Hydrogen sulphide and its therapeutic potential

Csaba Szabó

Abstract | Hydrogen sulphide (H_2S) is increasingly being recognized as an important signalling molecule in the cardiovascular and nervous systems. The production of H_2S from L-cysteine is catalysed primarily by two enzymes, cystathionine γ-lyase and cystathionine β-synthase. Evidence is accumulating to demonstrate that inhibitors of H_2S production or therapeutic H_2S donor compounds exert significant effects in various animal models of inflammation, reperfusion injury and circulatory shock. H_2S can also induce a reversible state of hypothermia and suspended-animation-like state in rodents. This article overviews the physiology and biochemistry of H_2S, summarizes the effects of H_2S inhibitors or H_2S donors in animal models of disease and outlines the potential options for the therapeutic exploitation of H_2S.

Hydrogen sulphide (H_2S) is a colourless, flammable, water-soluble gas with the characteristic smell of rotten eggs. For many decades, H_2S received attention primarily as a toxic gas and as an environmental hazard, but it is also produced in mammals including humans, and it can be detected in significant amounts[1–3] (FIG. 1).

H_2S is synthesized endogenously in various mammalian tissues by two pyridoxal-5′-phosphate-dependent enzymes responsible for metabolizing L-cysteine: cystathionine β-synthase (CBS, EC 4.2.1.22) and cystathionine γ-lyase (CSE, EC 4.4.1.1). The substrate of CBS and CSE, L-cysteine, can be derived from alimentary sources or can be liberated from endogenous proteins. It can also be synthesized endogenously from L-methionine through the trans-sulphuration pathway, with homocysteine being an intermediate in the process[1,2]. In tissue homogenates, rates of sulphide production are in the range of 1–10 pmoles per second per mg protein[4]. This results in low micromolar extracellular concentrations of sulphide. Sulphide can be rapidly consumed and degraded by various mammalian tissues.

The organ-specific expression and molecular regulation of CBS and CSE have been characterized by multiple groups. CBS is the predominant H_2S-generating enzyme in the brain and nervous system and is highly expressed in liver and kidney. CSE is mainly expressed in the liver and in vascular and non-vascular smooth muscle. Low levels of CSE are also detectable in the small intestine and stomach of rodents[2]. The activity of CBS is regulated presumably at the transcriptional level by glucocorticoids and cyclic AMP. The activity of CBS can be directly inhibited by nitric oxide (NO) and carbon monoxide (CO)[5]. It can be activated by the NO donor sodium nitroprusside in a manner that paradoxically does not involve NO, but involves a chemical modification of the enzyme[6]. The regulation of CBS has been overviewed recently[7]. The regulation of CSE is less understood, but there is evidence that myeloid zinc finger 1 (MZF1) and specificity protein 1 (SP1; also known as Sp1 transcription factor) play roles in its basal transcriptional activity[8], and the enzyme can be upregulated by bacterial endotoxin[7,8]. It is important to note that substantial differences exist between the human and the mouse CBS and CSE enzymes[2,7,8].

The enterobacterial flora is another source of H_2S. The intestinal epithelium expresses specialized enzyme systems that efficiently degrade sulphide to thiosulphate and sulphate — presumably to protect itself against high local concentrations of sulphide, and to prevent an excessive entry of H_2S into the systemic circulation[2,9]. There are also several inorganic sources of H_2S in the body, including a non-enzymatic reduction of elemental sulphur using reducing equivalents obtained from the oxidation of glucose, as described in erythrocytes[10]. For an in-depth overview on the source and metabolism of elemental sulphur in the mammalian body and for the overview of the fate of labile sulphur molecules in the mammalian body, the reader is referred to REFS 11–17. This article, after overviewing the biological effects of H_2S and evidence for the role of sulphide in various diseases, discusses strategies, results in disease models and challenges for exploiting the potential of therapeutic interventions related to H_2S.

Sulphide
In this article, the term sulphide is used to collectively describe all biologically active sulphide-related species, including HS^-. When referring to the chemical compounds, the chemical terms H_2S, Na_2S or NaHS are used.

Endotoxin
A toxin produced by Gram-negative bacteria and released from the bacterial cell.

Ikaria Inc., 1616 Eastlake Ave East, Suite 340, Seattle, Washington 98102, USA.
e-mail: csaba.szabo@ikaria.com
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Biological effects of H_2S

H_2S, together with NO and CO, belongs to a family of labile biological mediators termed gasotransmitters, which share many similarities (TABLE 1). As a gasotransmitter, H_2S rapidly travels through cell membranes without using specific transporters. H_2S exerts a host of biological effects on various biological targets (FIG. 2), resulting in responses that range from cytotoxic effects to cytoprotective actions[18–32], some of which are summarized in TABLE 2.

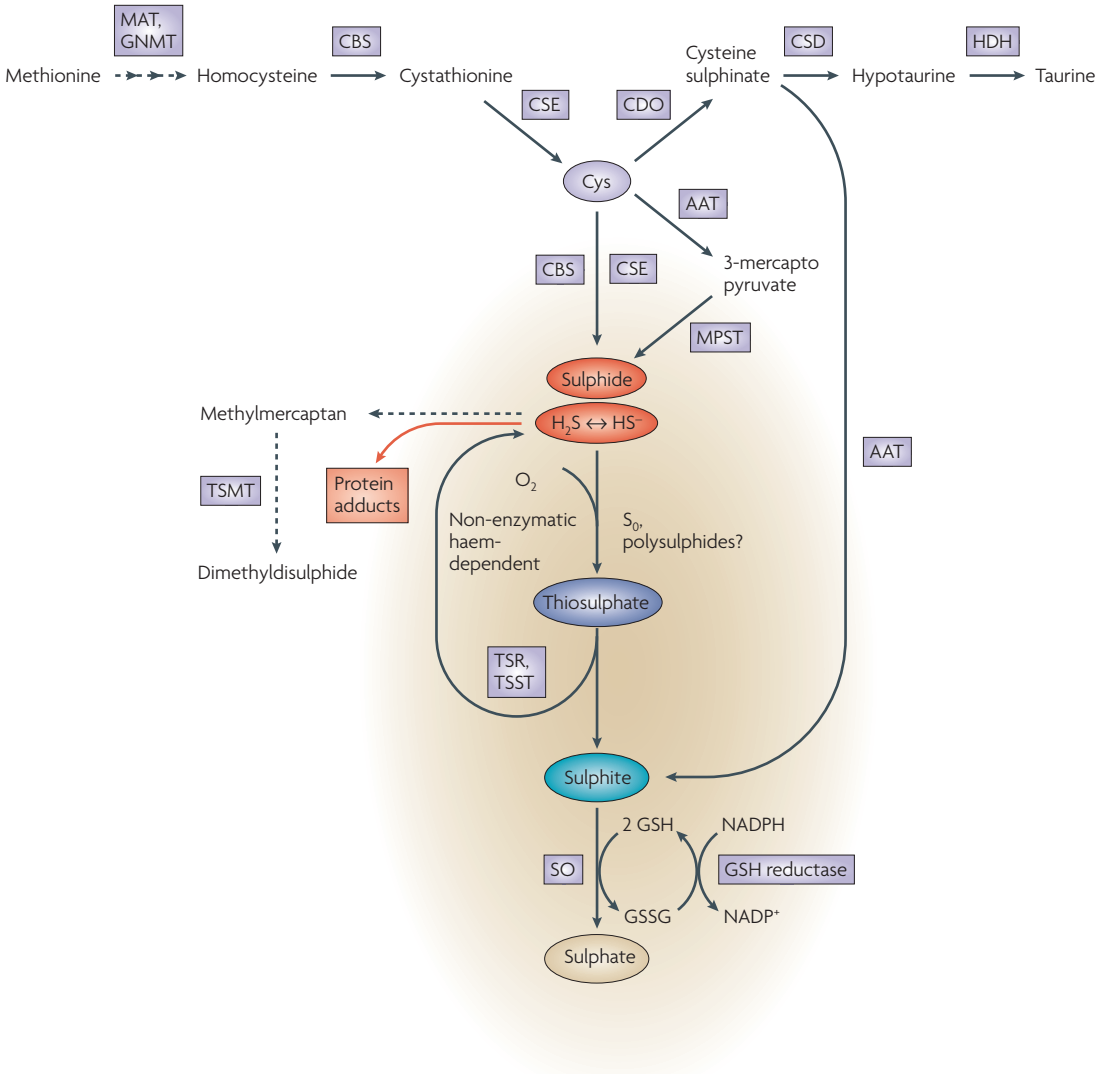

Figure 1 | **Enzymatic pathways of H_2S production in mammalian cells.** Methionine, which is derived from alimentary sources, is converted to *S*-adenosylmethionine by methionine adenosyltransferase (MAT). *S*-adenosylmethionine is subsequently hydrolysed to homocysteine by glycine *N*-methyltransferase (GNMT). Cystathionine β-synthase (CBS) catalyses the production of cystathionine by transferring serine to homocysteine. Cystathionine γ-lyase (CSE), a pyridoxal 5′-phosphate-dependent enzyme, subsequently converts cystathionine to cysteine (Cys). CSE catalyses a β-disulphide elimination reaction that results in the production of pyruvate, NH_4^+ and thiocysteine. Thiocysteine may react with cysteine or other thiols to form hydrogen sulphide (H_2S). One pathway of cysteine metabolism involves its oxidation to cysteine sulphinate by cysteine deoxygenase (CDO), which then gets further converted to hypotaurine by cysteine sulphinate decarboxylase (CSD), and subsequently to taurine by a non-enzymatic reaction or by hypotaurine dehydrogenase (HDH). The above reactions predominantly take place in the cytosol. In the mitochondria, cysteine can get converted to 3-mercaptopyruvate by aspartate aminotransferase (AAT), which can then be converted to H_2S by 3-mercaptopyruvate sulphur transferase (MPST). Sulphide, via non-enzymatic reactions, gets metabolized to thiosulphate (one molecule of sulphide yields two molecules of thiosulphate), which then gets converted to sulphite by thiosulphate reductase (TSR) — for instance in liver, kidney or brain tissues — or thiosulphate sulphurtransferase (TSST), which is predominantly expressed in the liver. The conversion of cysteine sulphinate to sulphinyl pyruvate by AAT, followed by a non-enzymatic reaction, can also yield sulphite. Sulphite gets oxidized to sulphate by sulphite oxidase (SO) by a glutathione (GSH)-dependent process. H_2S can also yield protein adducts, and can be converted to methylmercaptan and dimethyldisulphide by thiol-*S*-methyltransferase (TSMT). Non-enzymatic oxidation of sulphide can also yield the generation of polysulphides and elemental sulphur (S_0).

At micromolar concentrations, multiple studies have demonstrated the cytoprotective (antinecrotic or anti-apoptotic) effects of H_2S — typically generated *in vitro* from Na_2S or NaHS[26–29], which may be related to its ability to neutralize a variety of reactive species including oxyradicals[33] and peroxynitrite[26], hypochlorous acid[27] and homocysteine[28]. Some of these cellular effects are related to the modulation of intracellular caspases or kinase pathways, as detailed in TABLE 2. Multiple studies have also demonstrated that low levels of H_2S can upregulate endogenous antioxidant systems and can exert additive effects with known antioxidants, including *N*-acetylcysteine, glutathione and superoxide dismutase. Inhibition of endogenous H_2S production has been shown to enhance the cytotoxic effect of exogenous H_2S, which points towards a cytoprotective effect of low (physiological) levels of endogenous sulphide[21]. Higher (millimolar) H_2S exposure tends to be cytotoxic to the cells; this is due to free radical and oxidant generation, calcium mobilization, glutathione depletion, intracellular iron release, as well as induction of mitochondrial cell death pathways (TABLE 2).

It has been reported that relatively low concentrations of H_2S increase the production of cAMP, selectively enhance *N*-methyl-D-aspartate (NMDA)-receptor-mediated responses and facilitate the induction of hippocampal long-term potentiation in the CNS[24]. As with the other gasotransmitters, there is not a single sulphide receptor that is responsible for the biological actions of sulphide. Sulphide can bind to haem proteins as an axial ligand for the prosthetic group. Hence, sulphide has the potency to modulate the activities of haem-containing enzymes *in vitro,* which contributes to some of its (patho)physiological effects. Sulphide, as a thiol with strong reducing activities, may also be an important redox-controlling molecule similar to other small thiols, such as cysteine and glutathione. There are, indeed, indications for reactions of sulphide that are consistent with a direct antioxidant effect[26] — which is consistent with its cytoprotective effects in cell-based experimental systems[23,24]. Increasing cellular glutathione levels by the activation/expression of γ-glutamylcysteine synthetase (also known as glutamate cysteine ligase, EC 6.3.2.2) is another mechanism by which sulphide can exert cytoprotective effects, as demonstrated in primary cortical neurons exposed to the excitotoxin NMDA[24].

H_2S can also induce an upregulation of anti-inflammatory and cytoprotective genes including haem oxygenase 1 (*HO1*; also known as *HMOX1*) in pulmonary smooth muscle cells *in vivo*[34], in macrophages *in vitro*[22] and in rat nasal tissues *in vivo* (D. Dorman, personal communication). Microarray analysis in an intestinal cell line identified a substantial number of H_2S-regulated genes, including the gene for cytochrome c oxidase subunit V (with a simultaneous downregulation of the other subunits of the enzyme), vascular endothelial growth factor (*VEGF*), the insulin-like growth factor (*IGF*) receptor and several genes associated with the transforming growth factor-β (TGFβ) receptor pathway[20].

By upregulating HO1, H_2S can trigger the production of CO, another gasotransmitter with well-documented cytoprotective and anti-inflammatory effects[35], including inhibition of the nuclear factor-κB (NFκB) pathway and downregulation of inducible NO synthase (iNOS) expression and NO production by inflammatory stimuli[22]. The modulation of NFκB and iNOS by sulphide may be cell-type and stimulus dependent; for example, a potentiation of these responses has been reported in interleukin 1β (IL1β)-stimulated rat vascular smooth-muscle cells[36]. The expression of HO1 is probably the result of the activation by sulphide of the ERK pathway (TABLE 2).

A distinct pharmacological effect of H_2S relates to the opening of potassium-opened ATP channels (K_{ATP} channels)[37]. Some of the pharmacological actions of H_2S — including some of the vasodilatory effects as well as some of the preconditioning and cardiac protective effects — can be prevented by pretreatment with K_{ATP}-channel inhibitors such as glibenclamide[1,37]. Because of the crucial role of K_{ATP} channels in the regulation of pancreatic insulin secretion, multiple studies have examined the effect of H_2S on β-cells. In the INS-1E cells, H_2S was shown to open the K_{ATP} channels and inhibited insulin secretion[38]. In isolated mouse islets and in MIN6 cells, H_2S also suppressed insulin release. This effect, however, was not prevented by the K_{ATP}-channel blocker tolbutamide[39]. Interestingly, the inhibitory effect of L-cysteine on insulin secretion is also mediated by the endogenous release of H_2S[39].

The effects of H_2S in isolated organ systems usually include smooth-muscle relaxation, and (at high concentrations) suppression of the metabolic rate of the affected cell or organ. The smooth-muscle relaxing effects of sulphide (for example, in stomach or in blood vessels) are well characterized *in vitro*[1,2] and seem to involve a small and variable component of NO-related mechanisms, as well as K_{ATP}-channel opening and possibly an endothelium-dependent hyperpolarizing factor-like effect. It is noteworthy that recent studies in rat aortic rings have questioned the universal applicability of a K_{ATP}-channel-dependent action in the vasorelaxing effect of sulphide[40] (L. Kiss and C.S., unpublished observations). Even the universal applicability of H_2S as a vasorelaxant has been called into question: the vascular effect of sulphide is dramatically dependent on the ambient oxygen concentration, also the vascular effect of sulphide appears to be a rapid vasoconstrictor in well-oxygenated vascular ring systems[41]. A recent report points to the involvement of intracellular pH changes and the Cl^-/HCO_3 exchanger in the process of sulphide-induced vascular relaxation[171]. Endogenous sulphide production — via a K_{ATP}-channel-independent mechanism — seems to have a role in the hypoxic relaxation of the urinary bladder smooth muscle[42].

Injection of donors of H_2S to animals induces a transient hypotensive response[1,37]. It is unclear, however, whether this effect is physiologically relevant, as pharmacological inhibitors of H_2S production do not appear to exert haemodynamic effects in normal animals — as opposed to inhibitors of NO biosynthesis, which produce significant vasoconstrictor and pressor effects in normal animals and result in a reduction in the blood flow to vital organs[43,44]. A recent study implicates H_2S in blood-pressure regulation via its interaction with the NO pathway[45].

Long-term potentiation
The prolonged strengthening of synaptic communication, which is induced by patterned input and is thought to be involved in learning and memory formation.

Haem proteins
Haem proteins contain an iron complex of porphyrin, usually protoporphyrin IX, and function as catalysts in many biological processes.

Cytochrome c oxidase
A component of the oxidative phosphorylation machinery within the cell that normally binds oxygen.

Table 1 | **Comparison of nitric oxide, carbon monoxide and hydrogen sulphide**

Aspect	Nitric oxide	Carbon monoxide	Hydrogen sulphide
Initially characterized as a toxic gas or an environmental hazard?	Yes, it was known to be produced in exhaust fumes from internal combustion engines	Yes, it has long been known as a toxic gas emitted from the partial combustion of organic molecules, for example from internal combustion engines	Yes, it has been recognized as a toxic gas emanating from sewers, swamps and as a toxic by-product of industrial processes
Subsequently discovered as a molecule produced by mammalian cells?	Yes, it is now known to be produced from L-arginine by a class of enzymes (nitric oxide synthases), as well as various non-enzymatic processes (for example, from nitrite under acidic conditions)	Yes, it is now recognized to be synthesized from haem as a product of the enzyme haem oxygenase	Yes, it is now recognized to be synthesized from L-cysteine as a product of CBS and CSE, as well as a product of non-enzymatic processes in mammalian cells
Pharmacological inhibitors of its production available?	*N*-substituted L-arginine derivatives, for example, N^G-methyl-L-arginine (L-NMMA), guanidine derivatives (for example, aminoguanidine) and many others	Inhibitors of haem oxygenase, typically Zn-protoporphyrin-IX	Inhibitors with limited potency and utility include BCA and PAG
Diffusible, labile gas, rapidly eliminated by mammals?	Yes, breakdown products include nitrite and nitrate; half-life of seconds and eliminated as nitrite and nitrate in urine	Yes, half-life of minutes as carboxy-haemoglobin and eliminated via exhaled air	Yes, breakdown products include thiosulphate, sulphite and sulphate; half-life of minutes and eliminated mainly as free and conjugated sulphate in the urine
Reactions with haemoglobin?	Yes, to yield nitrosylhaemoglobin and methaemoglobin	Yes, to yield carboxyhaemoglobin	Yes, to yield sulphaemoglobin
Free radical?	Yes	No	No
Its 'receptor'?	cGMP with high affinity; also thiol groups, haem groups; K_{Ca} channels and others	cGMP with lower affinity; K_{Ca} channels	K_{ATP} channels and perhaps others
Does it affect cellular energetics?	Yes, via cytochrome c oxidase and other mitochondrial enzymes and the nuclear enzyme poly(ADP-ribose) polymerase	Yes, via cytochrome c oxidase and others	Yes, via cytochrome c oxidase and others
Vascular effects?	Yes, vasodilatation; mainly produced in endothelial cells under physiological conditions	Yes, vasodilatation; mainly produced in smooth-muscle cells, less so in endothelial cells	Yes, vasodilatation; only produced in smooth-muscle cells, not in endothelial cells
Anti-inflammatory effects?	Yes, at low (physiological) concentrations; higher concentrations can be toxic in its own right or via toxic metabolites	Yes, at low concentrations, involving MAP kinases and other pathways	Yes, at low concentrations; at higher concentrations it exerts pro-inflammatory effects

BCA, β-cyanoalanine; CBS, cystathionine β-synthase; cGMP, cyclic GMP; CSE, cystathionine γ-lyase; MAP, mitogen-activated protein; PAG, DL-propylargylglycine.

H_2S is an inhibitor of carbonic anhydrase (also known as carbonate dehydratase, EC 4.2.1.1)[46]. The physiological or pathophysiological effect of this reaction is unknown at present, although it has been linked to changes in the intrapulmonary CO_2 receptors and subsequent respiratory alterations in response to inhaled sulphide exposure[47]. H_2S can also inhibit cellular respiration, at least in part by acting as an inhibitor of cytochrome c oxidase (EC 1.9.3.1) via a reaction with its copper centre[48]. Inhibition of cytochrome c oxidase is a key factor in the regulation of cellular respiration[49,50]; all three gasotransmitters, NO, CO and H_2S, have effects on this enzyme, most probably in competition with each other (as well as with molecular oxygen). Inhibition of cytochrome c oxidase is a likely mechanism for the regulation of cellular oxygen consumption by H_2S[49,51], and has been implicated in the pharmacological effects of H_2S together with induction of suspended animation[52], as well as in some of its toxicological responses[53]. Exposure of experimental animals to inhaled H_2S resulted in an inhibition of cytochrome c oxidase in the brain, reduction of tissue oxygen uptake and inhibition of the reuptake of the excitatory neurotransmitter L-glutamate, followed by elevated extracellular glutamate concentrations[54].

Suspended animation
A state of temporary and reversible slowing down or cessation of life functions by external means.

Sulphide levels in disease

The plasma concentration of sulphide is regulated at the level of its generation and its consumption. Several reports describe significant changes in its plasma levels in various disease states. In patients with coronary heart

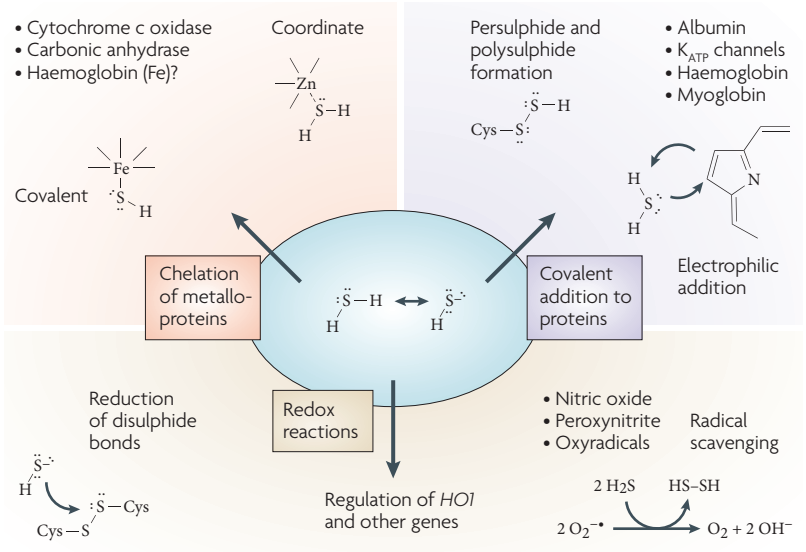

Figure 2 | **Some biological effects of H_2S in mammalian cells.** Top left: known cellular targets of sulphide include cytochrome c oxidase and carbonic anhydrase. Top right: sulphide can participate in reactions yielding persulphide and polysulphide. Sulphide can also bind to plasma proteins such as albumin, and it can activate ATP-activated potassium (K_{ATP}) channels in the myocardium, vascular smooth muscle and cardiac myocytes. The binding of sulphide to haemoglobin or myoglobin forms sulphaemoglobin or sulphmyoglobin. Bottom panel: Some of the redox reactions that sulphide participates in can result in the reduction of disulphide bonds, as well as reactions with various reactive oxygen and nitrogen species, resulting in free-radical scavenging and antioxidant effects. Sulphide has also been demonstrated to regulate cellular signal transduction pathways, resulting in alterations the expression of various genes and gene products including thioredoxin reductase and interleukin-1β (IL1β). *HO1*, haem oxygenase 1 (also known as *HMOX1*).

disease, plasma sulphide levels are reduced from 50 μM to ~25 μM[55]. Spontaneously hypertensive rats (SHR) also have substantially lower plasma levels of sulphide than normotensive control Wistar Kyoto (WKY) animals (WKY = 48 μM; SHR = 20 μM)[56]. In many diseases, including circulatory shock and diabetes mellitus, increases in circulating sulphide concentrations were reported. For example, in a carrageenan model of paw oedema, an ~40% increase in H_2S-synthesizing activity was reported in paw homogenates[57], whereas in a mouse model of pancreatitis, plasma levels of sulphide increased from 23 μM to 31 μM[58]. In haemorrhagic shock, plasma levels of sulphide increased from ~30 μM to 40 μM[59], and in liver and kidney homogenates from rodents with endotoxic shock, there were ~30–60% increases in sulphide production as compared with normal control animals[60,61]. In the plasma of endotoxin-treated mice, sulphide levels increased from 34 μM to 41 μM[61], whereas in a polymicrobial sepsis model elicited in mice with cecal ligation and puncture, plasma levels of sulphide elevated from ~10 μM to 20 μM[62]. In the endotoxic, septic and haemorrhagic shock models, there were upregulations of the mRNA of the H_2S-generating enzymes CSE and CBS[59–62].

Although we do not question the validity of the relative increases or decreases in circulating sulphide levels, we must note that there are substantial differences in the absolute values of baseline endogenous circulating concentrations of sulphide, which are most likely due to the substantial differences in the methods used by various groups. Many methods used to measure circulating levels of sulphide actually do not detect any baseline 'free' sulphide, and the various derivatization methods that are part of the assays (using various drastic assay conditions) are likely to liberate sulphide from its bound forms, thereby producing concentrations that are likely to represent a mixture of free and bound sulphide[64–66]. In summary, even though many reviews mention generous basal sulphide levels in the 50–150 μM range[1,2], baseline blood samples do not emit the characteristic unpleasant H_2S smell (whereas similar concentrations of H_2S in physiological buffers do). The determination and quantification of the exact free and bioavailable baseline sulphide levels in blood and tissues is probably lower than the levels usually mentioned in the literature and therefore requires additional clarification.

Sulphide inhibitors in models of disease

Pharmacological inhibitors of H_2S biosynthesis include DL-propargylglycine (PAG) and β-cyanoalanine (BCA)[67,68] (FIG. 3). These compounds, although of low potency, of low selectivity and of limited cell-membrane permeability[69,70], have been used in several studies to test the biological effect of inhibition of endogenous sulphide production (see below). When interpreting the data, the severe limitations of these experimental compounds must always be kept in mind.

As elevated levels of sulphide have been demonstrated in various models of disease, Moore and colleagues explored the effect of pharmacological inhibition of sulphide production in rats subjected to haemorrhagic shock[59], and reported that PAG or BCA (50 mg per kg) accelerated the recovery of mean arterial blood pressure. As the hypotension associated with haemorrhagic shock is due to the activation of K_{ATP} channels in the vascular smooth muscle[71], it was suggested that endogenous sulphide is responsible for the activation of the K_{ATP} channels. Indeed, inhibitors of sulphide production lost their pressor effect when the K_{ATP} channels were blocked by glibenclamide treatment[59]. In contrast to haemorrhagic shock, PAG (10–100 mg per kg) did not affect the haemodynamic responses in a rat model of endotoxin shock[60]. However, PAG prevented the increases in the plasma levels of liver and pancreas injury markers in the same model and reduced the tissue content of myeloperoxidase, consistent with an inhibition of adhesion and infiltration of the tissues by activated neutrophil granulocytes[60]. In a model of cecal ligation and puncture, PAG treatment reduced tissue neutrophil infiltration and improved liver and lung histology, and significantly prolonged the survival of the animals[62].

Two reports have investigated the effect of inhibitors of sulphide production in rodent models of local inflammation. In a carrageenan-induced inflammation model in the rat, PAG treatment (25–75 mg per kg) dose-dependently reduced paw oedema and paw neutrophil infiltration[57], whereas in a cerulein model

Cecal ligation and puncture
An experimental model of polymicrobial sepsis that is generally considered more relevant to the human disease than rodents injected with bacterial lipopolysaccharide (endotoxin).

of pancreatitis, PAG (100 mg per kg) attenuated pancreatic necrosis and reduced the degree of acute lung injury (which develops secondary to the pancreatic damage)[58].

In rat models of myocardial infarction, acute or subchronic treatment with PAG increased myocardial infarct size[72,73], consistent with a protective role of endogenous sulphide in myocardial infarction, and also

Table 2 | **Cytotoxic or cytoprotective effects of H_2S or its donors in cultured cells**

Cell type	H_2S concentration	Cellular response elicited by sulphide	Refs
Primary hippocampal astrocytes from rats	60–300 μM	• Increased intracellular calcium concentration • Induction of calcium waves	18
Human aortic smooth muscle cells	200–500 μM	• Induction of DNA fragmentation due to caspase activation, development of apoptotic phenotype • However, only minimal degree of cell death when assessed by the trypan blue exclusion assay • Induction of LDH release to the culture medium • Activation of the ERK and p38 MAP kinase pathways	19
IEC-18 rat intestinal crypt cells	1–5 mM	• Reduction of the intracellular reduced environment, increase in NADPH/NADP ratio • Promotion of early cell-cycle entry • Increased *Pcna* expression, apoptosis (only at 5 mM) • Increased Jun mRNA, alterations in the expression of various genes	20
HEK-293 endothelial kidney cells	100 μM (or CSE overexpression)	• Inhibition of cell proliferation • Increase in ERK and $p21^{Cip/WAK1}$ activity	21
RAW 264.7 mouse macrophages	200–500 μM	• Inhibition of NFκB activation and iNOS expression and nitric oxide production • Upregulation of *Ho1* expression and carbon monoxide production • Cytotoxicity (necrosis or apoptosis) was not observed, unless protein synthesis of the cells was inhibited by cycloheximide • Increase in cellular GSH levels	22
HCT116 human colon cancer cells	125 μM – 1 mM	• Prevention of β-phenylethyl isothiocyanate-induced caspase activation and associated apoptotic cell death • No cytotoxic effects	23
Rat primary cortical neurons and HT22 immortalized hippocampal cells	30–200 μM	• Increased cellular GSH and GSSG levels by activation/expression of γ-glutamylcysteine synthetase • Protection against NMDA (1 mM) induced excitotoxicity	24,25
SH-SY5Y human neuroblastoma cells	25–250 μM	• Protection against the peroxynitrite (300 μM) or hypochlorous acid (300 μM) induced decrease in cell viability • Reduction in intracellular tyrosine nitration and protein carbonyl formation	26,27
J774 mouse macrophages	10–1,000 μM	• Protection against nitric oxide or peroxynitrite-induced decrease in cell viability	32
A-10 rat myoblasts	30–50 μM	• Protection against homocysteine-induced cytotoxicity and reduction of the endogenous formation of hydrogen peroxide, peroxynitrite and superoxide • Enhancement of the cytoprotective effect of *N*-acetylcysteine and GSH	28
Human blood neutrophils, lymphocytes, eosinophils	200 μM – 3 mM	• Inhibition of spontaneous apoptosis in neutrophils • Acceleration of cell death in lymphocytes • No effect on the viability of eosinophils	29
Human pulmonary fibroblasts	10–75 μM	• Decrease in the viability of the cells over an incubation period of 12–47 hours • Increase in micronuclei formation, and increase in apoptotic signalling proteins	30
Rat primary hepatocytes	500 μM	• Decrease in the viability of the cells, this effect was more pronounced when endogenous GSH pools had been depleted • NaHS induced a depletion of intracellular GSH levels, especially at acidic pH • Intracellular iron chelation attenuated the cytotoxicity of NaHS	31

CSE, cystathionine γ-lyase; GSH, glutathione, GSSG, oxidized glutathione; Ho1, haem oxygenase 1 (also known as Hmox1); iNOS, inducible nitric oxide synthase; LDH, lactate dehydrogenase; NFκB, nuclear factor-κB; NMDA, *N*-methyl-D-aspartate; *Pcna*, proliferating cell nuclear antigen.

consistent with the protective effect of exogenous sulphide administration in the same experimental models (see below). TABLE 3 gives an overview of the experimental findings obtained with pharmacological inhibitors of sulphide production in animal models of disease[32,62–84,172,173].

Sulphide as an inducer of suspended animation

The discovery that H_2S can induce suspended animation began with experiments showing that hibernation-like states can be induced on demand in animals that do not naturally hibernate. In the first set of such studies, an experimental model of developing *Caenorhabditis elegans* embryos was used to mimic anoxic or ischaemic conditions for humans[85]. Anoxia induced a suspended-animation-like state in nematodes, which was a result of specific gene regulation processes[86,87]. Exposure of embryos to hypoxic oxygen concentrations (between 0.01% and 0.1% oxygen) did not result in suspended animation, and they exhibited signs of cellular damage and death after 24 hours. When the oxygen concentration in the embryos' atmosphere was slightly increased (to 0.5% oxygen), they progressed normally through embryogenesis, just like embryos in normoxia. Thus, even though the nematodes are capable of surviving in anoxia by entering into suspended animation and can develop normally in as little as 0.5% oxygen, nematodes do not survive in the tenfold range of oxygen concentrations in between[85].

In the next set of experiments, CO was used to compete with oxygen for binding to cytochrome c oxidase. CO protected the *C. elegans* embryos from the ischaemic damage when they were placed in intermediate oxygen concentrations — most probably by simulating anoxia via the blockade of the small amount of remaining oxygen available to the embryos[88].

Subsequent studies focused on the effects of H_2S, which — similar to CO — acts as an inhibitor of cytochrome c oxidase. When mice were placed in an atmosphere containing relatively low concentrations of H_2S gas (20–80 ppm), a dose-dependent reduction in the core body temperature and metabolic rate of the animals were observed[52]. The animals ceased all movement and appeared to lose consciousness. Over the course of several hours in this environment, the metabolic rate continued to decrease, as measured by their carbon dioxide output, ultimately falling tenfold. Their breathing rate slowed from 120 breaths per minute to fewer than 10. When the chamber of the animals was cooled, body temperature reached as low as 15°C (a level ~2°C above the air temperature) (FIG. 4). These findings support the hypothesis that H_2S can induce a suspended-animation-like state in a mammal that does not normally hibernate[52,89]. These mouse studies were recently repeated by another group[90]. It was confirmed that H_2S induces cardiovascular responses that are consistent with the physiology of hibernation. Although 6 hours of 80 ppm H_2S in the breathing air of the mice induced a decrease in heart rate from over 600 beats per minute to 130 beats per minute, and decreased the body temperature from 38°C to 30°C, mean arterial blood pressure and stroke volume remained unchanged[90].

Hibernation
It is well known that various organisms can reversibly arrest their essential life processes, in some cases for several years at a time. This phenomenon is known as quiescence, torpor or hibernation. An important aspect of this process is a significant reduction in both energy production and energy consumption. Organisms in this state are resistant to environmental stresses including temperature extremes and oxygen deprivation.

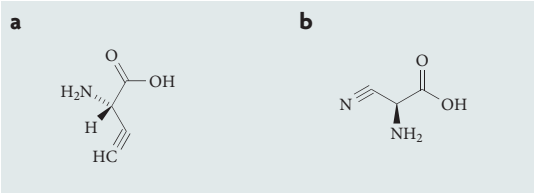

Figure 3 | **Structures of PAG and BCA.** PAG (DL-propylargylglycine) (**a**) and BCA (β-cyanoalanine) (**b**) are commonly used as inhibitors of H_2S biosynthesis, although they have low potency and selectivity, and limited cell-membrane permeability.

Whether the administration of H_2S in experimental models of disease (see below) is protective by producing certain aspects of suspended animation in cells and organs remains to be directly tested. In principle, hibernation is known to confer a cytoprotective phenotype, that is, tissues from hibernating animals are remarkably resistant to various hypoxic and ischaemic insults[89]. In addition to producing protective effects in whole animal models, the effects of sulphide may also be explored in the context of preservation of transplantable organs. Indeed, organs from hibernating animals are protected against the injury associated with storage and transplantation[91]. The possibility that the presence of H_2S in the storage fluid of transplantable organs may extend their storability is an attractive and testable hypothesis. In this context, it is noteworthy that CO has recently been shown to improve the function of the stored organs after transplantation[92,93].

Mouse models of metabolism are criticized by many investigators as poor models for the study of human metabolic disease. Because of their relatively large surface area to mass ratio, rodents are capable of effecting a rapid reduction in core temperature when challenged with a toxic insult. This degree of protective response is much smaller in larger mammals (and humans) with large thermal inertia and highly effective thermo-effectors that resist reductions in body temperature. As previously noted, one needs to take into account that mice have a body mass 3,000-times smaller than that of adult humans, as well as heart rates 14-times greater and respiratory rates 10-times faster than a human[94]. A human typically consumes approximately 3 ml per min per kg oxygen at rest, whereas a mouse consumes approximately 10-times more, and much of the oxygen consumed in a mouse is used for heat generation. It is possible, therefore, that by reducing oxygen consumption with sulphide by ~90%, ATP-requiring processes (for example, ion homeostasis) are not dramatically compromised in the mouse but rather sulphide may selectively influence the component of mitochondrial oxygen consumption connected with mitochondrial uncoupling. The window of opportunity to compromise oxidative phosphorylation in a human, therefore, may be much smaller than in the mouse. Nevertheless, reports demonstrate that sulphide can affect cellular metabolism in animals (and possibly humans), at least in part by inhibiting cytochrome c oxidase[53]. In fact, a slight but detectable inhibition of

Table 3 | **Effects of inhibitors of H_2S biosynthesis in animal models of disease**

Experimental model	Effect of inhibition of sulphide production	Refs
Haemorrhagic shock in the rat	• PAG or BCA (50 mg per kg i.p.) exerted pressor effects in animals subjected to haemorrhagic shock	59
Acute cerulein-induced pancreatitis and associated pulmonary injury in mice	• PAG (50 mg per kg i.p.) reduced pancreatic injury as assessed by circulating amylase levels • PAG reduced pancreatic and pulmonary myeloperoxidase levels, indicative of reduced tissue neutrophil infiltration • PAG treatment improved morphology of the pancreas and the lung	58
Metabolic inhibition preconditioning elicited by sodium cyanide and 2-deoxyglucose in cultured rat ventricular myocytes	• PAG or BCA (2 mM) prevented the cardioprotective effect of metabolic preconditioning	74
Endotoxin-induced inflammation in mice	• PAG (50 mg per kg i.p.) reduced renal, hepatic and pulmonary myeloperoxidase levels and improved the morphology of multiple organs	61
Endotoxin-induced haemodynamic alterations and inflammation in rats	• PAG (50 mg per kg i.p.) reduced hepatic myeloperoxidase levels and attenuated the plasma markers of organ failure (CK, ALT, AST, lipase)	60
NSAID (aspirin, indomethacin, ketoprofen or diclofenac) induced gastropathy in rats	• BCA (50 mg per kg i.p.) enhanced gastric injury, increased neutrophil adhesion, and increased inflammatory mediator (TNF, COX2, ICAM1) production	75
Carrageenan-induced hindpaw oedema in the rat	• PAG (50 mg per kg i.p.) prevented the carrageenan-induced increase oedema and the increase in the myeloperoxidase content of the hindpaw	57
Carrageenan-induced hindpaw oedema in the rat	• BCA (50 mg per kg i.p.) enhanced the carrageenan-induced oedema in the hindpaw	76
Sepsis induced by cecal ligation and puncture	• PAG (50 mg per kg i.p.) reduced mortality, reduced pulmonary and hepatic myeloperoxidase levels and improved the histological picture of the organs	62
Myocardial infarction induced by transient coronary artery ligation in the rat	• PAG (50 mg per kg i.p. pretreatment, given once or as a daily dosing for 7 days) increased myocardial infarct size	72,73
Hypoxic or endotoxin-induced myocardial ischaemic preconditioning	• PAG (50 mg per kg i.p.) attenuated the endotoxin-induced myocardial preconditioning but did not affect hypoxic myocardial preconditioning	72
Inflammatory mononuclear cell infiltration induced by carrageenan in the mouse air pouch model	• BCA (50 mg per kg i.p.) increased mononuclear cell infiltration into the pouch	76
Aspirin-induced leukocyte adherence in mesenteric venules of the rat	• BCA (50 mg per kg i.p.) increased mononuclear cell adhesion to the venules	76
Rat model of middle cerebral artery occlusion stroke	• BCA, PAG and hydroxylamine (0.25–2 mmol per kg i.p.) decreased stroke volume	83

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCA, β-cyanoalanine; CK, creatine kinase; COX2, cyclooxygenase 2; ICAM1, intracellular adhesion molecule 1; i.p., intraperitioneal; NSAID, non-steroidal anti-inflammatory drug; PAG, DL-propylargylglycine; TNF, tumour necrosis factor.

cytochrome c oxidase can be achieved in the lungs of animals exposed to as low as 50 ppm H_2S for 4 hours[53], which is consistent with irreversible inhibition of cytochrome c oxidase by sulphide.

Whether H_2S can induce a hibernation-like state in larger animals (or humans) remains to be seen. Humans can enter hibernation-like states and there are anecdotal reports from skiing accidents and other accidents showing that humans can tolerate long periods of hypoxia/hypothermia and can be rescued subsequently[89]. Further studies are required to determine whether pharmacological, on-demand induction of certain pharmacologically desirable aspects of hibernation is feasible in large animals and in humans.

Sulphide donors in models of disease

The ability of H_2S to influence cellular metabolism has been exploited for the purpose of metabolic suppression and protection from an otherwise lethal hypoxic insult. Normally, mice cannot survive for longer than 20 minutes when exposed to 5% oxygen. However, when mice were first put into a suspended-animation-like state by a 20-minute pretreatment with H_2S, and then exposed to low oxygen, their survival was extended to more than 6.5 hours in 5% oxygen with no apparent detrimental effects[95]. Moreover, when mice were exposed to a 20-minute pretreatment with H_2S followed by 1 hour at 5% oxygen, they survived for several hours at oxygen tensions as low as 3% (FIG. 5a). Thus, it appears that prior exposure to H_2S

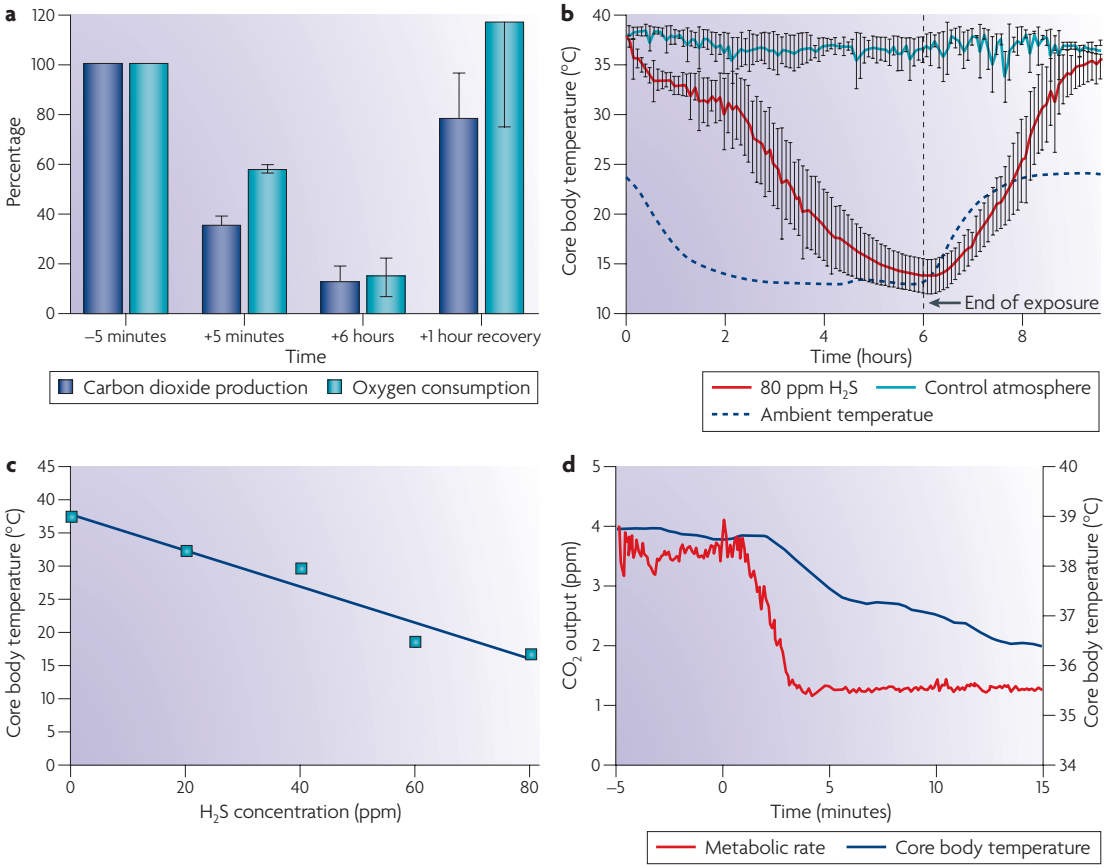

Figure 4 | **Induction of a suspended animation-like state in mice by H_2S inhalation.** **a** | Relative CO_2 production and O_2 consumption of mice exposed to 80 ppm of H_2S. **b** | Core body temperature of mice during 6 hours of exposure to either 80 ppm of H_2S (red line) or the control atmosphere (green line). The dotted line indicates ambient temperature. Values in (**a**) and (**b**) are means ± one standard deviation. **c** | Linear relationship between H_2S concentration and core body temperature (R^2 = 0.95) after 6 hours of exposure. **d** | CO_2 output and core body temperature of mice (time = 0 at the start of H_2S exposure). Image reproduced with permission from REF. 52 © (2005) American Association for the Advancement of Science.

reduces oxygen demand, making it possible for the mice to survive with low oxygen supply[95]. These results suggest that H_2S may be useful to prevent damage associated with hypoxia. Some of the protective effects of sulphide may be related to the ability of the compound to induce hypothermia, as hypothermia — which can be elicited by various pharmacological means — is known to confer resistance to hypoxia in the mouse[96]. Whether treatment with H_2S could be used to beneficially affect various forms of diseases associated with local or systemic tissue hypoxia needs to be explored. Indeed, in a recent report, Roth and colleagues reported that H_2S treatment markedly improves survival in a rat model of rapid lethal haemorrhage[174].

Over the past 2 years, a number of independent groups have reported the beneficial effects of H_2S or sulphide-donor compounds in animal models of disease[32,61,62,71–82,95,97,98] (TABLE 4). Some of these protective effects (for example, protection from lethal hypoxia; see above) occurred at hypothermia-inducing doses, whereas other protective effects — for example the protection from myocardial reperfusion injury — were found at lower doses, which were not associated with any apparent intrinsic physiological responses. Many of these studies focus on myocardial protection, and the cardioprotective effects of H_2S have been demonstrated in cultured myocytes[74], isolated perfused hearts[78] and in rodent and large animal models of coronary artery ligation and reperfusion[72,73,79,80,172] (FIG. 5b). The mechanism of this protection may be — at least in part — related to the ability of H_2S to activate myocardial K_{ATP} channels: in cells or animals in which the K_{ATP} channels are pharmacologically inhibited, the cardioprotective effect of sulphide is diminished[72,74]. However, definite conclusions on the exclusive role of K_{ATP} channels in the cardioprotective effect of H_2S cannot be made, as pharmacological inhibitors of K_{ATP} channels also increase myocardial infarct size in normal (that is, not H_2S-treated) animals[79,99]. The ERK and phosphatidylinositol 3-kinase (PI3K)/AKT pathways may also be involved in sulphide-induced cardiac protection mechanisms[175]. Interestingly, the therapeutic effect of sulphide-mediated cardioprotection is bell-shaped, both in perfused hearts and in whole animals: raising the dose of sulphide above the optimal dose results in diminished therapeutic efficacy[78,172].

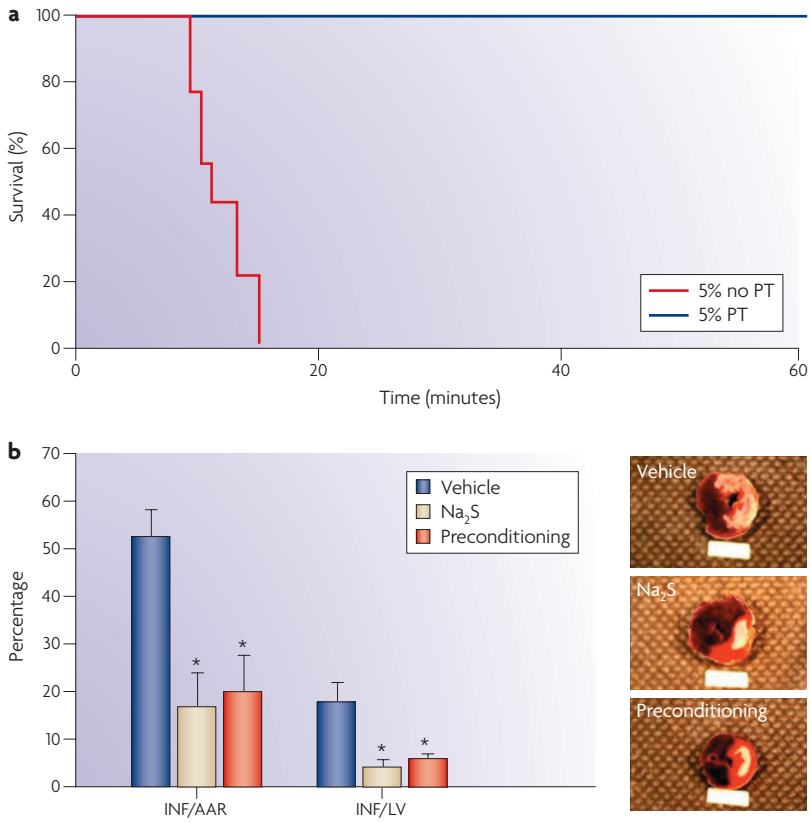

Figure 5 | **Protective effects of sulphide donors in disease models. a** | Protection of mice from lethal hypoxia by H_2S inhalation. Kaplan–Meyer curve for untreated (red line) and pretreated (blue line) mice exposed to 5% O_2 at T_a = 23°C. 5% PT = 20 minute 150 ppm H_2S pretreatment followed by 1 hour at 5% O_2; 5% no PT = 20 minutes of room air followed by 1 hour at 5% O_2. **b** | Protection from myocardial ischaemia–reperfusion injury in rats by intravenous injection of sodium sulphide. Male CD rats were anaesthetized with ketamine-xylazine and the heart was exposed through a left lateral thoracotomy while being mechanically ventilated. Regional myocardial ischaemia was produced by occlusion of the left main coronary artery for 30 minutes. In one group of animals, sodium sulphide was administered intravenously at a slow bolus dose of 3 mg per kg through a jugular catheter. Another group of animals received ischaemic preconditioning consisting of 3 cycles of 5 minutes ischaemia and 5 minutes reperfusion before the 30 minute coronary occlusion. Infarct size was quantitated at 24 hours after reperfusion by the Evans Blue method[49,76] and is expressed as percent of area at risk (INF/AAR) or as percent of left ventricle (INF/LV). Sulphide and preconditioning exerted myocardial protective effects of comparable degree. The right panels show representative cross-sections of the heart, where white areas represent the necrotic tissue, blue areas the normal tissue and red areas the viable cardiac tissue contained within the area at risk. *p <0.05 versus the corresponding vehicle-treated group. n = 5, 8 and 3 in the vehicle, sulphide and preconditioning groups, respectively. Image 5a reproduced with permission from REF. 95 © (2006) Lippincott Williams & Wilkins.

The effects of H_2S have also been attributed to an ability of the compound to induce myocardial preconditioning[72,74,77]. This effect of H_2S may, again, be linked — at least in part — to (mitochondrial?) K_{ATP} channels, as these channels seem to be relevant for the preconditioning process[100,101]. K_{ATP}-channel activation can also induce local vasodilatation, which may also contribute to its cardioprotective effects.

Another mechanism by which H_2S exerts its cardioprotective effects may relate to the ability of sulphide to reduce neutrophil adhesion; a K_{ATP}-channel-dependent response[76] (FIG. 6). Additional effects of H_2S whereby it may beneficially affect the outcome of ischaemia reperfusion may relate to its ability to react and neutralize cytotoxic reactive species, such as peroxynitrite[26], as this reactive nitrogen species plays an important pathogenic role in myocardial infarction[102]. As patients with coronary disease have reduced endogenous sulphide levels[103], sulphide treatment may be, in a way, considered as sulphide replacement therapy in cardiac diseases. So far, there are no published reports in the literature as to whether H_2S can exert beneficial effects in ischaemic organs other than the heart.

An additional area in which H_2S has been shown to exert protective effects is in animal models of inflammation, and inflammation-related pain (TABLE 4). Carrageenan-induced paw oedema and air pouch inflammation are simple test systems that are commonly used to explore the anti-inflammatory effects of experimental compounds. H_2S has been effective in reducing paw oedema and inflammatory cell infiltration in these models[72,76]. The anti-inflammatory effect of H_2S was attenuated by pretreatment with glibenclamide, thereby pointing to the involvement of K_{ATP} channels[72,76].

Sulphide may also have therapeutic potential in the angiogenesis/wound healing area. *In vitro* studies demonstrate that sulphide induces angiogenesis in chicken chorioallantoid membranes, stimulates endothelial cell proliferation, migration and tube formation[176,177]. Furthermore, sulphide donors stimulate gastric ulcer healing in rodent models[178,179].

More complex models in which H_2S has demonstrated efficacy include a rodent experimental model of colitis-associated colorectal distension[81] and a non-steroidal anti-inflammatory agent induced gastropathy model[76]. The gastroprotective effects are mediated, at least in part, by the vasodilatory effect of H_2S. The effects of H_2S also include antinociceptive effects, which may be, at least in part, mediated by the ability of H_2S to downregulate *FOS* expression in spinal-cord neurons, but may also involve effects on NO production and/or the K_{ATP}-channel pathway[81].

Several studies demonstrated that chemically linking an NO-donor species to known anti-inflammatory drugs can improve the therapeutic profile of the compound. This line of research has resulted in the discovery and clinical development of nitro-statins, nitro-nonsteroidals and nitro-steroids[104–106]. A similar approach has recently been introduced in conjunction with H_2S: the H_2S-releasing derivative of mesalamine has demonstrated superior anti-inflammatory and antinociceptive efficacy compared with the base mesalamine molecule in a rodent experimental model of colitis-associated colorectal distension[82]. Li and colleagues have recently reported on the biological effects of another sulphide-releasing compound, ACS 15, or *S*-diclofenac (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid 4-(3H-1,2,dithiol-3-thione-5-yl)phenyl ester)[84]. The compound was effective in endotoxin-induced inflammation models in rodents, and exhibited less gastric toxicity than the parent molecule[84]. The compound also reduced interleukin-1β (IL1β) production

Table 4 | **Effects of H_2S or its donors in animal models of disease**

Experimental model	Effect of sulphide/sulphide donor	Refs
Hypoxic pulmonary vasoconstriction in the rat	• NaHS (0.8 mg per kg per day i.p. once a day) attenuated the hypoxic vasoconstrictor response	34
Metabolic inhibition elicited by sodium cyanide and 2-deoxyglucose in cultured rat ventricular myocytes	• NaHS (10–100 μM) pretreatment (1 h or 16–24 h) protected against the loss of cell viability during metabolic inhibition	74
NSAID (aspirin, indomethacin, ketoprofen or diclofenac) induced gastropathy in rats	• NaHS (1.6–8 mg per kg i.p.) protected against gastric injury, reduced neutrophil adhesion, attenuated inflammatory mediator (*Tnf*, *Cox2*, *Icam1*) expression	75
Myocardial ischaemia-reperfusion in the isolated perfused rat hearts	• NaHS (0.1–1 μM) provided a concentration-dependent reduction in myocardial infarct size and reduced the duration and severity of arrhythmias	77
Myocardial ischaemia-reperfusion in the rat	• NaHS (3 mg per kg i.v.) given before the start of the coronary occlusion reduces myocardial infarct size	78
Myocardial ischaemia-reperfusion in the mouse	• NaHS (50–200 μg per kg i.v.) given before the start of the reperfusion reduces myocardial infarct size	79, 172
Myocardial ischaemia-reperfusion in the rat	• NaHS (0.8 mg per kg per day) given for 7 days before myocardial infarction, and continued for 2 days after infarction reduced myocardial infarct size	73
Myocardial ischaemia-reperfusion in the pig	• NaHS (100 μg per kg bolus, followed by 1 mg per kg per h infusion for 2 h) reduced myocardial infarct size, improved myocardial contractility and reduced neutrophil infiltration and myocardial cytokine expression	80
Myocardial injury in the rat induced by isoproterenol	• NaHS (160 or 800 μg per kg per day) attenuated the release of cardiac enzymes, reduced conjugated diene formation from myocardial samples and reduced plasma and cardiac malondialdehyde formation	33
Visceral pain model induced by colorectal dystension in the rat; TNBS-induced colitis in the rat	• NaHS (2–4 mg per kg i.p.) resulted in an attenuation of the nociceptive responses • A sulphide-releasing derivative of the anti-inflammatory drug mesalamine (100 mg per kg i.p.) exerted more pronounced anti-inflammatory effects than mesalamine alone	81,82
Mouse air pouch model	• NaHS (8 mg per kg i.p.) reduced mononuclear cell infiltration	75,76
Aspirin-induced leukocyte adherence in rat mesenteric venules	• NaHS (0.6–6 mg per kg i.p.) reduced leukocyte adhesion	76
Carrageenan-induced hindpaw oedema in the rat	• NaHS or H_2S (8 mg per kg i.p.) reduced the carrageenan-induced oedema in the hindpaw	76
Lethal hypoxia (5%) in mice	• H_2S (150 ppm) inhalation extended the survival of the mice subjected to lethal hypoxia	95
Shock induced by cecal ligation and puncture or by endotoxin in the mouse	• NaHS (0.8–10 mg per kg i.p.) further increased pulmonary and hepatic myeloperoxidase levels and aggravated the histological picture	61,62
Bleomycin-induced pulmonary fibrosis in the rat	• NaHS (50 or 250 μg per kg i.p. twice a day for 28 days) attenuated pulmonary malondialdehyde and hydroxyproline levels	62
Rat model of middle cerebral artery occlusion stroke	• NaHS (11 mg per kg i.p.) increased stroke volume	83
Endotoxin-induced lung and liver inflammation model in the rat	• A sulphide-releasing diclofenac derivative reduced tissue neutrophil infiltration, reduced IL1β levels, upregulated IL10 levels, and attenuated the activation of NFκB and AP1 and downregulated *Nos* and *Cox* expression	84
A dog model of cardiopulmonary bypass	• NaHS prevented the deterioration of cardiac function, improved haemodynamic parameters and coronary artery reactivity	97
A rat model of balloon-induced intimal hyperplasia	• NaHS (1.8 mg per kg per day for 7 days) reduced restenosis and vascular inflammation and improved coronary artery reactivity	98
Arterial hypertrophy associated with spontaneous hypertension in the rat	• NaHS (0.6–1.8 mg per kg per day for 3 months) reduced medial thickening in the arteries, reduced reactive oxidant production and the degree of atrial fibrosis	173
Rat model of lethal haemorrhage	• Inhaled H_2S (300 ppm) or intravenous Na_2S (1 mg/kg) improved survival	174

AP1, activator protein 1; *Cox2*, cyclooxygenase 2; *Icam1*, intracellular adhesion molecule 1; IL1β, interleukin 1β; IL10, interleukin 10; i.p., intraperitioneal; i.v. intravenous; NFκB, nuclear factor-κB; *Nos*, nitric oxide synthase; NSAID, non-steroidal anti-inflammatory drug; *Tnf*, tumour necrosis factor.

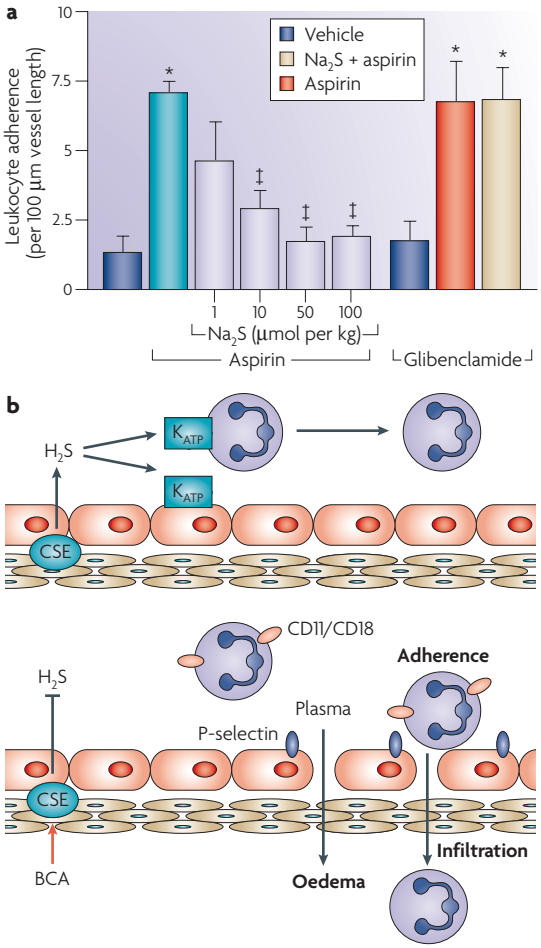

Figure 6 | **Potential mechanisms of protective effects of H_2S.** Sodium hydrogen sulphide inhibits the adhesion of neutrophils to endothelial cells. **a** | Hydrogen sulphide (H_2S) inhibits aspirin-induced leukocyte adherence in mesenteric venules through activation of ATP-activated potassium (K_{ATP}) channels. Sodium sulphide (Na_2S) dose-dependently suppressed leukocyte adherence induced by intragastric aspirin (50 mg per kg). The inhibition of aspirin-induced adherence by Na_2S was abolished by pretreatment with glibenclamide (10 mg per kg), implicating the role of K_{ATP}-channel activation in the effect of sulphide. *p<0.05 versus the corresponding vehicle-treated group. ‡p <0.05 versus the corresponding group receiving aspirin alone. Each group consisted of at least five rats. The results are plotted as mean ± SE. **b** | A schematic representation of the proposed mechanism. H_2S modulates inflammatory processes at the leukocyte–endothelial interface. Under normal conditions, H_2S is synthesized in blood vessels primarily by cystathionine γ-lyase (CSE), which is expressed in endothelial cells and smooth-muscle cells. H_2S tonically downregulates leukocyte adherence via activation of K_{ATP} on leukocytes and the endothelium. When endogenous H_2S synthesis is inhibited (for example with β-cyanoalanine; BCA), leukocyte rolling and adherence to the vascular endothelium increase, probably due in part to elevated expression of adhesion molecules on leukocytes (CD11/CD18) and endothelial cells (P-selectin). Marked increases in endothelial permeability, resulting in oedema formation, also occur when H_2S synthesis is suppressed. Image reproduced and adapted with permission from REF. 76 © (2006) The Federation of American Societies for Experimental Biology.

and upregulated the anti-inflammatory cytokine IL10 (REF. 84). Just like in the case of the NO-releasing derivatives, the mode of the enhancement of the therapeutic efficacy of H_2S-releasing moieties may be more than a local vasodilatory response alone, and may involve regulation of neutrophil functions, gene expression and other pathways. For instance, although mesalamine did not inhibit the expression of the inducible form of cyclooxygenase (COX2) and of IL1β in the colon and of FOS in spinal-cord neurons, sulpho-mesalamine exerted significant inhibitory effects[82].

Thus, H_2S or H_2S-donor compounds exert beneficial effects in multiple models of disease. The mechanisms of action are multiple and range from inhibition of cellular metabolism (in the lethal hypoxia model) to vasodilatation, K_{ATP}-channel activation, upregulation of anti-inflammatory genes (for example, *HO1*) and downregulation of inflammatory genes (*COX2*, *FOS*, *IL1β*). H_2S can inhibit the adhesion and activation of neutrophil granulocytes[76], and also has a slight inhibitory effect on platelet aggregation[107]. The relative contribution of these effects (as well as other, not yet identified protective mechanisms) to the pharmacological effects of H_2S in various disease models remains to be defined. It must also be noted that there are several disease models (stroke, sepsis) in which administration of H_2S donors has resulted in the exacerbation of the condition (TABLE 4).

Interestingly, inhibition of H_2S production and H_2S donors exert beneficial effects in the same experimental model of disease — for instance in the carrageenan paw oedema model[57,76]. In some cases, the experimental findings are difficult to reconcile (as one study demonstrates anti-inflammatory, another one pro-inflammatory effects in comparable rodent models of paw oedema). It is possible that endogenous sulphide at low and high local concentrations exerts opposing effects, which may be responsible for these findings. It is also conceivable that non-sulphide related (that is, nonspecific) effects of the pharmacological inhibitors used may contribute to these effects. It is interesting to note that a similar paradox has been previously noted with inhibitors versus donors of NO — both of them being effective in the carrageenan paw oedema models, owing to distinct pharmacological actions[108,109]. Clearly, there is an exquisite balance and a complex regulation of pathophysiological responses by endogenous and exogenous gasotransmitters.

Development of therapeutics related to H_2S

The emerging data on the biological effects of H_2S support two basic approaches for the development of sulphide-based therapeutics: inhibitors of CSE or CBS, leading to the suppression of endogenous sulphide formation, and H_2S gas or H_2S-releasing compounds (sulphide donors). It is also conceivable, but remains to be tested, whether modulation of endogenous H_2S pathways may contribute to the pharmacological actions of some of the already used or experimental drugs.

For inhibitors of H_2S formation, the experimental compounds currently available in the literature are of rather low potency and of questionable selectivity (see also above). In rat liver homogenates, BCA and PAG

exerted a concentration-dependent inhibition of sulphide production *in vitro*, with 50% inhibition achieved around 100 μM concentration of the compound, and 90% inhibition seen around 1 mM[60]. Consequently, the doses of BCA and PAG required to inhibit endogenous sulphide are in the high mg per kg range: many studies use 50 mg per kg in rodent experiments (TABLE 3). Clearly, at this dose the compound suppresses plasma sulphide concentrations (as supported by direct measurements in animals or in tissue homogenates), but it also exerts additional nonspecific effects. Indeed, when a number of structurally unrelated H_2S inhibitors are compared for potency (for example, in brain homogenates) versus biological efficacy (for example, as agents to reduce stroke volume in a rat model), several discrepancies become apparent. The inhibitor that is the least potent *in vitro* in the series (PAG) is the most effective neuroprotectant *in vivo*, and the most potent inhibitor tested (aminooxyacetic acid) produces some protection against stroke at low doses, whereas it exacerbates the pathology at higher doses[83].

When considered from the traditional drug development standpoint, the currently used tools to inhibit mammalian H_2S production barely qualify as true 'hit molecules'. Nevertheless, identification of novel hit molecules is probably reasonably straightforward when using high-throughput screening approaches utilizing sufficiently high numbers and diverse sets of chemical libraries.

When attempting to optimize and further develop such compounds, however, it is important to remember that CSE and CBS serve important roles in mammalian cysteine metabolism. Indeed, genetic CBS deficiency is known to lead to homocysteinaemia, a condition that is associated with elevated serum levels of homocysteine and homocystine (as well as related mixed disulphides), and leads to endothelial dysfunction and hypertension[110]. Genetically modified animals with CBS deficiency have impaired vascular function, and show a dysregulation of genes that are involved in hepatic lipid metabolism[111]. The risk of homocysteinaemia (and therefore endothelial dysfunction, hypertension, atherosclerosis or cardiovascular diseases) would make it unlikely that pharmacological inhibition of CBS could be developed for anything other than the short-term therapy of acute life-threatening indications. Based on the available *in vivo* data, circulatory shock or possibly stroke may be such possible indications (TABLE 3) that could utilize a potent parenteral inhibitor of sulphide production.

Over the past two decades, many 'magic bullets' have failed in the experimental therapy of circulatory shock[112], and a similar string of therapeutic failures pertains to stroke. A new development candidate for shock would be expected to show marked efficacy in preclinical models — not only in a pretreatment scenario, but also in a post-treatment paradigm: treatment with the compound would need to be delayed to a time when clinical signs of the disease are already present. An ideal development candidate would be required to be effective against multiple pathophysiological aspects of the disease. For instance, in the case of circulatory shock, it would preferably include haemodynamic changes and organ injury. The preclinical data available with pharmacological inhibitors of sulphide so far do not appear to meet such stringent requirements. Any potential development candidate compound would have to undergo the usual series of preclinical safety studies and would have to be well tolerated — at least in the short term — in healthy human volunteers.

Another potential area is the development of H_2S as an inhaled gas or as a parenteral injectable, as well as the development of modified drugs containing a H_2S-releasing moiety. Currently, H_2S is considered a toxic gas; an environmental hazard. This designation, however, has not posed an insurmountable problem in the past for the development of medical gases. An appropriate parallel is the example of inhaled NO, which was first known as a toxic gas (present in polluted air as a product of internal combustion engines), and which is currently approved for use in infants with primary pulmonary hypertension[113,114]. However, in contrast to NO, H_2S has an unpleasant odour. This characteristic of sulphide would not necessarily be a problem in anaesthetized patients, but would necessitate the implementation of appropriate trapping systems to prevent exposure of medical personnel. With enteral or parenteral formulations, odour would not create a problem (except perhaps in exhaled breath at high doses), but several challenging issues would have to be overcome. Some of the possible hurdles for the therapeutic development of H_2S may include inferior efficacy, when compared with already existing therapies; low therapeutic index; dose-limiting side effects; and manufacturing and formulation issues. Even though sulphide is an endogenous compound, all exogenous sulphide delivery systems would be required to pass stringent safety and efficacy in preclinical animal studies before progression into human studies.

As mentioned earlier, another avenue for pharmaceutical development involves the modification of existing drugs to link them with a sulphide-releasing moiety. Such compounds show preclinical efficacy in various models of inflammation[81,82] (TABLE 4). Another potential area may be the exploitation of 'sulphide-precursors' or 'prodrugs' that induce the production of sulphide by endogenous metabolic pathways. The biologically active garlic component *S*-allylcysteine is such an example: the cardioprotective effects and the vascular effects of this compound are related to its ability to yield biologically active sulphide[180,181].

Because of the history of H_2S as an environmental hazard, an extensive body of literature is available on its toxicology profile, most of which is via the inhaled route[115–123] (Supplementary information S1 (table), S2 (table)). A significant body of human data has also accumulated with inhaled H_2S in conjunction with human pharmacological and toxicological studies and environmental surveys (for reviews, see REFS 115–118; see also Supplementary information S2 (table)), ranging from physiological studies conducted at low levels of H_2S in the inhaled air[124,125] and neurobehavioural alterations[126] to case reports describing severe, sometimes lethal outcome in response to toxic doses of H_2S[127,128].

Because the toxicology of H_2S is an entire field, we only mention a few essential findings in this respect, and the reader is referred to detailed, expert reviews on this subject[115–118]. Toxic effects of inhaled H_2S at relatively high acute exposure levels or at chronic lower-level exposure levels include olfactory epithelial toxicity and a transient loss of olfaction, some pulmonary inflammation and toxicity, and possible effects on blood pressure and cardiovascular responses. There is also a clear neurological toxicity of inhaled H_2S at high doses. H_2S-associated neurotoxicity may involve inhibition of glutamate reuptake, as well as inhibition of monoamine oxidase (also known as amine oxidase, EC 1.4.3.4)[129]. The pulmonary toxicity of H_2S appears to involve substance P (TAC1) production from sensory neurons[130] and may involve the vanilloid receptor 1 (TRPV1)[130,131].

Dorman and colleagues have conducted thorough, recent evaluations on the toxicity of inhaled H_2S and have published several reports on the subject[122,132,133]. In adult male and female Fischer-344 and Sprague-Dawley rats and B6C3F1 mice undergoing whole-body exposure to 10, 30 or 80 ppm H_2S for 6 hours per day for at least 90 days, no adverse effects were found in the 10 ppm dosing group. Increasing doses of H_2S resulted in olfactory neuronal loss and rhinitis and, at higher doses, bronchiolar epithelial hypertrophy and hyperplasia. At the highest dose tested, reduced feed consumption was also noted[132]. Perinatal exposure by inhalation to H_2S (doses and daily exposure times as above) for approximately 1 month did not affect the number of females with live pups, litter size, average length of gestation, the average number of implants per pregnant female, pup growth, development or performance on any of the behavioural tests[133].

Animal safety data with respect to parenteral or enteral administration of H_2S or its donors are less abundant. It appears that injection of high doses of intravenous or intraperitoneal NaHS or Na_2S causes pulmonary neutrophil recruitment, inflammatory mediator production and histopathological alterations of the lung[61,131]. However, the doses (for example, 10 mg per kg) at which these pulmonary adverse effects were reported are generally higher than the doses at which therapeutic effects of sulphide were noted in animal models of disease (compare Supplementary information S1 (table) and S2 (table)). Intraperitoneal injection of Na_2S produces dose-dependent hypotensive responses and is associated with an LD_{50} of approximately 100 mg per kg in unventilated male Wistar rats[116]. Given the efficacy of inhaled NO, there may be no immediate advantage of inhaled H_2S over inhaled NO for acute pulmonary hypertension indications. However, various other indications (from induction of suspended-animation-like states to cardiac protection) may be conceivable for inhaled sulphide or for parenteral compounds that deliver sulphide.

The development of H_2S or H_2S-releasing drugs as pharmaceuticals would require a detailed understanding of the toxicological profile, as well as its pharmacokinetics, distribution and metabolism. In most *in vitro* studies (none of them conducted in validated test systems), no evidence for genotoxicity has been noted[115]. In a modified experimental system in which DNA repair was inhibited, H_2S administration did induce genotoxicity[134]. Additionally, Baskar demonstrated that H_2S induces concentration-dependent increase in micronuclei formation in human pulmonary fibroblasts co-treated with 5 μg per ml cytochalasine[30].

The metabolism and disposition of sulphide in the body is reasonably well characterized, at least for exogenously administered (high) doses[115–118]. A qualitative disposition study demonstrates that inhaled H_2S distributes mainly to the brain, liver, kidneys, pancreas and small intestine. A fraction of the intravenously injected H_2S donor exits as H_2S gas via exhaled air (but only during the first minutes after injection). The major metabolic pathway involves oxidation of sulphide to thiosulphate and sulphate, which are cleared from the body mainly via the urine in free and conjugated forms[135,136]. Although endogenous sulphide does not appear to be a major source of urinary sulphate (high levels of sulphate are excreted together with cysteine oxidation), thiosulphate is considered by many investigators as a relatively specific biomarker of endogenous sulphate levels. The exact mechanism of oxidation of sulphide to sulphate in biological systems is incompletely understood, even though it has been studied for ~50 years. The oxidation of sulphide to thiosulphate is increased in the presence of mammalian cells, clearly indicating the presence of an active process. Different tissues have different capacity to oxidize sulphide to sulphate and thiosulphate. The cecal and the colonic mucosa is highly active in this respect and some conversion has been reported in liver and plasma as well, while the skeletal muscle and the erythrocytes appear to be devoid of significant converting activity[137]. The role of a sulphide oxidase (possibly a mitochondrion-associated enzyme) and/or glutathione in catalysing the conversion of sulphide to thiosulphate have been proposed. An additional pathway may involve methylation of sulphide by cytosolic *S*-methyltransferase enzymes, although the importance of this pathway is debated by many investigators. There are also suggestions that haem compounds (peroxidase, catalase, ferritin) can catalyse the oxidation of sulphide. When blood was incubated with sulphide *in vitro*, its binding to proteins has been detected, presumably via sulphide participating in the formation of persulphides. This bound sulphide can experimentally be liberated by appropriate chemical reactants, such as dithiothreitol ((2*S*,3*S*)-1,4-bis-sulphanylbutane-2,3-diol). Similarly, significant amounts of H_2S can be liberated by dithiothreitol from brain tissues of animals previously exposed to toxic doses of H_2S[138,139]. Biological formation of sulphmyoglobin and sulphaemoglobin has also been described. Thiosulphate seems to be relatively stable in the blood and urine; its measurement has been used to confirm sulphide intoxication in humans[127,128,135].

Future studies

H_2S-related pharmacological research is a rapidly emerging field, which is likely to yield a number of therapeutic possibilities, and early stage drug candidates are now in development (TABLE 5). H_2S-related future therapeutic avenues might also include genetic approaches. Mice

Table 5 | **Selected H_2S-related compounds in development**

Compound (company)	Characteristics	Potential applications	Development stage
ACS-15 (CTG Pharma)	A sulphide-releasing diclofenac derivative	Arthritis	Preclinical
ATB-429 (Antibe)	A sulphide-releasing measalamine derivative, with antinociceptive and anti-inflammatory effects	Inflammatory bowel disease	Preclinical
ATB-346 (Antibe)	A sulphide-releasing NSAID?	Acute and chronic joint pain	Preclinical
ATB-284 (Antibe)	Composition not disclosed	Irritable bowel syndrome	Preclinical
IK-1001 (Ikaria)	Injectable	Suspended animation and multiple hypoxic/ischaemic conditions	Phase I

NSAID, non-steroidal anti-inflammatory drug.

that have a heterozygous deficiency of CBS have been established[111], and are expected to serve as a useful experimental tool in future studies, and overexpression of H_2S-producing enzymes as a therapeutic modality might be explored. A transgenic mouse with selective cardiac overexpression of CSE has recently been used to confirm the cardioprotective effect of endogenously produced sulphide in myocardial infarction[172]. A similar approach has already been explored with enzymes producing NO: gene transfer of NO synthases has been shown to be of significant benefit in multiple animal models of disease[140–142].

Other questions relate to the biological metabolism of sulphide and the fate of sulphide in biological systems. It seems that thiosulphate and urinary excretion of thiosulphate and sulphate are major part of the metabolism of sulphide[136]. Sulphide can also react with haemoglobin to form sulphaemoglobin. This reaction has been well characterized *in vitro*[143], and sulphaemoglobin has been detected in animals and humans after high doses of sulphide exposure in some of the published reports, but it was not found in other studies[144–148].

The biological mechanisms and pathways of sulphide detoxification have not yet been characterized in sufficient detail. Rhodanese (also known as thiosulphate sulphurtransferase, EC 2.8.1.1) is an enzyme that merits detailed investigation, especially in light of recent studies questioning its sulphide-metabolizing role[149]. A related subject is the identification of a possible antidote for H_2S overdosing/intoxication. *In vitro*, the cytotoxic effects of sulphide may be, at least in part, related to endogenous production of free radical and oxidant species[150], which may or may not be relevant to the *in vivo* toxicity of H_2S. *In vivo*, there are data to demonstrate that induced methaemoglobinaemia, that is, the administration of sodium nitrite protects against H_2S toxicity[115]. Also, $NaHCO_3$ treatment prevents the toxic effects of H_2S, possibly via affecting the inhibition of carbonic anhydrase by H_2S[121]. There is evidence that oxidized glutathione can reduce the toxicity of H_2S, possibly by interruption of hydrosulphide reactions of critical enzymatic disulphide groups. However, oxygen therapy (although it is recommended for subjects with sulphide intoxication) does not seem to produce consistent benefits in animals subjected to toxic doses of H_2S[115].

Direct measurements of basal levels of extracellular sulphide are in the low micromolar concentration range. If there is a substantial background level of sulphide, how is it possible that relatively small amounts of supplemental sulphide can exert biological effects? As discussed earlier, one possibility is that indirect means (electrodes or chemical reactants) of detection do not distinguish between free and bound pools of sulphide. Another possibility is that the biological sulphide-eliminating systems might get saturated by sudden exposure to sulphide, and the non-metabolized sulphide triggers a downstream biological effect (for instance on gene transcription, K_{ATP} channels, cytochrome c oxidase or other targets). In certain diseases, the local sulphide concentration may decrease[55,75], and supplementing the missing sulphide might have a beneficial effect.

Whether endogenous sulphide might have a role in regulating cellular energy production via its action on mitochondrial oxidative phosphorylation in mammals remains to be explored. It may make sense from an evolutionary standpoint, as it once played oxygen's molecular part in metabolism when our planet was young and oxygen was scarce. There are currently no published studies investigating the effect of inhibition of endogenous sulphide production on oxygen consumption and mitochondrial respiration *in vivo*. A suitable parallel is the regulation of cellular energy metabolism by NO: NO synthase inhibitors have been demonstrated to increase the oxygen consumption and the mitochondrial respiration of isolated organs and whole animals[151,152]. It is noteworthy that a recent report finds that H_2S is not only an inhibitor of the mammalian mitochondrial functions, but mitochondria can also use it as an electron donor (an inorganic substrate) that enters the mitochondrial respiratory chain between Complexes I and III (REF. 153). It remains to be determined in future studies whether this finding is a biochemical curiosity, or whether it also has (patho)physiological relevance.

As in the case of NO, the reactivity and the biological responses to sulphide are likely to be significantly influenced by the local milieu, such as the pH or the redox status of the cell. For example, acidic pH would be expected to shift the relative proportion of the three sulphide species (H_2S, HS^- and S^{2-}) in the cellular environment towards an abundance of H_2S gas, which is likely to affect the biological response. Regarding the role of oxidant stress, there are data demonstrating that the presence of superoxide (for example, in the vicinity of activated neutrophils and, quite possibly, in pathophysiological conditions associated with increased cellular superoxide generation) enhances the conversion of sulphite from sulphide, a response that can be suppressed by excess antioxidants in the medium[154]. Because sulphite has cytotoxic properties and is an inducer of neutrophil adhesion and aggregation[155–158], it is possible that the ambient level of oxidant species may

shift the biological response to sulphide from an anti-inflammatory, anti-adhesive phenotype to a cytotoxic, pro-adhesive phenotype. Because sulphite is cytotoxic and genotoxic[159,160], it may also mediate some of the cytotoxic or genotoxic effects in cells exposed to high concentrations of H_2S.

As sulphide is the third gasotransmitter, together with NO and CO (TABLE 1), it is not surprising that there are complex interactions between these mediators in regulating cell functions, cardiovascular responses and inflammatory/immune functions[1,2,161–164]. The endogenous production of H_2S appears to be enhanced by treatment with NO donors[37]. Low concentrations of H_2S were shown to markedly increase the vasorelaxant effect of the NO donor sodium nitroprusside. This effect may be because H_2S promotes the release of NO from vascular endothelium and sodium nitroprusside increases conversion of L-cysteine to sulphide. It may also be due to an increased expression of CSE in vascular smooth-muscle cells, increased uptake of cysteine by cells and/or the modulation of activity of cGMP-dependent protein kinase, which is a stimulant of CSE activity. On the other hand, infusion of low (10 μmol per kg) doses of H_2S donors exerts pressor effects due to direct physical interactions with basally produced NO[161], whereas higher doses (25 μmol per kg) produce hypotensive effects, presumably direct effects of sulphide on vascular K_{ATP} channels, leading to hyperpolarization and relaxation of the smooth muscle[45]. Thus, complex interactions exist between the direct and indirect vascular effects of H_2S. It is unclear at present how an additional, recently identified pharmacological effect of H_2S (direct inhibition of endothelial NO synthase)[165] is involved in the above observed findings.

Non-vascular aspects of NO–H_2S interactions have also been identified: it seems that NO-related effects contribute to the cytoprotective and antinociceptive effects of sulphide[2]. In macrophages, H_2S inhibits the expression of *iNOS* but upregulates the expression of *HO1* (REF. 22), thereby possibly shifting the balance of gasotransmitters from NO to CO. H_2S also acts as a scavenger and neutralizer of peroxynitrite[26], a key player in the cytotoxic effects of NO. As noted above, H_2S can also upregulate HO1, and can therefore induce a delayed production of CO.

As discussed by Fiorucci and colleagues[2], CO may be another factor capable of interacting with H_2S and NO, given that all three bind avidly to haem. Haemoglobin can, therefore, be viewed as a common sink for these three gasotransmitters, and saturation with one could lead to enhanced plasma levels, and biological effects, of the others. Indeed, saturation of erythrocytes with CO results in elevated plasma H_2S levels. Additional studies are needed to delineate the various interplays between the three gasotransmitters in health and disease, and to identify areas in which pharmacological modulation of these agents (alone or in combination) may provide therapeutic benefit.

As noted earlier, H_2S formed in enteric bacteria might affect cellular function in host organs. However, this pathway has not been investigated in sufficient detail. It appears that the colonic sulphide-detoxifying mechanisms are impaired in cancer and are upregulated in inflammation[166]. The hypothesis that endogenous sulphide production by the bacterial flora contributes to the pathogenesis of inflammatory bowel disease has been put forward, although experimental studies using bismuth — which has a capacity to bind enteric sulphide — speak against this hypothesis[167–170].

Last, but not least, we must point out that the molecular mechanisms of sulphide's cellular actions are poorly understood. Some of the effects are probably related to its effects on cytochrome c oxidase and mitochondrial functions (see above), while its effects on gene expression may be related to its effects on the NFκB and the ERK pathways[22], but the molecular details are unknown. As K_{ATP} channels are important mediators in many cardiovascular effects of sulphide, the in-depth delineation of the mode of the sulphide-mediated K_{ATP} channel activation[37] would also be of interest.

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Competing interests statement
The author declares competing financial interests: see web version for details.

DATABASES

SwissProt ENZYME: http://www.expasy.org/enzyme
Amine oxidase | carbonate dehydratase | cystathionine β-synthase | cystathionine γ-lyase | cystathionine γ-synthase | cytochrome c oxidase | glutamate cysteine ligase | thiosulphate sulphurtransferase

FURTHER INFORMATION

Ikaria Inc.: http://www.ikaria.com

SUPPLEMENTARY INFORMATION

See online article: S1 (table) | S2 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

Table S1 | Some pharmacological and toxicological effects of H_2S in animals.

Animal and treatment route	Effect of H_2S
Acute inhalation exposure in Fisher 344 rats for 4 hours (10, 200, 400, 1000 ppm).	Acute exposure to 400 ppm sulfide increased protein and LDH content and inflammatory cell content in nasal and the bronchoalveolar lavage fluids. A cytotoxic effect to the nasal epithelium was also observed. Acute exposure to 1000 ppm resulted in approximately 50% mortality.
Intravenous or intraperitoneal injection of NaHS (0.6-3 mg/kg) to rats.	Four phases of physiological responses are induced in a dose-dependent fashion: hyperpnea, unconsciousness (knockdown), apnea and death. The apnea response appears to have a peripheral sensor (lung?) and appears to be dependent on the vagus nerve. The LD_{50} of sulfide in rats is about 3 mg/kg i.v. and about 50 mg/kg i.p.
Naive mice subjected to hydrogen sulfide inhalation (20-80 ppm).	Mice subjected to inhaled H_2S (20-80 ppm) developed hypothermia within an hour, exhibited signs of suppressed metabolism, and entered a reversible, suspended animation-like state. Mice exhibited reduced heart rate but maintained blood pressure.
90-day inhalation toxicity study in B6C3F1/CrlBr mice (10, 30 or 80 ppm).	No adverse effects of hydrogen sulfide inhalation were seen at 10 and 30 ppm. At 80 ppm, reduced body weights, and reduced organ weights were observed, as well as a significant inflammatory responses in the nasal mucosa, coupled with a loss of olfactory epithelial cells.
90-day inhalation toxicity study in Fisher 344 rats (10, 30 or 80 ppm).	No adverse effects of hydrogen sulfide inhalation were noted at 10 and 30 ppm. At 80 ppm reduced body weights and reduced organ weights were observed, as well as an increased sulfhemoglobin blood level in male animals. Mild histological alterations were seen in the lung. Relative brain weights were decreased at 80 ppm. Mild to moderate inflammatory responses in the nasal mucosa were observed.
50-day inhalation toxicity study in dogs (15 ppm).	No adverse effects were seen at 15 ppm. The frequency of "abnormal blood pressure readings" increased.
10-week inhalation toxicity study in male SD rats (10, 30 or 80 ppm).	No adverse effects of hydrogen sulfide inhalation were noted at 10 ppm. At 30 and 80 ppm, olfactory mucosal lesions were observed, including olfactory neuron loss.
Rabbits exposed to 72 ppm H_2S for 1h – 1 day.	Repolarization disturbances in the heart, evidenced by flattened T-waves, ventricular extrasystoles, ECG changes.

Table S2 | Some pharmacological or toxicological effects of H_2S in humans

Exposure route and level	Effect of hydrogen sulfide
Levels in the breathing air of 0.02- 0.1 ppm	The concentration of sulfide reaches the level of odor detection.
Levels in the breathing air of 0.2-0.3 ppm	The concentration of sulfide reaches the level of nuisance, headaches, nausea and sleep disturbances.
Acute inhalation exposure during exercise in healthy men (0.5, 2 or 5 ppm).	Oxygen uptake tended to increase, and carbon dioxide output tended to decrease. Blood lactate concentrations significantly increased. Heart rate and ventilation were unaffected.
Acute inhalation exposure in healthy men (10 ppm).	Oxygen uptake tended to increase, and carbon dioxide output tended to decrease. Blood lactate concentrations increased. Heart rate and ventilation were unaffected.
Levels in the breathing air of 10-20 ppm	OSHA acceptable ceiling for H_2S concentration in the air at a workplace.
Levels in the breathing air of 30-50 ppm	Eye irritation.
Levels in the breathing air of 200 -700 ppm	Depression of the nervous system: nervousness, headache, lightheadedness, fatigue, extremity weakness, spasms.
Exposure to various levels of toxic concentrations of inhaled H_2S (500-1000 ppm [estimated levels])	Variable degree of discomfort, ranging (at 5-100 ppm) from eye irritation from shortness of breath, chest tightness, wheezes to "knockdown" (losing of consciousness) to fatalities.
1400 ppm in breathing air (acute exposure)	Severe intoxication, 2 out of 4 subjects died acutely, 1 died in hospital 22 hours later, 1 survived. High blood concentrations of thiosulfate (1-100 mg/l) were measured in autopsy samples.