

# EXPERT OPINION

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## Hydrogen sulfide as a therapeutic target for inflammation

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**Introduction:** The view of hydrogen sulfide has changed from a toxic by-product to a crucial signaling molecule, with enormous potential as a pharmacological target for diseases ranging from heart disease to sepsis. Despite this progression of ideas, there is still a large amount that is unknown about this gaseous signaling molecule. Hydrogen sulfide has been implicated as a tissue protectant in many pathological conditions, the mechanisms of tissue protection is a point of controversy, particularly distinguishing the direct actions from the indirect downstream effects of hydrogen sulfide. This point of controversy is particularly pertinent in inflammation research.

**Areas covered:** Current research into the pathways in which hydrogen sulfide can act as a pro-inflammatory molecule and as an anti-inflammatory molecule.

**Expert opinion:** How controversies regarding hydrogen sulfide may have occurred is discussed. Addressed are the direct and indirect pathways of hydrogen sulfide on inflammation, the effects of different concentrations of hydrogen sulfide and how the effects of hydrogen sulfide on the immune system vary with different delivery mechanisms. Furthermore, there is a discussion on what key gaps exist in current knowledge and must be addressed before hydrogen sulfide can be considered a valid pharmacological target.

**Keywords:** acute pancreatitis, hydrogen sulfide, inflammation, sepsis

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### 1. Introduction

Hydrogen sulfide has historically been thought of as a toxic gas with a foul odor. It elicits its toxic effects by reversibly inhibiting cytochrome *c* oxidase (complex IV in the mitochondrial electron transport chain), preventing oxidative phosphorylation and lowering the production of ATP [1]. Inhaled levels of over 500 p.p.m. can be lethal within 30 min [2]. However, over the last two and a half decades there has been growing evidence that not only is hydrogen sulfide produced by the body, that this production is crucial to many systems in the body including the cardiovascular system, CNS, immune system, pulmonary system and many more [3-6]. This has led to the acceptance of hydrogen sulfide as the third gaseous signaling molecule, along with carbon monoxide (CO) and nitric oxide (NO). This however, is still under contention by a minority in the field, primarily due to recent evidence that hydrogen sulfide may, under normal conditions, be present at levels below that believed to be of physiological significance [7]. However, it is clear that hydrogen sulfide production does occur in the body through many enzymes including cysteine aminotransferase, 3 mercaptosulfurtransferase (MST), cystathionine gamma lyase (CSE) and cystathionine beta synthase (CBS), which all involve the desulfhydration of cysteine (reviewed in [8]). Furthermore, the production and reception systems of hydrogen sulfide can be pharmacologically manipulated to alter many pathophysiology [1,9-11].

Hydrogen sulfide is an attractive pharmacological target, it is highly soluble in water, yet as a weakly polar small molecule it is also highly permeable in lipids and can cross cellular membranes easily, making it both easy to administer and highly bioavailable [2]. Unknowingly, the medicinal properties of hydrogen sulfide

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**Article highlights.**

- Hydrogen sulfide has a number of physiological effects that are beneficial during many pathologies.
- Because of the diversity of the effects of hydrogen sulfide it is difficult to elucidate which pathways are directly affected by hydrogen sulfide and which pathways are altered through the downstream effects of hydrogen sulfide.
- Exogenous and endogenous sources of hydrogen sulfide have been shown to be both pro-inflammatory and anti-inflammatory in a range of animal models of pathologies.
- The reason for these incongruities may be due to several factors including: tissue protection during a pathology resulting in a lower immune response, differing effects of high and low concentrations of hydrogen sulfide, at what concentrations hydrogen sulfide is endogenously produced during each pathology and difference between fast-releasing and slow-releasing hydrogen sulfide donors.
- There are currently several areas that need further research and corroboration before hydrogen sulfide can be developed as a therapeutic target. Key examples include further investigation into the endogenous concentrations of hydrogen sulfide, as well as the development of specific and safe inhibitors of endogenous hydrogen sulfide production.

This box summarizes key points contained in the article.

have already been in use for hundreds of years in the form of common natural remedies. Sulfur-donating compounds can be found in garlic (*Allium sativum*), in the form of diallyl thiosulfinate (allicin), and broccoli (*Brassica oleracea*), in the form of sulforaphane, these compounds have been found to be beneficial in many pathologies, such as hypertension and ischemic insult [12,13]. Research on hydrogen sulfide as a pharmacological target has grown quickly since the system's discovery. There are now many synthetic hydrogen sulfide donors that can be broken up into three broad categories, fast-releasing salts such as NaHS and Na<sub>2</sub>S, slow-releasing compounds including GYY4137 (morpholin-4-ium 4-methoxyphenyl phosphinodithioate) and S-propargylcysteine (SPRC), and existing drugs that have been modified to donate hydrogen sulfide such as NSAIDs including S-diclofenac. Further research into the development of drugs that intervene with the endogenous production of hydrogen sulfide by inhibiting the enzymes involved in its synthesis; have shown benefits in many animal models of human pathologies, especially models involving inflammation including acute renal damage and hemorrhagic shock [14,15]. The most commonly used inhibitors of hydrogen sulfide production is the moderately specific CSE inhibitor DL-propargylglycine (PAG) [14,16,17] and the CBS inhibitors hydroxylamine and aminoxyacetate [8].

The hydrogen sulfide field is not without controversy, many publications dramatically disagree with regard to the role of

hydrogen sulfide in animal models of human pathologies as well as the levels of hydrogen sulfide in the plasma and tissue. This review outlines the reported incongruities on the effects of hydrogen sulfide on the immune system and attempts to explain the cause of these differences. To discuss this it briefly addresses how hydrogen sulfide can act as a tissue protectant in many pathologies independent of its effects on the immune system, as well as the direct effects of hydrogen sulfide on the immune system, these effects may differ at different concentrations and different exposure times. We then review the difficulties of hydrogen sulfide research and the knowledge gaps in the field that must be investigated before hydrogen sulfide can fulfill its potential as a pharmacological target.

## 2. Tissue-protective effects of hydrogen sulfide

Tissue damage initiates an immune response; this response can, in some cases, become pathological inflammation causing further tissue damage. This is an important point when discussing the effects of hydrogen sulfide on inflammation. There is conflicting evidence in the literature that suggests that hydrogen sulfide is both anti-inflammatory and pro-inflammatory, often in very similar models of pathology [4,10]. It is important to assess whether the hydrogen sulfide has acted on preventing tissue damage and thus lowering the immune response, or conversely, hydrogen sulfide has acted by limiting inflammation and thus prevented further tissue damage. It is for this reason, this review briefly assesses the ways in which hydrogen sulfide may protect tissues during pathologies, independently from its direct effects on inflammation. It also discusses how hydrogen sulfide appears to affect multiple systems within the cell as well as multiple organ systems, and because of this it is very difficult to elucidate which effects of hydrogen sulfide are mediated directly by hydrogen sulfide or indirectly through downstream effects.

### 2.1 Protective effects as a vasodilator

Hypertension is often treated with vasodilators such as calcium antagonists, potassium channel openers, angiotensin-II antagonist, phosphodiesterase inhibitors, modulators of the adrenergic systems and NO donors [18]. Hydrogen sulfide acts a vasodilator; the exact mechanisms that it elicits this response are still being clarified, it appears to involve extracellular Ca<sup>2+</sup> and activating K<sub>ATP</sub> channels [19]. A few of the other potential mechanism include inhibiting phosphodiesterases [20], altering the oxidative state of vascular smooth muscle cells [21], inhibiting ACE [22], and interactions with NO, including forming a S-nitrosothiol that may have a separate vasodilation effects [23]. Modulation of hydrogen sulfide for the benefit of hypertension is a growing field; however this review addresses how this effect of hydrogen sulfide can act as a tissue protectant.

Vasodilators can act as a tissue protectant during ischemic reperfusion injury; hydrogen sulfide is not an exception [24].

It is difficult to separate vasodilation protection effects from the other cellular effects of the vasodilators themselves, as these may affect the pathophysiology independently of the vasodilation. However, the mechanisms of vasodilation-induced ischemia protection are thought to involve increased blood flow due to the dilation of the affected vessel and proximal vessels, allowing increased collateral blood flow, and increased blood vessel permeability that allows greater diffusion of nutrients and oxygen. Hydrogen sulfide has been found to be a vasodilator and this provided protection during ischemia through  $K_{ATP}$ -dependent mechanisms [25,26].

The vasodilation effects of hydrogen sulfide have also been found to be beneficial in NSAID-induced gastric pathologies. NSAIDs are one of the most commonly taken medications worldwide and are used to treat a number of conditions including mild chronic pain and prophylactically treating atherosclerosis. Yet, regular NSAID use is associated with many gastric pathologies, most commonly ulcers of the stomach and duodenum as well as gastric bleeding. The incidence of such complications is between 14 and 49% of regular users, with some of the risk factors being increased age, smoking, length of treatment and higher doses [27]. NSAIDs, by inhibiting the generation of eicosanoids and hydrogen sulfide, reduce the blood flow to the gastric mucosa, this increases the vulnerability of the mucosa to injury [28,29]. Hydrogen sulfide donating compounds, can through  $K_{ATP}$  channel-mediated vasodilation effects, prevent NSAID-induced vasoconstriction and its pathological effects [29].

## 2.2 Protective effects as a smooth muscle relaxant

A closely related effect to vasodilation is the smooth muscle relaxant effects of hydrogen sulfide. This effect has been seen in several organ systems in the body, most notably the gastrointestinal (GI) tract and the respiratory system. There is some research into the use of smooth muscle relaxants and  $K_{ATP}$  openers in treating spasm of the GI tract and diarrhea [30,31]. CBS and CSE are both expressed throughout the GI tract, hydrogen sulfide acts to relax the smooth muscle and reduce the frequency of intestinal contractions primarily through the opening of  $K_{ATP}$  channels [32]. This could lead to the development of hydrogen sulfide modulation in the treatment of diarrhea and spasms, however, it may also be important research as inhibition of GI function may be a negative side-effect of hydrogen sulfide modulation when targeting other chronic pathologies like hypertension.

The effect of hydrogen sulfide on respiratory smooth muscle is a promising pharmacological target in the treatment of airway constriction pathologies such as asthma. NaHS has been shown in mice, rats and guinea pigs to relax airway muscles, dilating the airways, increasing peak flow and reducing the effects of airway pathologies. The pathways of these effects are still to be elucidated research suggests that it is not due to modulations of  $K_{atp}$  channels, NK1 or NK2 tachykinin receptors, cGMP or COX-1 or COX-2, but may involve acidification of the cytosol of the cell and also cAMP pathways [4,33-36].

## 2.3 Protective effects of hydrogen sulfide as a suppressor of metabolic rate and hypothermia

Hydrogen sulfide can induce a hibernation-like state; this involves dropping the core body temperature and lowering the metabolic rate [1]. Hypothermia and the lowering of the metabolic rate can be therapeutic in a number of conditions ranging from ischemic insult to inflammation [37-40]. Hydrogen sulfide acts by competing with oxygen at the active site of cytochrome *c*, this inhibits the production of ATP without causing the breakdown of the electron transport chain that is seen during oxygen starvation [41]. These factors suggest that  $H_2S$  may be protective and beneficial during ischemia, multiple organ failure or during organ transplant where lactate buildup and oxygen demand can result in tissue damage or organ failure [42].

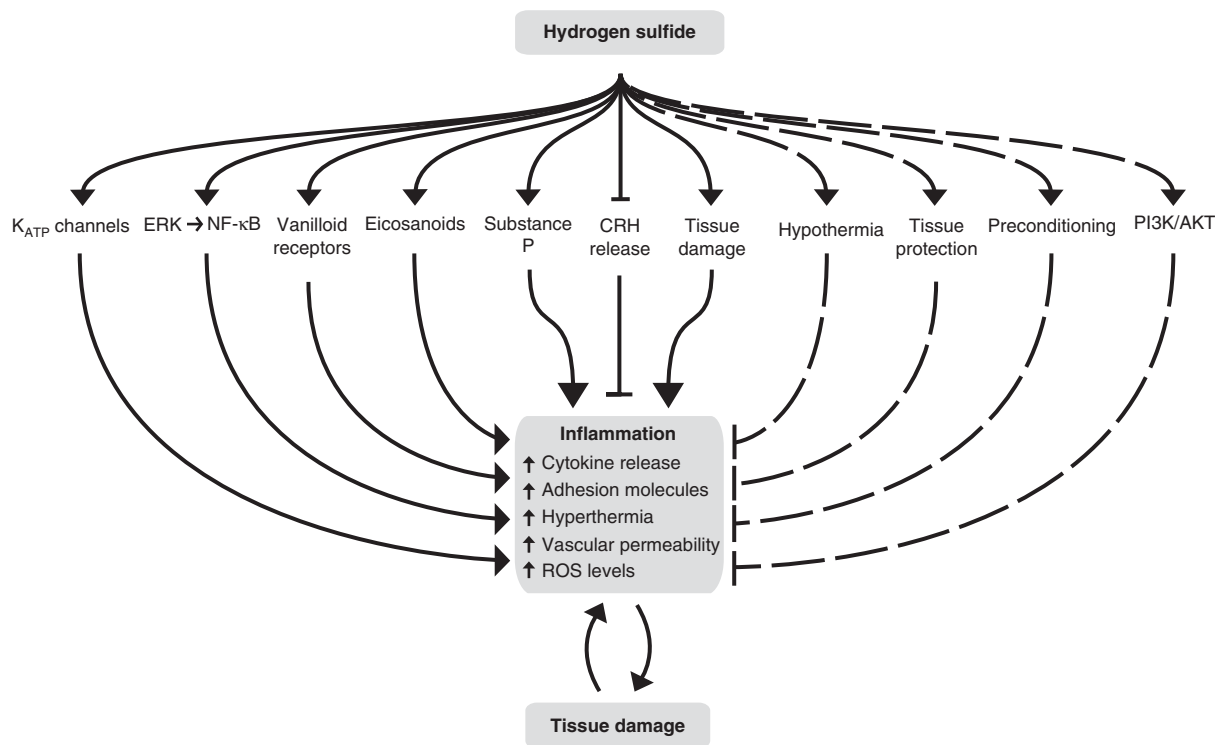
## 2.4 Hydrogen sulfide as an anti-oxidant

Reactive oxygen species (ROS) are highly reactive compounds that can react with cellular components causing dysfunction in the component such as increases in membrane permeability, loss of protein function and DNA mutation. During many diseases, such as pathological inflammation, ischemia and trauma, ROS levels increase and result in cytotoxicity [43-45]. Hydrogen sulfide can act as an antioxidant by both directly reacting with ROS and also restoring glutathione to its reduced state, increasing the cells antioxidant capacity [46-48]. There is some question as to whether hydrogen sulfide is produced endogenously at high enough levels and has appropriate reaction rates, for the antioxidant properties of hydrogen sulfide to be a significant mechanism of protection [7,49]. However, this does not question the therapeutic potential of exogenous sources of hydrogen sulfide that could result in much higher concentrations [49,50].

## 2.5 Hydrogen sulfide as an anti-apoptotic drug

In many pathological conditions cells undergo apoptosis and drugs that intervene in the process have been shown to protect tissue and improve outcome [51]. Hydrogen sulfide has been shown to modulate many of the cellular pathways of apoptosis including JNK inhibition, attenuation of caspase 9 and B cell leukemia/lymphoma 2 (Bcl2) activity [52,53]. Interestingly, the majority of studies that report anti-apoptotic effects, use models of ischemic injury [53,54].

Conversely, there is also evidence that hydrogen sulfide can be pro-apoptotic in a number of cell types such as human pulp stem cells, human gingiva epithelial cells, human aortic endothelial cells and mouse pancreatic cells [55-61]. This can increase the levels of cell death and be seen as a mechanism of hydrogen-sulfide-induced tissue damage. However, there is also evidence that induced apoptosis can protect the tissue in some pathologies, as programmed cell death is a controlled cell death and prevents the release of cytotoxic compounds from within the cell, as seen during necrosis [57,58,62,63]. More research is needed to fully clarify the direct interaction of hydrogen sulfide on apoptotic pathways and how these interactions affect different pathologies.



**Figure 1. The mechanisms in which hydrogen sulfide can act as a pro-inflammatory substance (solid black lines) and as an anti-inflammatory substance (dashed lines).** Hydrogen sulfide can act directly or indirectly to promote inflammation by: increased adhesion molecule expression resulting in leukocyte infiltration signaling via the opening of K<sub>ATP</sub> channels [87], upregulation of extracellular-signal-regulated kinase (ERK)-induced NF-κB activity [69,88-90], activation of vanilloid receptor-1 [6,10,72], generating higher levels of eicosanoids (such as prostaglandins) through the upregulation of phospholipase A<sub>2</sub> and COX enzyme activity [55,67], increased substance P production [66,91], suppression of corticotropin-releasing hormone (CRH) release [92], and increased levels of tissue damage [55]. Hydrogen sulfide can act as an anti-inflammatory substance by: Inducing hypothermia [1,81], protecting tissue from insult [13,25,44,45,47,93], preconditioning with slow release or low levels of hydrogen sulfide to induce tolerance and reduce hydrogen sulfide signaling during pathology [77,83], and activation of the PI3K/AKT pathway [82].

### 3. The role of hydrogen sulfide in inflammation

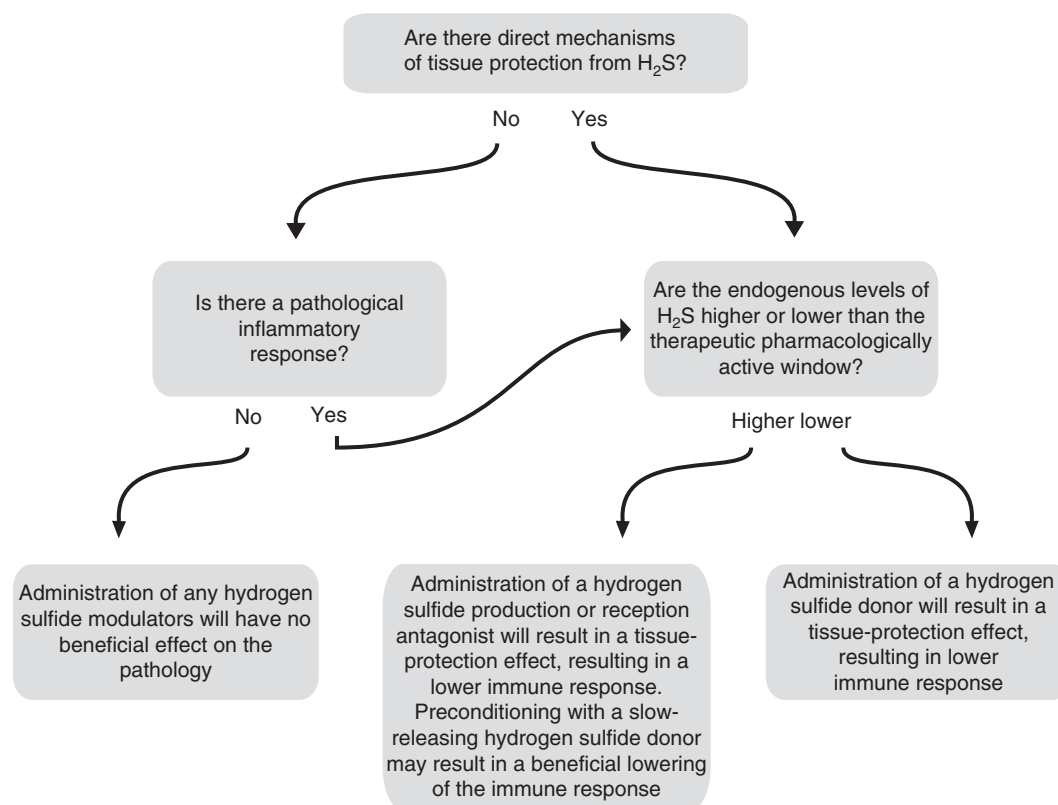
#### 3.1 Hydrogen sulfide as a pro-inflammatory compound

Hydrogen sulfide has been found to be pro-inflammatory when administered exogenously or produced at higher levels endogenously. Hydrogen sulfide has been found to be crucial in inflammation signaling in many animal models of pathologies including polymicrobial sepsis [6,64], lipopolysaccharide-induced inflammation [65], acute pancreatitis [11,66], paw edema [67], cisplatin-induced renal damage [14], hemorrhagic shock [15], airway inflammation [10] and burn injury [68], as well as cellular cultures including human monocytes [69] and mouse macrophages [70]. Several mechanisms have been theorized; however with so many potential effects of hydrogen sulfide during pathologies it is difficult to elucidate the exact pathways by which the hydrogen sulfide is eliciting its pro-inflammatory effects (Figure 1).

Hydrogen-sulfide-donating salts have been used to elucidate its function on the immune system during pathologies. Bianca *et al.* [67] administered NaHS and the precursor of hydrogen sulfide L-cysteine, to the hind paw of mice. Both induced pathological inflammation and edema. This was alleviated by inhibitors of the COX enzymes as well as inhibitors of phospholipase A<sub>2</sub>, this suggests either a direct or indirect role of eicosanoids in hydrogen-sulfide-induced inflammation (Figure 1) [67]. This research also showed that administration of L-cysteine, the substrate of the CSE and CBS enzymes, induces inflammation, suggesting, in this instance, that the supply of L-cysteine is the limiting factor of hydrogen sulfide production.

Hydrogen sulfide has also been shown to be produced at pathological levels during some pathologies. Blocking the production of hydrogen sulfide with the CSE inhibitor PAG has been shown to reduce inflammation, indicating the pathological role of endogenous hydrogen sulfide [71]. A study by Francescato *et al.* [14] induced nephrotoxicity in Wistar rats using the anticancer drug cisplatin, they saw a reduction of tissue





**Figure 2. A partial explanation of the differing results of hydrogen sulfide administration during pathologies.** Demonstrating, for each pathological state, the tissue-protection effects of hydrogen sulfide [94], endogenous production of hydrogen sulfide [34], the inflammatory response [11] and the method of hydrogen sulfide modulation [70], can alter how intervention in hydrogen sulfide signaling will affect the pathology.

damage as well as a reduction in inflammatory markers when casp1 was co-administered with PAG. Suggesting that hydrogen sulfide was being produced by CSE at levels that were either inducing tissue damage, signaling a pathological inflammatory response or both. Ang *et al.* [6] used a mouse model of sepsis to demonstrate that CSE-produced hydrogen sulfide may act through the vanilloid-1 receptor on sensory afferents, to induce an inflammatory response. They found that blocking either the production of hydrogen sulfide with PAG or the action of hydrogen sulfide on the vanilloid-1 receptor with an antagonist, improved the survival rates of the mice during sepsis (Figure 1). Furthermore, in a primary cell model of pancreatitis, Tamizhselvi *et al.* [66] demonstrated that PAG reduced pro-inflammatory marker levels following cerulean administration, these markers were also significantly lower when the cells were harvested from preprotachykinin-A gene-knockout mice, these mice cannot produce substance P, suggesting that hydrogen sulfide may be acting through this pathway to induce its pro-inflammatory effects (Figure 1) [72,73].

Hydrogen sulfide has also been shown to cause the sulfhydrylation of cysteine-38 of NF- $\kappa$ B. This sulfhydrylation promotes NF- $\kappa$ B binding with ribosomal protein S3 (RPS3). RPS3 is a co-activator that promotes DNA binding of

NF- $\kappa$ B, directly modulating transcription. This activation of NF- $\kappa$ B pathways, causes the upregulation of expression and secretion of pro-inflammatory cytokines [74].

Taken together it appears that two mechanism of hydrogen sulfide induced inflammation include: The activation of the vanilloid receptor 1 on sensory afferents, which then stimulates the release of substance P, this causes leukocyte adhesion, cytokine release and other process of inflammation through the activation of the NK-1 receptor [75]. Hydrogen sulfide also causes the direct activation of NF- $\kappa$ B through sulfhydrylation that results in NF- $\kappa$ B-induced upregulation of pro-inflammatory gene expression.

From this it is clear that there are direct pro-inflammatory effects of hydrogen sulfide on the immune system, understanding at what concentration this occurs and whether these concentrations can occur through endogenous production, is crucial to our understanding of hydrogen sulfide as a signaling molecule.

### 3.2 Hydrogen sulfide as an anti-inflammatory compound

Conversely, endogenous production and exogenous administration of hydrogen sulfide has also been found to be anti-inflammatory in many models of pathologies including

asthma [4], synovitis [76], LPS-induced inflammation [77], rhinitis [34], ischemia–reperfusion [78], neuropathologies [79] and gastric pathologies [29,80]. The mechanisms of hydrogen sulfide's anti-inflammatory properties are still to be elucidated and may be more indirect than the pro-inflammatory effects, and may occur primarily with administration of lower concentrations and/or slow releasing compounds (Figure 1).

An indirect mechanism of hydrogen sulfide being anti-inflammatory is the induction of hypothermia. Hypothermia is known to suppress inflammation in many pathologies resulting in less tissue damage and improved outcome [37,38,81]. Interestingly, hypothermia acts through downregulation of NF- $\kappa$ B, NF- $\kappa$ B is also key factor in hydrogen sulfide signaling, this complicates the research, making it difficult to separate the exact mechanisms of hydrogen sulfide in immune function (Figure 1) [81]. For this reason the body temperature of animals involved in mechanistic hydrogen sulfide studies, must be closely monitored in order to rule out the indirect effects of hypothermia.

A further indirect mechanism is tissue protection. As mentioned above there are many ways in which hydrogen sulfide has been implicated as a tissue protectant. Because the level of tissue damage and the corresponding inflammatory response are linked (Figure 1), it is difficult to separate the tissue protection effects and the subsequent drop in inflammatory response, from the potential direct effect hydrogen sulfide has on inflammation.

There is some research that establishes a direct anti-inflammatory effect of hydrogen sulfide. Tamizhselvi *et al.* [82] demonstrated that treatment of pancreatic acinar cells with lower doses of NaHS (5  $\mu$ M), reduced the production of inflammatory cytokines. This was abated by inhibition of PI3K. Furthermore, Western blot analysis demonstrating the PI3K phosphorylation of protein kinase B (AKT) was crucial to the anti-inflammatory signaling of hydrogen sulfide. This demonstrates a clear direct link between hydrogen sulfide and anti-inflammatory effects, these and other pathways need further research at a range of hydrogen sulfide concentrations in several inflammatory models, to further corroborate these direct links.

#### 4. Conclusion

Hydrogen sulfide donors have been found to be beneficial in many pathological conditions. However, because of the broad range of its effects, it is difficult to elucidate the exact pathways in which hydrogen sulfide has been protective. Conversely, hydrogen sulfide has been found to be pro-inflammatory, evidence for this involves hydrogen-sulfide-donating compounds that induce inflammation, as well as inhibitors of endogenous hydrogen sulfide production decreasing inflammation. Hydrogen sulfide also, in some situations, appears to be anti-inflammatory, this may be indirectly through the many tissue-protective effects of hydrogen sulfide and the hypothermic effects of hydrogen

sulfide, or through direct effects of hydrogen sulfide on the immune system that appear to involve the PI3K/AKT signaling pathway.

#### 5. Expert opinion

One of the most controversial issues with hydrogen sulfide is its role in inflammation. A key cause of this controversy is that hydrogen sulfide can act on the immune system by many different pathways (Figure 1). Different pathologies will be affected by the modulation of these pathways in different ways, possibly resulting in either a pro- or anti-inflammatory effect. The hydrogen sulfide system is further complicated by endogenous production, with different pathologies under varying conditions producing different levels of hydrogen sulfide. It is possible that in some cases the body is producing hydrogen sulfide above the pharmacologically efficacious window and adding an exogenous source of hydrogen sulfide exacerbates the condition causing increased tissue damage and an increased inflammatory response, and in other pathologies the converse could be occurring (Figure 1).

##### 5.1 Tissue protection and inflammation

It is difficult to draw conclusion from hydrogen sulfide studies of inflammation *in vivo*, because to induce clinically applicable inflammation an injury or infection must be modeled. In doing so tissue damage will occur, this will most often involve not only an inflammatory response but an increase in ROS and apoptosis both of which hydrogen sulfide may affect independently of inflammation, and by doing so cause a reduction in tissue damage and a subsequent reduction in inflammation. Furthermore, ischemic pathologies would greatly benefit from a reduction of metabolism and body temperature as well as vasodilation, therefore the anti-inflammatory properties seen maybe again indirectly through tissue protection [78]. Too often papers are concluding that the primary mode of action of hydrogen sulfide in tissue protection is through anti-inflammatory properties, justifying the conclusion through a reduction in inflammatory markers, without properly investigating the many other tissue-protecting effects of hydrogen sulfide. This also occurs with other mechanisms of tissue protection, where conclusions are drawn on how hydrogen sulfide is acting as a tissue protectant, without proper investigation. As an example the suggestion that the anti-oxidant capacity of hydrogen sulfide is the primary method of protection in ischemia, due to the lowering of markers of oxidative stress, without giving proper consideration to the hypothermic, vasodilation and metabolic effects of hydrogen sulfide, which may be the primary mechanisms of protection, with the drop in oxidative markers merely being a symptom of the tissue protection. Proper controls are needed for any study with hydrogen sulfide as inflammation and oxidative stress are a result of tissue damage as well as a cause, therefore any tissue protection may reduce inflammation and oxidative stress markers without any direct

effect, alternatively a direct effect on reducing inflammation and oxidative stress may result in a tissue protection effect.

## 5.2 Hydrogen sulfide concentrations and cellular studies

Cellular studies of hydrogen sulfide are helping clarify its role in inflammation. Zhi *et al.* [69] demonstrated that NaHS induced human monocytes to produce pro-inflammatory cytokines; however, the concentrations used showed no significant effect until 100  $\mu\text{M}$ , these concentrations may turn out to be too high to be considered endogenously applicable concentrations, these concentrations would then only be reached under pharmaceutical intervention [7]. A similar study by Whiteman *et al.* [70] on a mouse macrophage cell line, saw a similar result to that of the Zhi *et al.* [69] paper with NaHS, however, they found that when administering a compound that slowly released hydrogen sulfide, macrophage activation was suppressed. Some theorize that fast-release hydrogen sulfides compounds, such as sulfide salts (NaHS and  $\text{Na}_2\text{S}$ ), are pro-inflammatory possibly because of the brief periods of high concentration of hydrogen sulfide, and that compounds that slowly release hydrogen sulfide such as GYY4137, SPRC and Lawessons's reagent are anti-inflammatory. Furthermore, slow release is more similar to endogenous production of hydrogen sulphide, suggesting that the true physiological function of hydrogen sulfide on the immune system is an inhibitory one [70,83].

It has been well established that the immune system can undergo tolerance-like effects, that is, exposure to a pro-inflammatory substance at low levels prior to a high level exposure can reduce the inflammatory response to the high-level stimulus [84]. It is possible that this is a mechanism by which slow-releasing hydrogen sulfide compounds are acting as anti-inflammatories (Figure 2), however, more research is needed.

Cellular studies cannot elucidate the exact function of hydrogen sulfide endogenously until the precise concentrations of hydrogen sulfide in healthy and pathological tissue are established. Differing levels of hydrogen sulfide are found using different techniques. Predicted levels are considerably higher with colorimetric methods (10 – 100  $\mu\text{M}$  in tissue), than the voltametric methods (< 100 nM) [2,7]. This illustrates the need for further corroboration on hydrogen sulfide levels in tissue and plasma.

## 5.3 Endogenous production and the pharmacologically active window

The division of hydrogen sulfide's effects solely as fast-releasing compounds being pro-inflammatory and slow-releasing compounds being anti-inflammatory may be inaccurate. Endogenous enzymatic production can be pro-inflammatory and would not be considered equivalent to fast-releasing compounds. The blocking of endogenous production has been found to suppress inflammation, indicating that endogenous hydrogen sulfide has a role in immune cell recruitment and activation signaling [14,15,43,68]. However, this research

is clouded with issues of non-specificity and potential toxicity of hydrogen-sulfide-producing-enzyme inhibitors, such as PAG [85].

A further difficulty in investigating hydrogen sulfide's function in the immune system, is the potential of a pharmacologically active window in which hydrogen sulfide may be beneficial and how this compares with endogenous production of hydrogen sulfide (Figure 2). Some studies indicate the pharmacologically active window for hydrogen sulfide is very narrow with a study by Yenari and Han [81] on pancreatic acinar cells showing a reduction in inflammatory markers at 5 and 10  $\mu\text{M}$  but not at 30  $\mu\text{M}$ . This narrow window is of significance when considering endogenous production, in some pathologies, such as rhinitis, the endogenous production has been found to be lower than that of healthy controls, and increasing hydrogen sulfide levels with donors was beneficial. This may be because low levels of hydrogen sulfide induce oxidative stress and subsequent inflammation (Figure 2) [34]. In other pathologies the endogenous concentrations may be too high, inducing pathological inflammation and possibly toxic effects (Figure 2) [14,55]. In these pathologies there is potential to use hydrogen sulfide as a biomarker for the severity of the disease. Indeed, preliminary results have shown that circulating levels of  $\text{H}_2\text{S}$  are elevated in patients with severe acute pancreatitis as compared with control population and patients with mild acute pancreatitis [86]. These factors emphasize the need to develop specific and non-toxic antagonist of CBS, CSE and MST, not only for research purposes but also for their potential as pharmaceuticals, as well as highly accurate methods of measuring the concentrations of hydrogen sulfide in serum and tissue.

## 5.4 Summary

It is clear that hydrogen sulfide modulation could have huge medical potential in many pathological conditions. Its multifaceted action makes it a difficult compound to study, however; it also widens its potential as a pharmacological target. For this field of research to move forward several steps need to be taken: We must attempt to isolate the exact function of hydrogen sulfide in each pathological system and attempt to separate the indirect effects from the direct effects of hydrogen sulfide on tissue damage and the immune system, additional research is needed in assessing the exact levels of hydrogen sulfide in the healthy and pathological tissue and plasma, we also must develop specific and non-toxic inhibitors of the enzymes involved in hydrogen sulfide production and, finally, research is needed to establish the differing effects of slow-releasing and fast-releasing compounds. Only once this knowledge is gained, may hydrogen sulfide become a valuable pharmacological target for treating conditions in clinical settings.

## Declaration of interest

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