Hydrogen sulfide-based therapeutics and gastrointestinal diseases: translating physiology to treatments

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Abstract
Hydrogen sulfide (H2S) is commonly known for its characteristic “rotten egg” smell and its infamous use as a toxic gas causing respiratory failure. Therefore, it may seem unlikely that this gas could play important roles as a mediator of many physiological processes. Much like the other gaseous mediators, so has it in the case of nitric oxide and carbon monoxide. H2S is a signaling molecule (68) with vasodilatory (4, 78) and neuromodulatory (31, 35) properties, and it can bind to hemoglobin (51, 69). Indeed, just as cross talk has been identified between these earlier recognized gaseous mediators, so has it in the case of H2S and nitric oxide (8).

Reported levels of H2S in serum and tissues vary with differing methods of measurement, but concentrations are generally reported to be between 50 and 160 μM in the brain (1, 26) and 50 μM in serum (19, 77). It has been argued that these are overestimates, the true concentrations of H2S being three orders of magnitude lower, because of very efficient catabolism of H2S (23). In the small and large intestine, the mucosa itself produces H2S (44, 61) and many of the bacteria within the lumen can produce H2S in close proximity to the epithelium (22, 25). The colonic mucosa may be especially well adapted to “detoxify” the H2S that it is exposed to (24). Thus we and others have suggested that the intestinal epithelium may serve as a “metabolic barrier” to the diffusion of bacteria-derived H2S into the lamina propria (24, 62). Within the epithelium, H2S is rapidly inactivated by a complex of mitochondrial enzymes collectively referred to as the “sulfide oxidation unit” (37, 46). In vitro, colonocytes can deactivate H2S at concentrations as high as 50 μM (46). Moreover, colonocytes have been described as the best adapted cells for utilizing H2S as an energy source, generating adenosine triphosphate (27, 36, 37, 46). This action of H2S may underlie at least some of the beneficial effects of H2S donors in the gastrointestinal (GI) tract and in other organs, particularly in circumstances of anoxia/hypoxia (15, 21, 36, 37).

In mammalian tissues, H2S is synthesized from cysteine (32, 34) either via pyridoxal-5′-phosphate (PSP)-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (38, 70), or the more recently described, P5P-independent cysteine aminotransferase (CAT)-3-mercaptopyruvate sulfurtransferase (3-MST) pathway (52) (Fig. 1). We have recently reported that this latter pathway is the major pathway...
source of H$_2$S production in the rat colon in health and during colitis (21). Interestingly, synthesis of H$_2$S was found to be upregulated specifically at sites of mucosal ulceration, and a marked decrease in the rates of inactivation of H$_2$S was also demonstrated at these sites (21). This is consistent with a previous report of diminished capacity for H$_2$S catabolism in colitis (10), although in the study of Flannigan et al. (21), the diminished inactivation of H$_2$S at sites of ulceration appeared to be due to marked downregulation of expression of sulfide quinone reductase, the rate-limiting mitochondrial enzyme for oxidation of H$_2$S (73).

CBS-deficient mice do not survive long after birth and have severe growth retardation (48). They exhibit hyperhomocysteinemia, consistent with the crucial role of hepatic CBS in converting homocysteine to cysteine (48). CSE-deficient mice exhibit significantly elevated systemic blood pressure and diminished endothelium-dependent relaxation (74). The impact of genetic deficiencies of the CSE and CBS on the digestive system have not been described, other than a marked decrease in colonic H$_2$S synthesis in CSE-deficient mice (61).

Here we review some of the evidence of a role for H$_2$S in modulating GI inflammation and repair and discuss the potential key role of H$_2$S-releasing drugs in the treatment of inflammatory bowel disease (IBD) and the gastroenteropathy associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs).

**Glossary**

**Chemokines**
A family of cytokine signaling molecules that induce chemotaxis (attraction) of neighboring responsive cells

**Cytokines**
Cell-signaling molecules, which can be proteins or glycoproteins. Pro-inflammatory cytokines include IL-1β and TNF-α.

**Mucosal defense**
The components of the GI system through which the integrity of the mucosal “barrier” is maintained in the face of potential harmful luminal contents.

**Myeloperoxidase**
An enzyme found in all cells of myeloid origin (particularly high content in neutrophils), whose activity is used as a biochemical marker of granulocyte infiltration into tissue.

**NSAIDs**
Nonsteroidal anti-inflammatory drugs, which act by inhibiting the cyclooxygenase (COX) enzymes, thereby suppressing synthesis of prostaglandins and thromboxanes.

**Vascular permeability**
The capacity of a blood vessel to allow the passage of fluid, molecules and, sometimes, of whole cells through the vessel wall.

**H$_2$S in inflammation**

As in the case of nitric oxide, H$_2$S can exert pro- or anti-inflammatory effects depending on its concentration and the particular experimental model and design (34, 55, 72). However, the balance of evidence points to H$_2$S exerting anti-inflammatory effects at physiological concentrations (Fig. 2). H$_2$S plays a key role in vasodilation, one of the cardinal hallmarks of inflammation, but also an effect crucial to repair of damaged tissue (63, 65). H$_2$S can also reduce edema formation (56, 76). For example, in a rat model of carrageenan-induced hind paw edema, administration of a H$_2$S donor (NaHS) resulted in a significant reduction of paw volume. In contrast, administration of an inhibitor of CSE (β-cyanoaniline) significantly increased the degree of swelling (76). These effects may have been partly due to the ability of H$_2$S to inhibit leukocyte adherence to the vascular endothelium, as first demonstrated in mesenteric venules (76). Zenardo et al. (76) showed that administration of H$_2$S donors (NaHS, Na$_2$S, or Lawesson’s reagent) inhibited leukocyte adherence to the vascular endothelium and migration of leukocytes to sites of inflammation. In addition, CBS heterozygous mice, in which H$_2$S synthesis is reduced, exhibit increased vascular permeability, reduced leukocyte rolling velocity, and increased leukocyte adherence to the endothelium (33).

H$_2$S has also been shown to reduce production of proinflammatory cytokines, chemokines, and enzymes, and to induce apo-
ptosis of neutrophils (Fig. 2) and a shift of macrophages to an anti-inflammatory phenotype (13, 59). GYY4137, a slow-releasing H2S donor, was reported to inhibit the secretion of TNF-α and IL-1β in an endotoxic shock model (40, 72). As described in more detail below, an H2S-releasing derivative of mesalamine significantly reduced mucosal expression of several proinflammatory cytokines and chemokines in a mouse model of colitis (20). Inhibition of mucosal TNF-α levels was also observed in a rat model of colitis following treatment with either of two H2S donors (61). Moreover, allyl disulfide, a H2S-releasing garlic constituent (4), suppressed TNF-α expression and NF-κB activation in samples of colonic tissue from patients with ulcerative colitis (2).

H2S has also been shown to activate the Nrf2 stress response pathway, upregulating an array of detoxifying proteins and antioxidant enzymes (49, 75). The underlying mechanism for this effect may be the sulfhydration, by H2S, of a protein (Keap1) that tonically suppresses Nrf2 activity (49, 75). These effects are consistent with an important role of H2S in preserving mitochondrial integrity and function, particularly in circumstances of hypoxia or anoxia (15, 36, 37).

H2S in Gastrointestinal Mucosal Defense

The GI mucosa is relentlessly exposed to potentially erosive substances such as acid, bile, digestive enzymes, ethanol, and various drugs, such as NSAIDs, the latter being one of the major causes of gastric ulceration (63, 67). The processes that allow the GI mucosa to resist injury in the face of such challenges are collectively referred to as “mucosal defense” (62). One of the key elements of mucosal defense is maintenance of a high level of mucosal blood flow, which helps to minimize tissue damage and also facilitates repair when damage occurs (45, 64). As mentioned above, H2S is a vasodilator
and it has been shown to contribute significantly to mucosal defense (18, 44). Inhibition of H2S synthesis results in greater damage when an NSAID is subsequently administered (57). On the other hand, H2S donors can prevent the decrease in gastric mucosal blood flow caused by NSAIDs and markedly reduce the severity of NSAID-induced gastric injury (18, 68). The protective effects of H2S in this setting are likely attributable in part to the inhibition of NSAID-induced leukocyte adherence to the vascular endothelium (18, 76), which has been shown to be a critical event in the pathogenesis of NSAID-induced GI injury (60). This effect of H2S appears to be due to a downregulation of expression of ICAM-1 on endothelial cells and LFA-1 on leukocytes (18).

H2S also influences nonvascular factors that contribute to mucosal defense. For instance, it can stimulate duodenal bicarbonate secretion (30), which helps to protect the stomach and small intestine from the damaging effects of gastric acid. As mentioned above, H2S can be utilized by mitochondria to generate ATP, particularly in settings of hypoxia (27, 36). In these circumstances, H2S may reduce tissue injury and accelerate repair processes (15, 36). Ischemia-reperfusion injury in the stomach has been shown to be significantly reduced by pretreatment with an H2S donor (43).

Further evidence of a crucial role of H2S in maintaining GI mucosal defense has come from studies in which inhibitors of endogenous H2S synthesis have been administered to rats. Administration of inhibitors of CSE or CBS over the course of a week resulted in significant inflammation along the length of the GI tract (61). The tissue was atrophic and there were significant elevations of granulocytes in the tissue, a marked reduction in mucosal expression of COX-2, and a parallel reduction in mucosal PGE2 synthesis (61). These results suggest that H2S tonically downregulates GI mucosal inflammation, in part via upregulation of COX-2-mediated prostaglandin synthesis. Of course, one cannot rule out the possibility that the observed detrimental effects on the GI tract were a secondary effect of the inhibitors on other tissues or organs. However, further evidence for a role of H2S in maintenance of mucosal integrity and downregulation of inflammation comes from studies using local administration of iodoacetamide to induce severe inflammation in the colon (50) or stomach (3, 68). Iodoacetamide avidly binds l-cysteine, therefore making it unavailable for conversion to H2S (68). Its administration leads to a marked increase in granulocyte infiltration into the mucosa, which in the colon is accompanied by epithelial destruction (50).

**H2S in Gastric and Colonic Ulcer Healing**

H2S has been shown to accelerate the healing of gastric (58) and colonic (20, 61) ulcers in rodents. Shortly after induction of an ulcer in the stomach (by serosal application of acetic acid), there is a marked upregulation of expression of CSE and CBS at the site of injury (58). Elevated capacity for H2S synthesis has also been observed (58). Treatment with l-cysteine, the precursor for H2S synthesis, or with H2S donors, markedly accelerated the healing of the gastric ulcers (58). On the other hand, administration of an inhibitor of CSE activity (l-propargylglycine) significantly delayed gastric ulcer healing and abolished the beneficial effects of administration of l-cysteine (58, 61). Likewise, administration of inhibitors of H2S synthesis to rats with hapten-induced colitis resulted in exacerbation of the ulcers (more expensive and more penetrating), often leading to perforation and death (61). On the other hand, intracolonic administration of H2S donors led to a marked acceleration of the healing of ulcerated colonic tissue (61).

The beneficial effects of H2S on gastrointestinal ulcer healing may be in part due to enhancement of mucosal blood flow at the ulcer margins, where the most active proliferation and angiogenesis occurs (42, 58). Other studies have shown that H2S can directly promote angiogenesis, which is vital to ulcer repair (8, 54, 66). The ability of H2S to drive mitochondrial ATP production in circumstances of reduced mucosal blood flow may also contribute to the prohealing effects of H2S (15, 34).

**Translation to Therapies**

The therapeutic potential of H2S has been exploited in novel drug design (6, 7, 55, 66). Some examples pertinent to the digestive system are described below.

**Inflammatory bowel disease**. Mesalamine (5-aminosalicylic acid) is the first-line therapy for IBD, the collective term for Crohn’s disease and ulcerative colitis. However, it is a relatively weak drug, requiring doses as high as 6 g per day, and is only effective in cases of IBD of mild-to-moderate severity (29). ATB-429 is an H2S-releasing derivative of mesalamine and was developed with the notion that the H2S released from the compound would provide additional anti-inflammatory and ulcer-healing effects to those of mesalamine. This approach appears to have been successful, at least in preclinical studies. In one such study, mice with hapten-induced colitis were treated orally twice daily with vehicle, mesalamine or ATB-429 (20). After 3 days of treatment, there was a significant reduction in the colitis disease activity score and in tissue granulocyte numbers (myeloperoxidase activity), which was not seen with mesalamine. In another study, mice with hapten-induced colitis received twice-daily oral treatments with mesalamine, ATB-429, or vehicle for 1 wk. Mesalamine had a marginal beneficial effect, whereas ATB-429 significantly reduced the severity of colitis as well as the expression of the inflammatory markers TNF-α, IFN-γ, IL-1, IL-12 p40, IL-2, RANTES, and iNOS (20).

ATB-429 also exhibited significant antinociceptive effects, in both healthy rats and rats with hapten-induced colitis (8). Graded colorectal distention resulted in parallel increases in pain perception (abdominal withdrawal responses). Pretreatment with mesalamine did not alter the pain responses compared with vehicle, whereas a marked reduction in pain responses was observed in rats pretreated with ATB-429 (11). It has been previously shown that H2S has neuromodulatory effects in the brain (1, 31) as well as antinociceptive effects (11), and this may be an additional benefit of ATB-429 in treating IBD. Abdominal pain is a common symptom of IBD, and there are limited options for treatment. Although opioids are effective in reducing visceral pain, they also inhibit intestinal motility, which can be problematic in some IBD patients. It is noteworthy that an H2S-releasing salt of trimebutine (an antispasmodic) is in phase 2 clinical trials for its use as a colonic analgesic, to facilitate colonoscopy (9).
Arthritis. NSAIDs are a mainstay of treatment of the symptoms of arthritis, but such use is limited by their adverse effects on the GI tract (67). The GI-protective actions of H₂S have been exploited in the design of novel NSAIDs that, in preclinical studies, exhibit much greater safety than conventional NSAIDs (Fig. 3). For example, ATB-346 is an H₂S-releasing naproxen derivative (57) that suppresses COX-1 and COX-2 activities at least as effectively as naproxen (5, 57). ATB-346 also exhibits comparable (or improved) anti-inflammatory activity to naproxen, including when examined in a rat model of arthritis (57). Despite the suppression of COX activity, ATB-346 produces little or no gastric damage even at exceptionally high doses (57). ATB-346 also produces no small intestinal damage (5, 57). H₂S-releasing derivatives of other NSAIDs (e.g., diclofenac, indomethacin, aspirin) have also been developed and have been shown to elicit negligible damage in the GI tract, in sharp contrast to the parent drugs, while still producing significant anti-inflammatory effects (39, 56, 66). Several H₂S-releasing NSAIDs have also been shown to be very effective, more so than the parent drugs, in various in vitro and in vivo cancer models (7, 17, 47, 53).

As mentioned above, mice deficient in one of the key enzymes for H₂S synthesis (CSE) exhibit marked hypertension (74). Delivery of H₂S such as via H₂S-releasing NSAIDs may therefore adversely affect systemic blood pressure. A single-bolus administration of a high dose (90 μmol/kg) of ATB-346 did not significantly affect systemic blood pressure in rats (57). The same was observed with an H₂S-releasing derivative of diclofenac (60 μmol/kg). Indeed, this could be seen as a beneficial effect of these compounds, since administration of equimolar doses of naproxen or diclofenac elicited significant increases in blood pressure. NSAIDs can significantly exacerbate hypertension and this likely contributes to the significant elevation of serious cardiovascular adverse events in chronic NSAID users (28).

Studying the GI safety of novel NSAIDs in healthy rats can give a false signal of safety (bearing in mind that these drugs are used mainly to be people with systemic inflammatory disease and often other comorbidities). ATB-346 was therefore tested in several models in which gastric mucosal defense was impaired through pharmacological manipulation. This included ablation of sensory afferent nerves with capsaicin, pretreatment with an inhibitor of nitric oxide synthesis (L-NAME), pretreatment with an inhibitor of H₂S synthesis (β-cyanoalanine), pretreatment with an antagonist of ATP-sensitive potassium channels (glibenclamide), or coadministration with low-dose aspirin. In each of these cases, gastric damage caused by naproxen was significantly worse than when naproxen was given to healthy rats (57). However, in all cases, ATB-346 did not elicit significant gastric damage.

ATB-346 was also studied in rat models that mimicked some of the comorbidities that are relevant to human NSAID-induced gastroenteropathy, such as arthritis, obesity, advanced age, hypertension, and polypharmacy (NSAID treatment combined with a proton pump inhibitor and low-dose aspirin). In each of these cases, gastric PGE₂ and whole blood thromboxane levels in ATB-346-treated rats were reduced to an equivalent extent as in rats treated with naproxen. However, whereas extensive gastric and/or intestinal damage was observed in rats treated with naproxen, such damage did not develop in the rats treated with ATB-346 (5).

In addition to causing the formation of ulcers in the GI tract, NSAIDs can interfere with the healing of preexisting ulcers. Gastric ulcers can be induced in rats by serosal application of acetic acid, and the healing of these ulcers can be modulated by traditional and COX-2 selective NSAIDs (42). Thus treatment with naproxen or celecoxib, at anti-inflammatory doses, results in a significant impairment of ulcer healing compared with treatment with vehicle (57). In contrast, ATB-346 significantly enhanced ulcer healing compared with vehicle and compared with the other NSAIDs (57).

**Final Perspectives**

H₂S is an endogenous anti-inflammatory agent that promotes mucosal integrity, repair, and resolution of inflammation. It is, therefore, a strong candidate to be exploited as a therapeutic agent. A particularly attractive application is the coupling of an H₂S-releasing group to drugs that can cause damage to the GI tract (NSAIDs being one example). Other promising applications focus on the antinociceptive properties of H₂S. The ability of H₂S to serve as a “fuel” for mitochondria, particularly in circumstances of low oxygen, appears to be a particularly exciting mechanism through which this gasotransmitter can limit tissue injury and promote repair.
REFERENCES


