Latest updates on SARS-CoV-2 (Corona Virus)

Chapter 2

Possible Use of Hydrogen Sulfide Donors to Treat The Consequences of SARS-Cov-2 Infection

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Abstract

Intoduction: In the last decade, the gasotransmitter hydrogen sulphide (H_2S) has received a lot of attention as a biological mediator of great importance in plant and animal kingdoms. It was demonstrated by numerous studies that H_2S may participates in several vital processes in humans under normal conditions and in the case of various pathologies and stresses. H_2S alongside with other signal molecules may provide neuroprotection, anti-inflammation, and importantly regulates cardiovascular state in humans.

Objective: The major objective of this chapter is to review recent advances in our understanding of molecular mechanisms underlying anti-inflammatory effects of H_2S in biology and medicine with special emphasis made on possible application of H_2S donors to fight with SARS-CoV-2 infection. This chapter summarizes the results of various studies demonstrated protective anti-inflammatory and antiviral effects of H_2S in various model systems and in humans.

Results: Here we describe donors producing H_2S with different kinetics and possible mechanisms of their highly diverse protective effects at cellular and organismal levels. The review of accumulated data suggests a high therapeutic potential for application of H_2S donors and specifically sodium

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thiosulfate ($Na_2S_2O_3$) to treat COVID-19 patients because of several protective properties of this gasotransmitter. Importantly, sodium thiosulfate is an approved human drug and is already widely used to treat various diseases in humans including acute pneumonia.Based on accumulated data we strongly recommend to use harmless FDA approved H_2S donor sodium thiosulfate in COVID-19inhalation-based therapy at all stages of the disease progression in particular in severe cases of the disease instead of mechanical ventilation.

Keyword: Hydrogen Sulphide; GYY4137; Heat Shock Proteins; Inflammation; Transsulphuration Pathway; Sodium Thiosulfate; Corona Virus

Abbreviations: TSP: transsulphuration pathway; STS: sodium thiosulfate; CBS: cystathionine β-synthase; CSE: cystathionine γ-lyase; MST: 3-mercaptopyruvate sulphur transferase; CVD: cardiovascular disease; Hsp: heat shock proteins; IL: interleukin; TNF-α: tumor necrosis factor; SAAs: sulfur-containing amino acids (SAAs); CDO: cysteine dioxygenase; CAT: cysteine aminotransferase; rHsp70: recombinant heat shock protein 70.

1. Introduction

Hydrogen sulphide (H_2S) was known for many years and sulfide rich water was routinely used in sanatoriums and clinics in baths to treat multiple skin and cardiovascular-related diseases in Russia and other countries for ages. Initially, H_2S has been considered an environmental poisonous gas able to inhibit cytochrome *c* oxidase exhibiting effects similar to cyanide [1,2]. Modern concept based on H_2S production in various tissues and organs and its signal role in fine regulation of numerous cellular metabolic processes under normal conditions and in various pathologies in humans has been developed and widely accepted by medical and biological communities only in the past decade [3,4,5]. Hydrogen sulphide (H_2S) along with carbon monoxide (CO) and nitric oxide (NO) belongs to the group of gasomediators or gasotransmitters [3,5].

In recent years, hydrogen sulfide (H_2S), which is formed in various mammalian tissues, has emerged as an important modulator of several biological functions including inflammation process and its deficiency has been associated with different disorders in humans [5]. Importantly, in the last decade multiple natural and synthetic donors of H_2S were discovered releasing the gas with different kinetics and efficacy [5,6,7]. Although a great deal of work has been done on the protective role of H_2S in cardiovascular diseases (CVD) and neurodegeneration exploring various *in vitro* and *in vivo* models medical application of H2S is hindered by the fact that the developed H_2S donors often used in experimental biology such as GYY4137 and NaHS are not human drugs and are not approved by FDA. This chapter is focused on recent advances in our understanding of signal and anti-inflammatory roles played by H_2S in CDV and respiratory diseases with special emphasis on possible application of harmless and widely used in medicine sodium thiosulfate to treat patients at various stages of COVID-19 disease.

2. H2S Production and Metabolism

The major enzymes and pathways involved in H_2S production and metabolism are known for many years [4,5,8]. The transsulphuration pathway (TSP) is well studied and apparently one of the most ancient reactions taking place in all studied organisms. This process leading to H_2S production includes the conversion of homocysteine to cysteine. In human, *Drosophila* and other eukaryotes H_2S is synthesized in the cells via endogenous three major enzymes and to less extent by nonenzymatic pathway. There are several excellent reviews describing basic aspects of cysteine sulfur metabolism and specific details of H_2S biosynthesis and degradation [4] Briefly, H_2S is synthesized inside the cells predominantly from cysteine by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulphur transferase (MST) which also participates in transsulfuration pathway. CBS in animals is found in various the brain and nervous system, while CSE which is also responsible for H_2S production is generated predominantly in the peripheral tissues including lungs via a reaction of L-cysteine and cystathionine. MST, which is localized mostly in mitochondria, acts as a sulphur carrier, rather than an H_2S producer. Importantly, interconversion of sulfur-containing amino acids is achieved by cysteine dioxygenase (CDO), cysteine aminotransferase (CAT), and cysteine lyase [5].

Besides well characterized enzymatic generation of H_2S from sulfur-containing amino acids (SAAs), the existence of non-enzymatic H_2S production from SAAs was also demonstrated. Non-enzymatic H_2S production *in vitro* and in blood via a reaction specific for the SAA cysteine serving as substrate also exists and under physiological conditions requires coordinated catalysis by Vitamin B_6, pyridoxal(phosphate), and iron [9].

It was demonstrated by different groups that defects of H_2S synthesizing enzyme system mentioned above are involved in a plethora of diseases in humans including atherosclerosis, ischemia, cancer and different neurodegenerative diseases [7,10-13]. The effects of H_2S administration are usually strictly time and dose-specific and sometime paradoxical. Thus, endogenous H_2S or a relatively low level of exogenous H_2S may exhibit a pro-cancer effect, whereas higher doses of H_2S led to the death of cancer cells *in vivo* and *in vitro*. This observation suggests that inhibition of H_2S biosynthesis or administration of H_2S donors may serve as two alternative novel approaches for cancer treatment [11].

It is of note, that since H_2S is a powerful reducing agent it should be rapidly consumed in the blood vessels by several endogenous oxidant species including hydrogen peroxide [5] to ensure that H_2S is quickly removed from the cells after it exerts its signal or protective role.

3. Different Donors of H_2S Used in Biology and Medicine

In the last years many series of H_2S prodrugs were developed and they could be divided in

three major classes: plant-derived natural prodrugs, hydrolysis-based prodrugs and controlled-release H_2S prodrugs [5,6,7]. While the number of H_2S donors is growing rapidly and they include ADT-OH; S-dielofenac, Naproxen derivative ATB-346 [5,7,14] and many other compounds at the present time in most of the studies with model organisms and cell cultures two donors, namely sodium hydrosulfide (NaHS) and synthetic compound GYY4137 are used. These two most popular donors differ significantly by kinetics of H_2S release and, hence, in several cases may differ in their protective effects. Sodium hydrosulfide gives a rapid bolus of H_2S, while GYY4137 is slow-releasing donor which is now widely used in *in vitro* and *in vivo* studies[4,5,8]. These two H_2S donors exhibit clear-cut anti-inflammatory effects in widely used rodent models of endotoxic shock, reducing the expression of several proinflammatory cytokines [5,15 ;16]. Thus, H_2S donors have been demonstrated to modulate the levels of LPS-induced inflammatory mediators in murine macrophages and human joint cells *in vitro* when added simultaneously or before toxin administration. Characteristically, the effect of GYY4137 on acute joint inflammation *in vivo* depends on the dose and timing of its administration [5]. On the other hand, it was shown that in mice intranasal administration of slow (GYY4137) and rapid (NaHS) hydrogen sulfide releasing donors modulates different manifestations of LPS-induced inflammation and importantly may efficiently prevent parenchymal inflammation and interalveolar thickening. Strikingly different H_2S releasing patterns of these donors may account for the differences sometimes observed in their protective effects in several rodent model systems of inflammation and CDV [3,15].

Collectively, anti-inflammatory role of H_2S-producing donors was demonstrated in numerous models of inflammation taking place in different organs. Thus, it was shown that both mentioned above H_2S donors ameliorate inflammation in the model of experimentally induced peritonitis in mice. Along these lines, it was demonstrated that GYY4137 administration modulate TNF-α and IFN levels and significantly reduces intestinal barrier injury in a mouse model of endotoximia [17]. Similarly, H_2S-releasing Naproxen derivative ATB-346 was able to reduce experimentally induced intestinal inflammation [14].

Characteristically, the above mentioned H_2S donors exerted their protective effects when applied by different routes. Thus, when injected intraperitoneally GYY4137 with high efficacy protects against myocardial ischemia and reperfusion injury by decreasing apoptosis and attenuating oxidative stress. Similarly introduced NaHS was shown to efficiently ameliorate sepsis-induced myocardial injury by activating biosynthesis of mitochondria in mice. In other studies H_2S donors were administrated intranasally, intravenously or even intragastrically. One major challenge in the development of H_2 S-based therapeutics is its efficient delivery and at the present time various drug delivery strategies are developed to use H_2S-releasing donors [18, 19, 20, 21]. In recent years we studied the effects of endogenous and exogenous H_2S at the cellular and organism levels in parallel with the investigation of protective roles of recombinant human

Hsp70 [22, 23, 24, 25]. In our studies we explored slow and fast-releasing H_2S donors in several human cell cultures [26, 27]. The performed broad scale analysis using various cell lines has shown a significant anti-inflammatory effects of H_2S which decreases major manifestations of LPS-induced inflammation such as ROS, NO, TNF-α etc. Surprisingly, similar results were obtained in our investigation of protective role of recombinant Hsp70 using the same cell cultures [25,26]. Moreover, intravenous administration of rHsp70 similar to effects of H_2S mentioned above normalized blood pressure in rats and restore several biochemical blood characteristics disturbed by LPS-challenge [28].

Interestingly, similar to our results accumulated in the studies of the effects of sub-chronic intranasal administration of recombinant human Hsp70 in two different mouse models of AD [29, 22] analogous treatment with NaHS also enhanced learning and memory of aged mice [30]. Moreover, in model mice NaHS exhibited modulatory effects on synaptic transmission in the neuromuscular junction [31] similar to neuroprotective effects of recombinant Hsp70 demonstrated in our studies [22]. Therefore, the application of major H_2S donors exerts diverse anti-inflammatory roles in various cellular and animal models that may partially coincide with well described protective effects of exogenous recombinant Hsp70 demonstrated in various models. These results suggest a cross talk between these very ancient adaptogenic systems (*hsps* and H_2S) in the fight against inflammation at different levels [32].

4. **Diverse Mechanisms Underly the Anti-Inflammatory Role of H_2S**

Various *in vitro* and *in vivo* studies clearly demonstrated that the anti-inflammatory effects of GYY4137 are realized mainly by inhibiting NF-κB signaling in the target cells. This inhibition results in the decreased secretion of major proinflammatory mediators [5, 16]. To this end, our transcriptomic analysis exploring a human cell culture and a combined effects of LPS and GYY4137 administration revealed a pronounced inhibition of many LPS-induced pro-inflammatory signal pathways. Interestingly, GYY4137 significantly ameliorated LPS-induced induction of various immune-related genes. We also demonstrated that pre-treatment with GYY4137 results in a significant inhibition of the over expression of many LPS-induced TNF effectors including several chemokines and major cytokines, colony stimulating factor 2 and transcription factor JunB [26, 27]. It is also known that H_2S affects the expression of several critical transcription factors besides NF-kB *in vivo* and *in vitro* [3,4,5]. It was also shown that H_2S administration protects primary cultures of neurons from cell death by increasing the level of reduced glutathione.Generally speaking the protection against LPS challenge may be realized by different ways and, hence, at different levels. Thus, our transcriptomic studies demonstrated a significant influence of GYY4137 on expression of multiple genes involved in immune response and inflammation. However, we are well aware that this observation by no means excludes other possible molecular targets of H_2S. It is well-known that there are different inflammatory pathways. Along these lines, it is clear that H_2S may not only act

directly on the cells but it may also trigger several indirect effects (e.g. by sulfarization etc.). Furthermore, H_2S is able to modulate the expression of numerous genes epigenetically by modifying chromatin state. It is of note that the pattern of transcriptomic changes described after GYY4137 pretreatment of human cell culture partially overlaps with that observed in the brain of mice after sub-chronic intranasal administration of recombinant human Hsp70 [22, 23, 24].To this end, we demonstrated that GYY4137 treatment *per se* induced the expression of several chaperones [26]. Furthermore, inhibitor analysis enabled us to conclude that both rHsp70 and GYY4137 exercise their protective effects against LPS-induced ROS and NO production exploring pinocytosis, as well as clathrin-; caveolin-; tubulin-and receptor-mediated types of endocytosis [27].

4. Hydrogen Sulfide Efficiently Ameliorates Ischemia and Respiratory Diseases in the Model Organisms

Pharmacological experiments using various H_2S donors as well as genetic studies using CSE knockout mice suggested the major role for this signal molecule in the regulation of blood vessel state and cardiac response to inflammation and ischemia [33, 19].While various protective effects of H_2S were demonstrated in several body systems its critical role in the cardiovascular system (CDV) functioning apparently attracted the most attention so far [4,5]. Interestingly, a deficiency in the H_2S predisposes mammals to hypertension and cardiovascular diseases. H_2S generated from sodium hydrogen sulphide (NaHS) or GYY dilates rat blood vessels by mechanisms providing the opening of vascular smooth muscle K-ATP channels [5, 13,34]. Multiple studies suggest that exogenous H_2S may play an important role in blood pressure control, angiogenesis and cardioprotection under normal conditions and in various pathologies including ischemia [3, 4]. Thus, in the rodent species administration of H_2S donors such as NaHS, S-diclofenal or GYY4137 by different routes dilate blood vessels and significantly reduced blood pressure [5, 20]. In several model system of myocardial ischemia and reperfusion highly efficient protective effects of H_2S donors were demonstrated [18]. In these models the donors were introduced by different means and were able to alleviate histological injury as well as significantly reduce the ischemia area [18, 19]. It is of note that rHsp70 was also highly efficient in ameliorating the consequences of stroke in model species including the reduction of the infarct volume. Overexpression or introduction of Hsp70 was shown to protect brain cells from ischemic damage, while the inhibition of Hsp70 synthesis, increased brain damage [35, 36, 37]. Specifically, it was shown that thiosulfate used as H_2S donor in experimentally induced ischemia exerts its antiapoptotic effects via persulfidation of caspase-3. The authors conclude that given the established safety track record of this substance, thiosulfate may be successfully used in clinics as therapeutic against ischemic brain injury [38]. H_2S was also found to be highly protective in several model systems of respiratory diseases. Application of H_2S donors protected human alveolar epithelial cells through regulating transforming growth

factor in the model of bleomycin-induced pulmonary fibrosis and restores the structure of pulmonary alveoli [39]. Similarly, in lungs sodium thiosulfate not only inhibited LPS-induced production of cytokines and nuclear factor-κB activation but also decreased permeability and lung injury. STS treatment was shown to efficiently prevent the induction of interleukin-6 in the lungs in the case of mouse caecal ligation and puncture (CLP)-induced sepsis model. Furthermore, in endothelial cells, STS increased intracellular levels of sulfide and ameliorated LPS-induced production of ROS and other mediators of inflammation. Collectively, the major protective effects of STS were probably associated with inhibition of the LPS-induced nuclear factor-κB activation [40]. Besides well-documented anti-inflammatory, concentration dependent effects of H_2S demonstrated in various systems in view of COVID-19 pandemic, special attention should be paid to antiviral properties of this gas. Thus, exploring an *in vitro* mouse model of human respiratory syncytial virus (RSV) infection, and newly developed H2S donor (TAGDD-1) treatment significant reduction of viral replication was demonstrated when the donor was added six hours after infection. In this model intranasal delivery of TAGDD-1 to infected mice significantly reduced viral replication and lung inflammation. Moreover, TAGDD-1 treatment dramatically improved several clinical disease parameters and ameliorated pulmonary dysfunction. Similarly, endogenous H_2S in the respiratory tract, was shown to participate in the regulation of several important functions including pulmonary circulation, airway tone and apoptosis [23, 41, 42]. Moreover, several groups demonstrated that besides well-documented anti-inflammatory activity, cellular H_2S exerts broad-spectrum antiviral activity both in *vitro* and *in vivo*. It has been recently demonstrated that exogenous H_2S generated by GYY4137 plays a protective role in infection of highly diverse groups of enveloped RNA viruses by inhibiting inflammatory responses and importantly viral replication. Thus, in this broad-scale study the antiviral activity of this H_2S donor was tested on enveloped RNA viruses from Filo-, Ortho-,Flavi- and Bunyavirus families. Notably, at the present time there are no FDA-approved vaccine or therapeutic available [43, 41]. The performed study demonstrated that GYY4137 application drastically reduced replication of all tested viruses probably inhibiting an early step of this process. Importantly, the observed antiviral activity exhibited by H_2S coincided with the down regulation of several major pro-inflammatory mediators and drop of viral-induced nuclear translocation of transcription factors from Interferon and Nuclear Factor (NF)-kB families. These findings definitely have important implications for the development of novel therapeutic strategies for viral respiratory infections in particular in light of COVID-19 pandemic and urge to generate harmless controlled-release H_2S donors for clinical therapeutic applications. The accumulated data suggest that application of H_2S donors may help to ameliorate the consequences of corona virus infection at all stages of the disease due to reported antiviral properties of this gas.

5. Application of Hydrogen Sulfide in Humans to Treat Various Diseases Including Acute Pneumonia

In model animal species H_2S donors are often introduced by inhalation to efficiently alleviate ventilation-induced lung injury and pneumonia often induced by bacteria or viruses [42, 44]. There are, however, several issues that hinder the wide application of H_2S therapies to treat corona virus infection in the clinics. Since hydrogen sulfide is a toxic gas its use "as is" for inhalation in humans is problematic. Theoretically water soluble sulfide salts such as Na_2S or NaHS that generate free H_2S in aqueous solutions may be used for inhalation with nebulizer, but first, there is no clinical data or any documented experience of sulfide inhalation, and second, it is important to note that pharma grade sulfides are not available. These are the reasons why clinicians focused on sodium thiosulfate ($Na_2S_2O_3$) which is FDA approved human drug. USP grade Sodium thiosulfate ($Na_2S_2O_3$) is available as Drug Substance and also as sterile solution for injections. Molecule of $Na_2S_2O_3$ contains divalent sulfur (S^{-2}) and could be a donor of hydrogen sulfide in aqueous solutions in humans. It was demonstrated by many studies that STS is quite harmless substance. It is rapidly cleared by kidney and was used for decades as an antidote against cyanide poisoning and as nephroprotectant during cisplatin administration [1]. STS pharmacokinetics has been established in healthy volunteers and hemodialysis patients [45]. This compound was also successfully used in humans to treat calciphylaxis, whichis a potentially life-threatening disease involving endovascular fibrosis, arteriolar media calcification and subcutaneous tissue thrombosis. This disease has a mortality rate of up to 80%.

Sodium thiosulfate has both antioxidant and cation-chelating properties and intravenous use of high doses of sodium thiosulfate in the treatment of this disease demonstrated significant beneficial effects in most patients [46].

As we mentioned above STS was widely used in mice and other model organisms to mitigate lung injury or inflammation [42,44]. Interestingly, in Russia in several hospitals sodium thiosulfate injections or inhalation were successfully used for many years to ameliorate the progression of lung injury and acute pneumonia even in children [47, 48, 49]. The literature available in Russia recommended inhalation treatment of patients with pneumonia with 2ml of 5% solution of sodium thiosulfate exploring individual nebulizer 2 times daily. Treatment course for pneumonia was 10-15 inhalations [50].

Stroke is emerging as a rather common recently documented complication of COVID-19 infection often observed in elderly patients with heart disease and obesity [51]. It is known that corona virus infection induces systemic inflammatory response often accompanied by severe endothelial disfunction and microthrombosis [52]. Apparently, lung microtrombosis which often resulted in stroke may contribute to the observed respiratory failure usually observed

in severe cases of corona virus infection. It is of note that H_2S was often used in clinical medicine and basic scientific research to treat stroke [53]. Since H_2S donors were successfully applied and were shown to impact stroke outcome in several stroke preclinical model systems the treatment with this gas should also be promising therapy for COVID-19 especially in patients with cardiovascular diseases. In the past few years, correlation between the level of the generation of endogenous H_2S in serum and the pathogenesis of a variety of acute and chronic inflammatory lung diseases such as asthma has been observed [23].To this end, recently direct clinical data have been obtained linking the level of H_2S in serum of SARS-CoV-2 patients and final outcome of pneumonia representing the major symptom of severe cases of the disease. Characteristically, survivors from the group with acute pneumonia had significantly higher H_2S levels on day 1 and 7 after admission. The authors conclude that H_2S level in the serum is a useful marker for severity and final outcome of the disease. As expected, based on the multiple studies of the model systems, in SARS-CoV-2 patients serum H_2S level negatively correlated with C-reactive protein and IL-6 and positively correlated with the absolute lymphocyte count in peripheral blood [54, 55].

At the present time in several Russian hospitals inhalation of STS is widely used in combination with hot helium to treat COVID-19 patients and the first results are highly promising and allow to decrease the number of patients requiring mechanical ventilation (Sergei Onikienko M.D., personal communication).

6. Conclusions

The demonstrated pivotal role of H_2S in ameliorating inflammation response and cytokine cascade as well as determination of several mechanisms of action of this gasotransmitter in the case of RNA viruses infection enable to recommend the application of harmless H_2S donor (Sodium thiosulfate) to treat the patients at any stage of COVID-19 disease. Importantly, this substance was successfully used in Russia to treat acute pneumonia resulted from bacteria and virus infection including SARS-CoV-2**.** At the first stages of the disease **t**hiosulfate applied by inhalation apparently exercises its antiviral potential to prevent corona virus amplification. At the later stages of the infection STS treatment should ameliorate lung inflammation and decrease chances of microthrombosis and stroke often associated with the disease progression. Additionally, to prevent often occurring ventilator-induced lung injury (VILI) pre- and post treatment (inhalation) with hydrogen sulfide should be routinely performed in the case of covid-19 patients requiring ventilation.

7. Conflict of interest

There is no conflict of interest with content of the book chapter.

8. Acknowledgement

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9. Compliance with Ethical Standards

The author declares that this chapter does not contain any studies with human participants.

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