_2\text{S}_2\text{O}_3$ injections for four days improved survival of wildtype mice, however, there was no survival benefit in $\text{CSE}^{-/-}$ mice in the same study [52]. These studies hint towards a critical role for CSE in mediating $\text{Na}_2\text{S}_2\text{O}_3$ effects. In contrast, in resuscitated $\text{CSE}^{-/-}$ mice with blunt chest trauma and hemorrhagic shock, $\text{Na}_2\text{S}_2\text{O}_3$ clearly reduced the vasopressor requirements needed to achieve hemodynamic targets, thereby attenuating lactic acidosis and both lung and kidney dysfunction [53]. However, in $\text{CSE}^{-/-}$ mice with diabetes type I as a co-morbidity undergoing the same kind of injury, this benefit of $\text{Na}_2\text{S}_2\text{O}_3$ was lost [62] (under review). Wildtype animals were not yet investigated in this context. Furthermore, in a translationally relevant porcine model of hemorrhagic shock with underlying atherosclerosis, $\text{Na}_2\text{S}_2\text{O}_3$ also exerted a benefit [13]. Here, it is important to point out that atherosclerosis is associated with a down-regulation of the endogenous H_2S enzymes, which worsens the outcome of these animals from critical injury [63–65]. Even though these animals can be considered a large-animal analog to $\text{CSE}^{-/-}$ mice, with respect to their lack of CSE, they have reduced CSE expression and not a global knock out as the $\text{CSE}^{-/-}$, thus the atherosclerotic pigs might still have residual CSE activity. Still, in the atherosclerotic pigs with hemorrhagic shock and subsequent intensive care management, i.v. administration of $\text{Na}_2\text{S}_2\text{O}_3$ during the first 24 h of resuscitation was associated with improved lung function at 48 and 72 h after hemorrhagic shock, which was associated with increased levels of glucocorticoid receptor expression in the lung [13]. Strikingly, the clinical benefit of $\text{Na}_2\text{S}_2\text{O}_3$ on lung function could not be observed in resuscitated cardiovascularly healthy pigs after hemorrhagic shock [66] (unpublished data), which, in contrast to some of the results from mouse experiments, suggests a particular benefit from $\text{Na}_2\text{S}_2\text{O}_3$ for subjects with impaired CSE/impaired endogenous H_2S availability.

3.5. Role of the Interplay of H_2S and Thiosulfate

Interestingly, in the atherosclerotic pigs, the beneficial effects manifested 24 h after the $\text{Na}_2\text{S}_2\text{O}_3$ had already been stopped, even though elevated sulfide levels were only detected immediately at the end of $\text{Na}_2\text{S}_2\text{O}_3$ administration, but not 24 h after the administration [13]. This begs the question of if the elevation of sulfide levels or the thiosulfate itself actually exerts the therapeutic benefit. In one of the abovementioned studies, the authors added $\text{Na}_2\text{S}_2\text{O}_3$ -treated groups only after observing that H_2S inhalation in their study elevated plasma sulfide as well as thiosulfate levels [48]. They hypothesized that thiosulfate is the actual beneficial molecule. This is in line with findings from Marutani et al., who detected elevated cerebral thiosulfate, but not sulfide levels, in their murine model of brain I/R [47]. In contrast, Ravindran et al. reported elevated H_2S

levels in the brain after $\text{Na}_2\text{S}_2\text{O}_3$ treatment and do not mention thiosulfate [45]. In murine LPS- and sepsis-induced acute lung injury, Sakaguchi et al. reported that $\text{Na}_2\text{S}_2\text{O}_3$ treatment elevated both plasma and lung tissue thiosulfate and sulfide levels [50]. Shirozu et al. detected elevated plasma and liver thiosulfate levels after $\text{Na}_2\text{S}_2\text{O}_3$ administration in their model, whereas sulfide levels were only elevated in the plasma for 6 h [49]. Thus, they also hypothesized that thiosulfate itself mediated the beneficial effects, in particular, that $\text{CSE}^{-/-}$ mice in their study also were characterized by elevated thiosulfate levels and mirrored the beneficial effects of $\text{Na}_2\text{S}_2\text{O}_3$ in wildtype animals [49]. Recent work from Marutani et al. also suggests that H_2S catabolism, i.e., high SQR activity, which would naturally contribute to elevated thiosulfate levels, can contribute to cerebral hypoxia tolerance [67]. For further information on the potential therapeutic perspective for sulfide catabolism and the sulfide/thiosulfate interplay, the reader is referred to a recent review by Marutani and Ichinose [68]. Further potential downstream cellular signaling pathways for therapeutic effects of $\text{Na}_2\text{S}_2\text{O}_3$, such as Nrf2 signaling, have been recently reviewed by Zhang et al. [69].

$\text{Na}_2\text{S}_2\text{O}_3$ is an example for a sulfane sulfur-containing compound, which are regarded as a form of H_2S storage, which can easily release this gasotransmitter in response to biological signals. Both reactive sulfur species (H_2S and sulfane sulfur) always coexist in a biological system. Toohey has indicated that H_2S is rather a biodegradation byproduct of sulfane sulfur-containing compounds. The author suggests that the sulfane sulfur compounds, which are present in cells at higher concentrations than H_2S , are responsible for the observed biological effects attributed to H_2S . [70,71], which strengthens the speculation that thiosulfate is “more important” than H_2S .

4. Clinical Perspective

Regardless of the underlying mechanism for the cytoprotective effects of $\text{Na}_2\text{S}_2\text{O}_3$, an open question still exists as to whether there is a future for this clinically available compound in critical care. The pre-clinical results are promising, but have certain limitations: (1) only three out of the 19 studies presented in Table 1 investigated a mixture of male and female animals [13,46,52], in fact most of the studies were limited to male animals; (2) old age as a complication, which can affect both the expression of the endogenous H_2S enzymes, as well as H_2S levels [72], was not considered in any of the pre-clinical studies presented here; (3) of 12 in vivo studies presented in Table 1, only 4 studies included basic fluid resuscitation [47–49,56] and only 2 studies included resuscitation strategies that are common practice in critical care [13,53]. Thus, the translational relevance of the other studies remains to be proven.

Already in 1966, Paris et al. were able to achieve an increase in urine output by $\text{Na}_2\text{S}_2\text{O}_3$ treatment in 16 out of 20 patients with severe burn-induced shock, which was even associated with alleviating the shock in these patients [73]. Currently (February 2022), 63 studies are listed on clinicaltrials.gov with the search term “sodium thiosulfate”. Most of these trials (34) look at $\text{Na}_2\text{S}_2\text{O}_3$ as an adjuvant in chemotherapy, as an antidote for cisplatin intoxication (see Figure 3). Another 20 trials deal with diseases tied into calcium dysregulations (calciophylaxis, vascular calcification, calcinosis cutis), trying to make use of the calcium chelating properties of $\text{Na}_2\text{S}_2\text{O}_3$ (see Figure 3). Three trials look at contact dermatitis and three further trials investigate endodontic disease each. One trial investigates the potential of $\text{Na}_2\text{S}_2\text{O}_3$ for kidney transplantation. Only two of the listed trials are directly relevant to critical care: NCT03017963 “Safety and Tolerability of Sodium Thiosulfate in Patients With an Acute Coronary Syndrome (ACS) Undergoing Coronary Angiography Via Trans-radial Approach.”, and NCT02899364 “Sodium Thiosulfate to Preserve Cardiac Function in STEMI (GIPS-IV)”.

The results of the ACS trial have already been published [74]. A dose-escalation study with i.v. $\text{Na}_2\text{S}_2\text{O}_3$ to a maximum dose of 15 g in two doses has been performed on 18 patients undergoing coronary angiography for ACS. A slight drop in systolic blood pressure was determined 1 h after the administration of the first dose, which could not be

observed after the second dose. Two patients experienced brief periods of hypotension, one accompanied by mild nausea. No serious adverse events were observed [74]. The same group will investigate the potential benefit of an intervention with $\text{Na}_2\text{S}_2\text{O}_3$ in a follow-up double-blind, randomized, placebo-controlled, multicenter trial (GIPS-IV), with a planned enrollment of 380 patients with ST-elevation myocardial infarction (STEMI) [75]. Patients will receive 12.5 g $\text{Na}_2\text{S}_2\text{O}_3$ directly after admission and a second dose 6 h later. The primary endpoint is myocardial infarct size after 4 months; secondary endpoints include the effects of $\text{Na}_2\text{S}_2\text{O}_3$ on peak CK-MB (creatin kinase muscle brain type, as an early measure of MI) during admission and left ventricular ejection fraction and NT-proBNP (N terminal pro brain natriuretic peptide, as a measure of heart insufficiency) levels at 4 months follow-up [75]. The group expects first results on the primary endpoint soon (“Q1 2022”), but no updates have been published yet.

Another interesting perspective for the clinical application of $\text{Na}_2\text{S}_2\text{O}_3$ is the treatment of pre-eclampsia. Preliminary results of our own observational study DRKS0001771 (“Role of the oxytocin-receptor and the H_2S -system in preeclampsia and HELLP-syndrome (NU-HOPE)”) confirm the dysregulation of endogenous H_2S availability in pre-eclampsia, which has also been published previously [76,77]. Pre-eclampsia is not necessarily a critical illness, but can develop into one, in particular in case of “Hemolysis Elevated Liver Enzymes Low Platelet count (HELLP)” syndrome, thus it is relevant to be considered in the context of this mini review. The interaction between the endogenous H_2S and oxytocin systems, which has recently been identified, might play a particularly important role in the molecular mechanism of pre-eclampsia and make the perspective of treatment with an H_2S donor even more relevant. $\text{CSE}^{-/-}$ and oxytocin receptor ($\text{OTR}^{-/-}$) mice show a reciprocal loss of other proteins in the heart [78,79]. Furthermore, resuscitated $\text{CSE}^{-/-}$ mice had a particularly pronounced loss of cardiac OTR expression after bunt chest trauma, which could be restored by the administration of an exogenous H_2S donor (GYY4137) [80]. Preliminary results from our observational trial confirm this interaction and dysregulation of the H_2S - and OT -systems during pre-eclampsia (see Figure 4A). Furthermore, psychological trauma might play an important role in this context: a dysregulation and interaction of the H_2S - and OT -systems has been shown in an animal model of early life stress [78], and placental CSE expression has been shown to be directly related to the patients’ childhood trauma load (see Figure 4B). Taken together, these results suggest that patients with underlying psychological trauma, in particular, might benefit from treatment with $\text{Na}_2\text{S}_2\text{O}_3$.

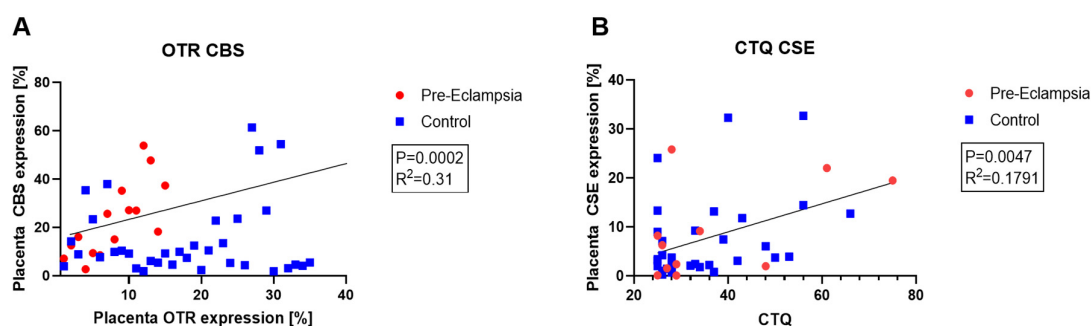


Figure 4. Preliminary results of the NU-HOPE observational trial. (A): Placental expression of the H_2S -producing enzyme cystathionine- β -synthase (CBS) and oxytocin receptor (OTR) directly correlate. (B): The childhood trauma questionnaire score (CTQ) directly correlates with placental expression of the H_2S -producing enzyme cystathionine- γ -lyase (CSE).

COVID-19 patients might be another group that could profit from $\text{Na}_2\text{S}_2\text{O}_3$ therapy. $\text{Na}_2\text{S}_2\text{O}_3$ is a recognized drug devoid of major side effects, which attenuated murine acute lung injury [50] and cerebral ischemia/reperfusion injury [47]. It was also shown that

$\text{Na}_2\text{S}_2\text{O}_3$ significantly attenuated shock-induced impairment of lung mechanics and gas exchange in pigs after hemorrhagic shock [13]. Plasma H_2S levels of survivors of COVID-19 pneumonia were significantly higher at day 1 and day 7 after admission in comparison to non-survivors [81]. These results suggest that H_2S might be a valuable biomarker for the severity of COVID-19 infection on the one hand [81], and that exogenous administration of H_2S might be a relevant therapeutic approach for these patients on the other hand [82]. Even though several groups have suggested $\text{Na}_2\text{S}_2\text{O}_3$ as a therapeutic adjuvant in the therapy of COVID-19 patients [82,83], there currently are no registered clinical trials on the subject. In contrast, clinicaltrials.gov lists 13 clinical trials for the therapeutic potential of N-acetyl-cysteine (NAC) for COVID-19 patients. NAC is an antioxidant molecule, also able to elevate sulfide levels, which might have various benefits for SARS-CoV-2 [84].

5. Conclusions

Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) is a clinically approved H_2S donor with a good safety profile. Pre-clinical experimental studies show that $\text{Na}_2\text{S}_2\text{O}_3$ can mediate cyto-protection in various types of ischemia reperfusion injury, physical trauma, and inflammation. The first clinical trials investigating the effects of $\text{Na}_2\text{S}_2\text{O}_3$ in myocardial infarct in humans are currently ongoing. Extending these clinical trials to other conditions would help uncover the full therapeutic potential for $\text{Na}_2\text{S}_2\text{O}_3$ in critical care.

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References

- Francis, R.C.; Vaporidi, K.; Bloch, K.D.; Ichinose, F.; Zapol, W.M. Protective and Detrimental Effects of Sodium Sulfide and Hydrogen Sulfide in Murine Ventilator-Induced Lung Injury. *Anesthesiology* **2011**, *115*, 1012–1021. <https://doi.org/10.1097/ALN.0b013e31823306cf>.
- Simon, F.; Giudici, R.; Duy, C.N.; Schelzig, H.; Öter, S.; Gröger, M.; Wachter, U.; Vogt, J.; Speit, G.; Szabó, C.; et al. Hemodynamic and Metabolic Effects of Hydrogen Sulfide during Porcine Ischemia/Reperfusion Injury. *Shock* **2008**, *30*, 359–364. <https://doi.org/10.1097/SHK.0b013e3181674185>.
- Derwall, M.; Westerkamp, M.; Löwer, C.; Deike-Glindemann, J.; Schnorrenberger, N.K.; Coburn, M.; Nolte, K.W.; Gaisa, N.; Weis, J.; Siepmann, K.; et al. Hydrogen Sulfide Does Not Increase Resuscitability in a Porcine Model of Prolonged Cardiac Arrest. *Shock* **2010**, *34*, 190–195. <https://doi.org/10.1097/SHK.0b013e3181d0ee3d>.
- Asfar, P.; Calzia, E.; Radermacher, P. Is Pharmacological, H_2S -Induced “suspended Animation” Feasible in the ICU? *Crit. Care* **2014**, *18*, 215. <https://doi.org/10.1186/cc13782>.
- Merz, T.; Denoix, N.; Wepler, M.; Gäßler, H.; Messerer, D.A.C.; Hartmann, C.; Datzmann, T.; Radermacher, P.; McCook, O. H_2S in Acute Lung Injury: A Therapeutic Dead End(?). *Intensive Care Med. Exp.* **2020**, *8*, 33. <https://doi.org/10.1186/s40635-020-00324-0>.
- Bracht, H.; Scheuerle, A.; Gröger, M.; Hauser, B.; Matallo, J.; McCook, O.; Seifritz, A.; Wachter, U.; Vogt, J.A.; Asfar, P.; et al. Effects of Intravenous Sulfide during Resuscitated Porcine Hemorrhagic Shock. *Crit. Care Med.* **2012**, *40*, 2157–2167. <https://doi.org/10.1097/CCM.0b013e31824e6b30>.

7. Wepler, M.; Merz, T.; Wachter, U.; Vogt, J.; Calzia, E.; Scheuerle, A.; Möller, P.; Gröger, M.; Kress, S.; Fink, M.; et al. The Mitochondria-Targeted H₂S-Donor AP39 in a Murine Model of Combined Hemorrhagic Shock and Blunt Chest Trauma. *Shock* **2019**, *52*, 230–239. <https://doi.org/10.1097/SHK.0000000000001210>.
8. Nußbaum, B.L.; Vogt, J.; Wachter, U.; McCook, O.; Wepler, M.; Matallo, J.; Calzia, E.; Gröger, M.; Georgieff, M.; Wood, M.E.; et al. Metabolic, Cardiac, and Renal Effects of the Slow Hydrogen Sulfide-Releasing Molecule GYY4137 during Resuscitated Septic Shock in Swine with Pre-Existing Coronary Artery Disease. *Shock* **2017**, *48*, 175–184. <https://doi.org/10.1097/SHK.0000000000000834>.
9. Nigwekar, S.U.; Thadhani, R.; Brandenburg, V.M. Calciphylaxis. *N. Engl. J. Med.* **2018**, *378*, 1704–1717. <https://doi.org/10.1177/1461444810365020>.
10. Tsang, R.Y.; Al-Fayea, T.; Au, H.J. Cisplatin Overdose: Toxicities and Management. *Drug Saf.* **2009**, *32*, 1109–1122. <https://doi.org/10.2165/11316640-000000000-00000>.
11. Hunt, G.M.; Ryder, H.F. Metabolic Acidosis after Sodium Thiosulfate Infusion and the Role of Hydrogen Sulfide. *Clin. Case Rep.* **2018**, *6*, 1595–1599. <https://doi.org/10.1002/ccr3.1673>.
12. McGeer, P. Medical Uses of Sodium Thiosulfate. *J. Neurol. Neuromedicine* **2016**, *1*, 28–30. <https://doi.org/10.29245/2572.942x/2016/3.1032>.
13. Datzmann, T.; Hoffmann, A.; McCook, O.; Merz, T.; Wachter, U.; Preuss, J.; Vettorazzi, S.; Calzia, E.; Gröger, M.; Kohn, F.; et al. Effects of Sodium Thiosulfate (Na₂S₂O₃) during Resuscitation from Hemorrhagic Shock in Swine with Preexisting Atherosclerosis. *Pharmacol. Res.* **2020**, *151*, 104536. <https://doi.org/10.1016/j.phrs.2019.104536>.
14. Olson, K.R.; DeLeon, E.R.; Gao, Y.; Hurley, K.; Sadauskas, V.; Batz, C.; Stoy, G.F. Thiosulfate: A Readily Accessible Source of Hydrogen Sulfide in Oxygen Sensing. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* **2013**, *305*, 592–603. <https://doi.org/10.1152/ajpregu.00421.2012>.
15. Goodwin, L.R.; Francom, D.; Dieken, F.P.; Taylor, J.D.; Warenycia, M.W.; Reiffenstein, R.J.; Dowling, G. Determination of Sulfide in Brain-Tissue by Gas Dialysis Ion Chromatography-Postmortem Studies and 2 Case-Reports. *J. Anal. Toxicol.* **1989**, *13*, 105–109.
16. Abe, K.; Kimura, H. The Possible Role of Hydrogen Sulfide as an Endogenous Neuromodulator. *J. Neurosci.* **1996**, *16*, 1066–1071. <https://doi.org/10.1523/JNEUROSCI.16-03-01066.1996>.
17. Mikami, Y.; Shibuya, N.; Kimura, Y.; Nagahara, N.; Ogasawara, Y.; Kimura, H. Thioredoxin and Dihydrolipoic Acid Are Required for 3-Mercaptopyruvate Sulfurtransferase to Produce Hydrogen Sulfide. *Biochem. J.* **2011**, *439*, 479–485. <https://doi.org/10.1042/BJ20110841>.
18. Abou-Hamdan, A.; Guedouari-Bounihi, H.; Lenoir, V.; Andriamihaja, M.; Blachier, F.; Bouillaud, F. Oxidation of H₂S in Mammalian Cells and Mitochondria. *Methods Enzymol.* **2015**, *554*, 201–228. <https://doi.org/10.1016/bs.mie.2014.11.042>.
19. Bouillaud, F. Sulfide Oxidation Evidences the Immediate Cellular Response to a Decrease in the Mitochondrial ATP/O₂ Ratio. *Biomolecules* **2022**, *12*, 361. <https://doi.org/10.3390/biom12030361>.
20. Szabo, C.; Ransy, C.; Módos, K.; Andriamihaja, M.; Murghes, B.; Coletta, C.; Olah, G.; Yanagi, K.; Bouillaud, F. Regulation of Mitochondrial Bioenergetic Function by Hydrogen Sulfide. Part I. Biochemical and Physiological Mechanisms. *Br. J. Pharmacol.* **2014**, *171*, 2099–2122. <https://doi.org/10.1111/bph.12369>.
21. Libiad, M.; Yadav, P.K.; Vitvitsky, V.; Martinov, M.; Banerjee, R. Organization of the Human Mitochondrial Hydrogen Sulfide Oxidation Pathway. *J. Biol. Chem.* **2014**, *289*, 30901–30910. <https://doi.org/10.1074/jbc.M114.602664>.
22. Kabil, O.; Banerjee, R. Enzymology of H₂S Biogenesis, Decay and Signaling. *Antioxid. Redox Signal.* **2014**, *20*, 770–782. <https://doi.org/10.1089/ars.2013.5339>.
23. Bilska-Wilkosz, A.; Iciek, M.; Górny, M.; Kowalczyk-Pachel, D. The Role of Hemoproteins: Hemoglobin, Myoglobin and Neuroglobin in Endogenous Thiosulfate Production Processes. *Int. J. Mol. Sci.* **2017**, *18*, 1315. <https://doi.org/10.3390/ijms18061315>.
24. Olson, K.R. Hydrogen Sulfide as an Oxygen Sensor. *Antioxid. Redox Signal.* **2015**, *22*, 377–397. <https://doi.org/10.1089/ars.2014.5930>.
25. Olson, K.R. Hydrogen Sulfide as an Oxygen Sensor. *Clin. Chem. Lab. Med.* **2013**, *51*, 623–632. <https://doi.org/10.1515/cclm-2012-0551>.
26. Cannon, J.W. Hemorrhagic Shock. *New Engl. J. Med.* **2018**, *378*, 370–379. <https://doi.org/10.1056/NEJMr1705649>.
27. Eltzschig, H.K.; Eckle, T. Ischemia and Reperfusion—from Mechanism to Translation. *Nat. Med.* **2011**, *17*, 1391–1401. <https://doi.org/10.1038/nm.2507>.
28. McCook, O.; Radermacher, P.; Volani, C.; Asfar, P.; Ignatius, A.; Kemmler, J.; Möller, P.; Szabó, C.; Whiteman, M.; Wood, M.E.; et al. H₂S during Circulatory Shock: Some Unresolved Questions. *Nitric Oxide-Biol. Chem.* **2014**, *41*, 48–61. <https://doi.org/10.1016/j.niox.2014.03.163>.
29. Whiteman, M.; Winyard, P.G. Hydrogen Sulfide and Inflammation: The Good, the Bad, the Ugly and the Promising. *Expert Rev. Clin. Pharmacol.* **2011**, *4*, 13–32. <https://doi.org/10.1586/ecp.10.134>.
30. Matallo, J.; Vogt, J.; McCook, O.; Wachter, U.; Tillmans, F.; Gröger, M.; Szabo, C.; Georgieff, M.; Radermacher, P.; Calzia, E. Sulfide-Inhibition of Mitochondrial Respiration at Very Low Oxygen Concentrations. *Nitric Oxide-Biol. Chem.* **2014**, *41*, 79–84. <https://doi.org/10.1016/j.niox.2014.06.004>.

31. Módis, K.; Bos, E.M.; Calzia, E.; van Goor, H.; Coletta, C.; Papapetropoulos, A.; Hellmich, M.R.; Radermacher, P.; Bouillaud, F.; Szabo, C. Regulation of Mitochondrial Bioenergetic Function by Hydrogen Sulfide. Part II Pathophysiological and Therapeutic Aspects. *Br. J. Pharmacol.* **2014**, *171*, 2123–2146. <https://doi.org/10.1111/bph.12368>.
32. Dyson, A.; Dal-Pizzol, F.; Sabbatini, G.; Lach, A.B.; Galfo, F.; dos Santos Cardoso, J.; Pescador Mendonça, B.; Hargreaves, I.; Bollen Pinto, B.; Bromage, D.I.; et al. Ammonium Tetrathiomolybdate Following Ischemia/Reperfusion Injury: Chemistry, Pharmacology, and Impact of a New Class of Sulfide Donor in Preclinical Injury Models. *PLoS Med.* **2017**, *14*, e1002310. <https://doi.org/10.1371/journal.pmed.1002310>.
33. Mendonça, B.P.; Cardoso, J.D.S.; Michels, M.; Vieira, A.C.; Wendhausen, D.; Manfredini, A.; Singer, M.; Dal-Pizzol, F.; Dyson, A. Neuroprotective Effects of Ammonium Tetrathiomolybdate, a Slow-Release Sulfide Donor, in a Rodent Model of Regional Stroke. *Intensive Care Med. Exp.* **2020**, *8*, 13. <https://doi.org/10.1186/s40635-020-00300-8>.
34. Kruihof, P.D.; Lunev, S.; Aguilar Lozano, S.P.; de Assis Batista, F.; Al-dahmani, Z.M.; Joles, J.A.; Dolga, A.M.; Groves, M.R.; van Goor, H. Unraveling the Role of Thiosulfate Sulfurtransferase in Metabolic Diseases. *Biochim. Biophys. Acta-Mol. Basis Dis.* **2020**, *1866*, 165716. <https://doi.org/10.1016/j.bbdis.2020.165716>.
35. Nguyen, I.T.N.; Wiggenhauser, L.M.; Bulthuis, M.; Hillebrands, J.L.; Feelisch, M.; Verhaar, M.C.; van Goor, H.; Joles, J.A. Cardiac Protection by Oral Sodium Thiosulfate in a Rat Model of L-NNA-Induced Heart Disease. *Front. Pharmacol.* **2021**, *12*, 650968. <https://doi.org/10.3389/fphar.2021.650968>.
36. Araya, C.E.; Fennell, R.S.; Neiberger, R.E.; Dharnidharka, V.R. Sodium Thiosulfate Treatment for Calcific Uremic Arteriolopathy in Children and Young Adults. *Clin. J. Am. Soc. Nephrol.* **2006**, *1*, 1161–1166. <https://doi.org/10.2215/CJN.01520506>.
37. Chen, K.; Rose, C. Nitrite and Thiosulfate Therapy in Cyanide Poisoning. *J. Am. Med. Assoc.* **1952**, *149*, 113–119. <https://doi.org/10.1001/jama.1952.02930190015004>.
38. Bauer, M.; Radermacher, P.; Wepler, M. Sodium Thiosulfate: A New Player for Circulatory Shock and Ischemia/Reperfusion Injury? In *Annual Update in Intensive Care 2019*; Vincent, J.-L., Ed.; Springer Nature: Cham, Switzerland, 2019; pp. 183–198, ISBN 978-3-030-06067-1.
39. Ravindran, S.; Boovarahan, S.R.; Shanmugam, K.; Vedarathinam, R.C.; Kurian, G.A. Sodium Thiosulfate Preconditioning Ameliorates Ischemia/Reperfusion Injury in Rat Hearts Via Reduction of Oxidative Stress and Apoptosis. *Cardiovasc. Drugs Ther.* **2017**, *31*, 511–524. <https://doi.org/10.1007/s10557-017-6751-0>.
40. Ravindran, S.; Kurian, G.A. Preconditioning the Rat Heart with Sodium Thiosulfate Preserved the Mitochondria in Response to Ischemia-Reperfusion Injury. *J. Bioenerg. Biomembr.* **2019**, *51*, 189–201. <https://doi.org/10.1007/s10863-019-09794-8>.
41. Kannan, S.; Boovarahan, S.R.; Rengaraju, J.; Prem, P.; Kurian, G.A. Attenuation of Cardiac Ischemia-Reperfusion Injury by Sodium Thiosulfate Is Partially Dependent on the Effect of Cystathione Beta Synthase in the Myocardium. *Cell Biochem. Biophys.* **2019**, *77*, 261–272. <https://doi.org/10.1007/s12013-019-00871-8>.
42. Boovarahan, S.R.; Venkatasubramanian, H.; Sharma, N.; Venkatesh, S.; Prem, P.; Kurian, G.A. Inhibition of PI3K/MTOR/KATP Channel Blunts Sodium Thiosulphate Preconditioning Mediated Cardioprotection against Ischemia-Reperfusion Injury. *Arch. Pharmacol. Res.* **2021**, *44*, 605–620. <https://doi.org/10.1007/s12272-021-01339-1>.
43. Ravindran, S.; Jahir Hussain, S.; Boovarahan, S.R.; Kurian, G.A. Sodium Thiosulfate Post-Conditioning Protects Rat Hearts against Ischemia Reperfusion Injury via Reduction of Apoptosis and Oxidative Stress. *Chem.-Biol. Interact.* **2017**, *274*, 24–34. <https://doi.org/10.1016/j.cbi.2017.07.002>.
44. Ravindran, S.; Kurian, G.A. Effect of Sodium Thiosulfate Postconditioning on Ischemia-Reperfusion Injury Induced Mitochondrial Dysfunction in Rat Heart. *J. Cardiovasc. Transl. Res.* **2018**, *11*, 246–258. <https://doi.org/10.1007/s12265-018-9808-y>.
45. Ravindran, S.; Gopalakrishnan, S.; Kurian, G.A. Beneficial Effect of Sodium Thiosulfate Extends beyond Myocardial Tissue in Isoproterenol Model of Infarction: Implication for Nootropic Effects. *J. Biochem. Mol. Toxicol.* **2020**, *34*, e22606. <https://doi.org/10.1002/jbt.22606>.
46. Oksman, T.M.; Levandovskii, I.V.; Epishin, N.Yu.; Vrana, M.; Blažek, Z. Sodium Thiosulfate in the Treatment of Early Postischemic Disorders. *Bull. Exp. Biol. Med.* **1982**, *92*, 275–278. <https://doi.org/10.1007/bf00829520>.
47. Marutani, E.; Yamada, M.; Ida, T.; Tokuda, K.; Ikeda, K.; Kai, S.; Shirozu, K.; Hayashida, K.; Kosugi, S.; Hanaoka, K.; et al. Thiosulfate Mediates Cytoprotective Effects of Hydrogen Sulfide against Neuronal Ischemia. *J. Am. Heart Assoc.* **2015**, *4*, e002125. <https://doi.org/10.1161/JAHA.115.002125>.
48. Tokuda, K.; Kida, K.; Marutani, E.; Crimi, E.; Bougaki, M.; Khatri, A.; Kimura, H.; Ichinose, F. Inhaled Hydrogen Sulfide Prevents Endotoxin-Induced Systemic Inflammation and Improves Survival by Altering Sulfide Metabolism in Mice. *Antioxid. Redox Signal.* **2012**, *17*, 11–21. <https://doi.org/10.1089/ars.2011.4363>.
49. Shirozu, K.; Tokuda, K.; Marutani, E.; Lefer, D.; Wang, R.; Ichinose, F. Cystathionine γ -Lyase Deficiency Protects Mice from Galactosamine/Lipopolysaccharide-Induced Acute Liver Failure. *Antioxid. Redox Signal.* **2014**, *20*, 204–216. <https://doi.org/10.1089/ars.2013.5354>.
50. Sakaguchi, M.; Marutani, E.; Shin, H.; Chen, W.; Hanaoka, K.; Xian, M.; Ichinose, F. Sodium Thiosulfate Attenuates Acute Lung Injury in Mice. *Anesthesiology* **2014**, *121*, 1248–1257. <https://doi.org/10.1097/ALN.0000000000000456>.
51. Acero, G.; Nava Catorce, M.; González-Mendoza, R.; Meraz-Rodríguez, M.A.; Hernández-Zimbron, L.F.; González-Salinas, R.; Gevorkian, G. Sodium Thiosulphate Attenuates Brain Inflammation Induced by Systemic Lipopolysaccharide Administration in C57BL/6J Mice. *Inflammopharmacology* **2017**, *25*, 585–593. <https://doi.org/10.1007/s10787-017-0355-y>.

52. Renieris, G.; Droggiti, D.E.; Katrini, K.; Koufargyris, P.; Gkavogianni, T.; Karakike, E.; Antonakos, N.; Damoraki, G.; Karageorgos, A.; Sabracos, L.; et al. Host Cystathionine- γ Lyase Derived Hydrogen Sulfide Protects against *Pseudomonas Aeruginosa* Sepsis. *PLoS Pathog.* **2021**, *17*, e1009473. <https://doi.org/10.1371/JOURNAL.PPAT.1009473>.
53. Gröger, M.; Hogg, M.; Abdelsalam, E.; Kress, S.; Hoffmann, A.; Stahl, B.; Saub, V.; Denoix, N.; McCook, O.; Calzia, E.; et al. Effects of Sodium Thiosulfate During Resuscitation From Trauma-and-Hemorrhage in Cystathionine Gamma Lyase (CSE) Knockout Mice. *Shock* **2022**, *57*, 131–139. <https://doi.org/10.1097/SHK.0000000000001828>.
54. Ravindran, S.; Ramachandran, K.; Kurian, G.A. Sodium Thiosulfate Mediated Cardioprotection against Myocardial Ischemia-Reperfusion Injury Is Defunct in Rat Heart with Co-Morbidity of Vascular Calcification. *Biochimie* **2018**, *147*, 80–88. <https://doi.org/10.1016/j.biochi.2018.01.004>.
55. Schulz, J.; Kramer, S.; Kanatli, Y.; Kuebart, A.; Bauer, I.; Picker, O.; Vollmer, C.; Truse, R.; Herminghaus, A. Sodium Thiosulfate Improves Intestinal and Hepatic Microcirculation Without Affecting Mitochondrial Function in Experimental Sepsis. *Front. Immunol.* **2021**, *12*, 2121. <https://doi.org/10.3389/fimmu.2021.671935>.
56. Broner, C.W.; Shenep, J.L.; Stidham, G.L.; Stokes, D.C.; Fairclough, D.; Schonbaum, G.R.; Reh, J.E.; Hildner, W.K. Effect of Antioxidants in Experimental *Escherichia Coli* Septicemia. *Circ. Shock* **1989**, *29*, 77–92.
57. Mohan, D.; Balasubramanian, E.D.; Ravindran, S.; Kurian, G.A. Renal Mitochondria Can Withstand Hypoxic/Ischemic Injury Secondary to Renal Failure in Uremic Rats Pretreated with Sodium Thiosulfate. *Indian J. Pharmacol.* **2017**, *49*, 317–321. https://doi.org/10.4103/ijp.IJP_751_16.
58. Snijder, P.M.; Frenay, A.R.; de Boer, R.A.; Pasch, A.; Hillebrands, J.L.; Leuvenink, H.G.D.; van Goor, H. Exogenous Administration of Thiosulfate, a Donor of Hydrogen Sulfide, Attenuates Angiotensin II-Induced Hypertensive Heart Disease in Rats. *Br. J. Pharmacol.* **2015**, *172*, 1494–1504. <https://doi.org/10.1111/bph.12825>.
59. Nguyen, I.T.N.; Klooster, A.; Minnion, M.; Feelisch, M.; Verhaar, M.C.; van Goor, H.; Joles, J.A. Sodium Thiosulfate Improves Renal Function and Oxygenation in L-NNA-Induced Hypertension in Rats. *Kidney Int.* **2020**, *98*, 366–377. <https://doi.org/10.1016/j.kint.2020.02.020>.
60. Rein, J.L.; Miyata, K.N.; Dadzie, K.A.; Gruber, S.J.; Sulica, R.; Winchester, J.F. Successfully Treated Calcific Uremic Arteriolopathy: Two Cases of a High Anion Gap Metabolic Acidosis with Intravenous Sodium Thiosulfate. *Case Rep. Nephrol.* **2014**, *2014*, 765134. <https://doi.org/10.1155/2014/765134>.
61. Sen, U.; Vacek, T.P.; Hughes, W.M.; Kumar, M.; Moshal, K.S.; Tyagi, N.; Metreveli, N.; Hayden, M.R.; Tyagi, S.C. Cardioprotective Role of Sodium Thiosulfate on Chronic Heart Failure by Modulating Endogenous H₂S Generation. *Pharmacology* **2008**, *82*, 201–213. <https://doi.org/10.1159/000156486>.
62. Gröger, M.; Hogg, M.; Abdelsalam, E.; Kress, S.; Hoffmann, A.; Stahl, B.; Calzia, E.; Wachter, U.; Vogt, J.A.; Wang, R.; et al. Effects of Sodium Thiosulfate during Resuscitation from Trauma-and-Hemorrhage in Cystathionine- γ -Lyase Knockout Mice with Diabetes Type 1. *Front. Med.* **2022**, *57*, 131–139.
63. Merz, T.; Denoix, N.; Wigger, D.; Waller, C.; Wepler, M.; Vettorazzi, S.; Tuckermann, J.; Radermacher, P.; McCook, O. The Role of Glucocorticoid Receptor and Oxytocin Receptor in the Septic Heart in a Clinically Relevant, Resuscitated Porcine Model With Underlying Atherosclerosis. *Front. Endocrinol.* **2020**, *11*, 299. <https://doi.org/10.3389/fendo.2020.00299>.
64. Merz, T.; Wepler, M.; Nußbaum, B.; Vogt, J.; Calzia, E.; Wang, R.; Szabo, C.; Radermacher, P.; McCook, O. Cystathionine- γ -Lyase Expression Is Associated with Mitochondrial Respiration during Sepsis-Induced Acute Kidney Injury in Swine with Atherosclerosis. *Intensive Care Med. Exp.* **2018**, *6*, 43. <https://doi.org/10.1186/s40635-018-0208-z>.
65. Merz, T.; Stenzel, T.; Nußbaum, B.; Wepler, M.; Szabo, C.; Wang, R.; Radermacher, P.; McCook, O. Cardiovascular Disease and Resuscitated Septic Shock Lead to the Downregulation of the H₂S-Producing Enzyme Cystathionine- γ -Lyase in the Porcine Coronary Artery. *Intensive Care Med. Exp.* **2017**, *5*, 17. <https://doi.org/10.1186/s40635-017-0131-8>.
66. Messerer, D.A.C.; Gässler, H.; Hoffmann, A.; Gröger, M.; Benz, K.; Huhn, A.; Calzia, E.; Radermacher, P.; Datzmann, T. The H₂S Donor Sodium Thiosulfate (Na₂S₂O₃) Does Not Improve Inflammation and Organ Damage after Hemorrhagic Shock in Cardiovascular Healthy Swine. *Front. Immunol.* **2022**, (under review).
67. Marutani, E.; Morita, M.; Hirai, S.; Kai, S.; Grange, R.M.H.; Miyazaki, Y.; Nagashima, F.; Traeger, L.; Magliocca, A.; Ida, T.; et al. Sulfide Catabolism Ameliorates Hypoxic Brain Injury. *Nat. Commun.* **2021**, *12*, 3108. <https://doi.org/10.1038/s41467-021-23363-x>.
68. Marutani, E.; Ichinose, F. Emerging Pharmacological Tools to Control Hydrogen Sulfide Signaling in Critical Illness. *Intensive Care Med. Exp.* **2020**, *8*, 5. <https://doi.org/10.1186/s40635-020-0296-4>.
69. Zhang, M.Y.; Dugbartey, G.J.; Juriasingani, S.; Sener, A. Hydrogen Sulfide Metabolite, Sodium Thiosulfate: Clinical Applications and Underlying Molecular Mechanisms. *Int. J. Mol. Sci.* **2021**, *22*, 6452. <https://doi.org/10.3390/ijms22126452>.
70. Toohey, J.I. Sulphane Sulphur in Biological Systems: A Possible Regulatory Role. *Biochem. J.* **1989**, *264*, 625–632. <https://doi.org/10.1042/bj2640625>.
71. Toohey, J.I.; Cooper, A.J.L. Thiosulfoxide (Sulfane) Sulfur: New Chemistry and New Regulatory Roles in Biology. *Molecules* **2014**, *19*, 12789–12813. <https://doi.org/10.3390/molecules190812789>.
72. Predmore, B.L.; Alendy, M.J.; Ahmed, K.I.; Leeuwenburgh, C.; Julian, D. The Hydrogen Sulfide Signaling System: Changes during Aging and the Benefits of Caloric Restriction. *Age* **2010**, *32*, 467–481. <https://doi.org/10.1007/s11357-010-9150-z>.
73. Paris, E.I.; Pelisov, M.G. Use of Sodium Thiosulfate in Shock Due to Burns. *Voen.-Meditsinskii Zhurnal* **1966**, *5*, 38–40.
74. de Koning, M.S.L.Y.; Assa, S.; Maagdenberg, C.G.; van Veldhuisen, D.J.; Pasch, A.; van Goor, H.; Lipsic, E.; van der Harst, P. Safety and Tolerability of Sodium Thiosulfate in Patients with an Acute Coronary Syndrome Undergoing Coronary

- Angiography: A Dose-Escalation Safety Pilot Study (SAFE-ACS). *J. Interv. Cardiol.* **2020**, *2020*, 6014915. <https://doi.org/10.1155/2020/6014915>.
75. de Koning, M.S.L.; van Dorp, P.; Assa, S.; Hartman, M.H.; Voskuil, M.; Anthonio, R.L.; Veen, D.; Pundziute-Do Prado, G.; Leiner, T.; van Goor, H.; et al. Rationale and Design of the Groningen Intervention Study for the Preservation of Cardiac Function with Sodium Thiosulfate after St-Segment Elevation Myocardial Infarction (GIPS-IV) Trial. *Am. Heart J.* **2022**, *243*, 167–176. <https://doi.org/10.1016/j.ahj.2021.08.012>.
 76. Wang, K.; Ahmad, S.; Cai, M.; Rennie, J.; Fujisawa, T.; Crispi, F.; Baily, J.; Miller, M.R.; Cudmore, M.; Hadoke, P.W.F.; et al. Dysregulation of Hydrogen Sulfide Producing Enzyme Cystathionine γ -Lyase Contributes to Maternal Hypertension and Placental Abnormalities in Preeclampsia. *Circulation* **2013**, *127*, 2514–2522. <https://doi.org/10.1161/CIRCULATIONAHA.113.001631>.
 77. Holwerda, K.M.; Bos, E.M.; Rajakumar, A.; Ris-Stalpers, C.; van Pampus, M.G.; Timmer, A.; Erwich, J.J.H.M.; Faas, M.M.; van Goor, H.; Lely, A.T. Hydrogen Sulfide Producing Enzymes in Pregnancy and Preeclampsia. *Placenta* **2012**, *33*, 518–521. <https://doi.org/10.1016/j.placenta.2012.02.014>.
 78. Wigger, D.C.; Gröger, N.; Lesse, A.; Krause, S.; Merz, T.; Gündel, H.; Braun, K.; McCook, O.; Radermacher, P.; Bock, J.; et al. Maternal Separation Induces Long-Term Alterations in the Cardiac Oxytocin Receptor and Cystathionine γ -Lyase Expression in Mice. *Oxidative Med. Cell. Longev.* **2020**, *2020*, 4309605. <https://doi.org/10.1155/2020/4309605>.
 79. Denoix, N.; McCook, O.; Ecker, S.; Wang, R.; Waller, C.; Radermacher, P.; Merz, T. The Interaction of the Endogenous Hydrogen Sulfide and Oxytocin Systems in Fluid Regulation and the Cardiovascular System. *Antioxidants* **2020**, *9*, 748. <https://doi.org/10.3390/antiox9080748>.
 80. Merz, T.; Lukaschewski, B.; Wigger, D.; Rupprecht, A.; Wepler, M.; Gröger, M.; Hartmann, C.; Whiteman, M.; Szabo, C.; Wang, R.; et al. Interaction of the Hydrogen Sulfide System with the Oxytocin System in the Injured Mouse Heart. *Intensive Care Med. Exp.* **2018**, *6*, 41. <https://doi.org/10.1186/s40635-018-0207-0>.
 81. Renieris, G.; Katrini, K.; Damoulari, C.; Akinosoglou, K.; Psarrakis, C.; Kyriakopoulou, M.; Dimopoulos, G.; Lada, M.; Koufargyris, P.; Giamarellos-Bourboulis, E.J. Serum Hydrogen Sulfide and Outcome Association in Pneumonia by the SARS-CoV-2 Coronavirus. *Shock* **2020**, *54*, 633–637. <https://doi.org/10.1097/SHK.0000000000001562>.
 82. Datzmann, T.; Merz, T.; McCook, O.; Szabo, C.; Radermacher, P. H₂S as a Therapeutic Adjuvant Against COVID-19: Why and How? *Shock* **2021**, *56*, 865–867. <https://doi.org/10.1097/SHK.0000000000001723>.
 83. Evgen'ev, M.B.; Frenkel, A. Possible Application of H₂S-Producing Compounds in Therapy of Coronavirus (COVID-19) Infection and Pneumonia. *Cell Stress Chaperones* **2020**, *25*, 713–715. <https://doi.org/10.1007/s12192-020-01120-1>.
 84. Bourgonje, A.R.; Offringa, A.K.; van Eijk, L.E.; Abdulle, A.E.; Hillebrands, J.L.; van der Voort, P.H.J.; van Goor, H.; van Hezik, E.J. N-Acetylcysteine and Hydrogen Sulfide in Coronavirus Disease 2019. *Antioxid. Redox Signal.* **2021**, *35*, 1207–1225. <https://doi.org/10.1089/ars.2020.8247>.