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FORUM REVIEW ARTICLE

Effects of Hydrogen Sulfide on the Microbiome: From Toxicity to Therapy

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Abstract

Significance: Hydrogen sulfide (H_2S), an important regulator of physiology and health, helps resolve inflammation and promotes tissue repair in the gastrointestinal tract.
Recent Advances: Gut microbiota live as a multispecies biofilm in close interaction with the upper mucus layer lining the epithelium. The relative abundance, spatial organization, and function of these microorganisms affect a broad range of health outcomes. This article provides a state-of-the-art review of our understanding of the cross talk between H_2S, the gut microbiota, and health. H_2S can have toxic or therapeutic effects, depending on its concentration and source. When produced at excessive concentrations by local microbiota, H_2S may cause mucus disruption and inflammation and contribute to development of cancer. In contrast, low levels of endogenous or exogenous H_2S directly stabilize mucus layers, prevent fragmentation and adherence of the microbiota biofilm to the epithelium, inhibit the release of invasive pathobionts, and help resolve inflammation and tissue injury. Although scarce, research findings suggest that dietary H_2S obtained from plants or ingestion of the H_2S precursor, L-cysteine, may also modulate the abundance and function of microbiota.
Critical Issues: A critical issue is the lack of understanding of the metagenomic, transcriptomic, and proteomic alterations that characterize the interactions between H_2S and gut microbiota to shape health outcomes.
Future Directions: The ambivalent roles of H_2S in the gut offer a fertile ground for research on such critical issues. The findings will improve our understanding of how H_2S modulates the microbiota to affect body function and will help identify novel therapeutic strategies. *Antioxid. Redox Signal*. 36, 211–219.

Keywords: hydrogen sulfide, microbes, inflammation

Introduction

THE BIOLOGICAL OUTCOMES of complex interactions among ~3 million genes of our gut microbiome, the host cells, and the environment implicate an immense variety of metabolites. The gastrointestinal microbiome, which comprises all the genetic material within a microbiota (collection of microorganisms in a given niche), contains complex microbial communities of bacteria, viruses, and Eukarya, including fungi. It plays key roles in health and disease and influences the pathogenesis of inflammatory bowel diseases (IBDs), cancer, metabolic diseases such as obesity, and brain disorders such as autism spectrum disorders and stroke (26, 43, 54). The gut microbiome is in turn affected by a diverse range of metabolites, including gaseous mediators such as hydrogen sulfide (H_2S) (Fig. 1). Sulfur is indispensable for all living organisms, whether prokaryotes or eukaryotes. In bacteria, sulfur can be used as an energy source when it is converted to

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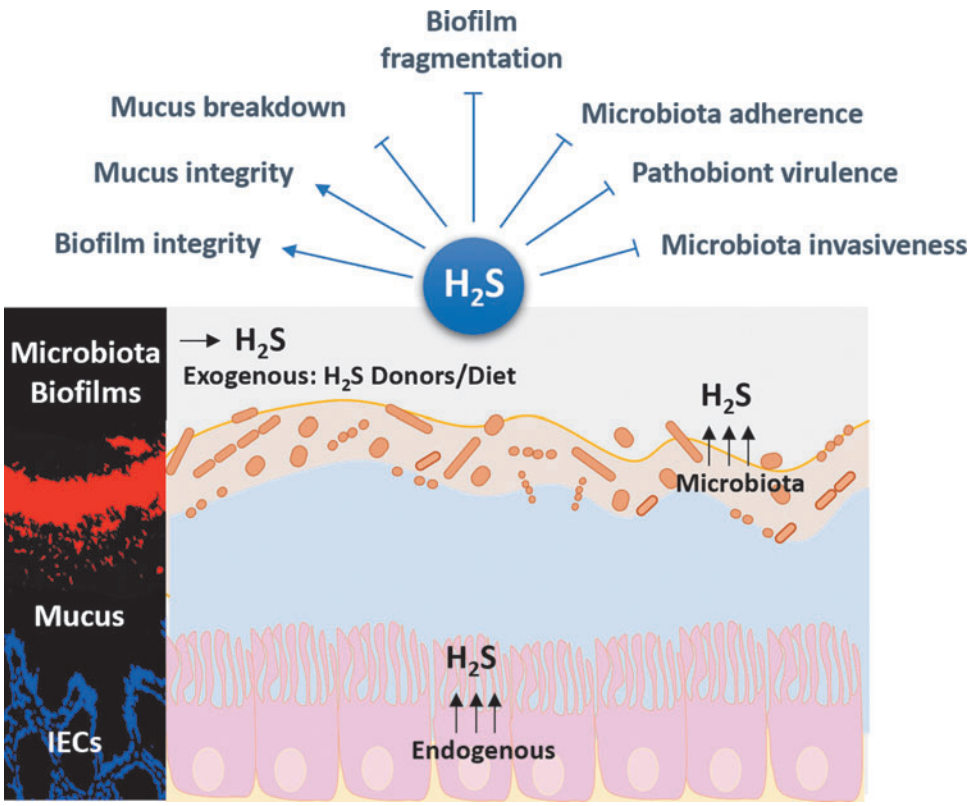

FIG. 1. Summary graphic illustration of the findings discussed in this review. Representative micrograph of the rat colonic mucosa (*left panel*), identifying the microbiota biofilm (Eubacterial stain; Molecular Probes) separated from the host epithelial cells (IECs, DAPI stain) by mucus layers. Endogenous and exogenous H_2S, as well as H_2S produced by microbiota, promotes (→) or inhibits (-I) a variety of characteristics in mucosal microbiota biofilms. It can alter the relative abundance, spatial organization, and function of bacteria in these biofilms. H_2S also prevents the fragmentation and epithelial adherence of these microbiota, while maintaining the integrity of mucus layers separating the microbiota from host tissues. H_2S inhibits the formation and invasiveness of pathobionts, which further contribute to the anti-inflammatory actions of this gaseous mediator. H_2S, hydrogen sulfide; IECs, intestinal epithelial cells.

H_2S. There is ever-increasing interest in H_2S and in how this ubiquitous gasotransmitter may affect gastrointestinal health and overall body homeostasis. In the gut, H_2S was first recognized as a bacterial metabolite produced *via* microbiota-driven anaerobic respiration of sulfite/sulfur-containing substrates (ingested with food or water), such as cysteine and taurine (2-aminomethanesulfonate) (31, 63). Anaerobic sulfur-reducing bacteria, such as those residing in the human large intestine, obtain energy by dissimilatory sulfate reduction following activation of the sulfate by adenosine-5-phosphosulfate reductase (31).

The reactions of the transsulfuration and reverse transsulfuration pathways allow the conversion of cysteine to homocysteine, and vice versa, respectively. Conversion of homocysteine to cysteine in the reverse transsulfuration pathway contributes to methionine-to-cysteine conversion by generating the necessary homocysteine. Recent findings indicate that in *Helicobacter pylori*, LuxS, which serves as a quorum-sensing molecule in many bacteria, plays a key role in the reverse transsulfuration pathway providing cysteine to the bacterium (14). Hence, as cysteine is key to the growth of many bacteria, these pathways help these microorganisms grow in the absence of exogenous cysteine. Clearly, LuxS serves both as a signaling and a metabolic molecule in bacteria. Recent studies also discovered that the metabolic conversion of taurine by gut bacteria was enabled by a previously uncharacterized glycyl radical enzyme (63). Together, these pathways are the major processes through which gut bacteria produce H_2S.

It was then found that a variety of cells in the intestine and elsewhere also produce H_2S *via* cystathionine beta-synthase (CBS), cystathionine gamma-lyase (CSE), or 3-mercaptopyruvate sulfurtransferase (3MST) (1, 32, 66, 80). In turn, cells obtain energy *via* sulfide oxidation of H_2S in the mitochondria (18). Excessive intracellular H_2S inhibits mitochondrial respiration and is therefore detrimental to host metabolism (32). Studies have reported that in humans, concentrations of H_2S were highest in the colon ($\sim$250 *M*) and may reach 40 *M* in the cecum and rectum (2, 25, 32). It has been suggested that H_2S through high intestinal concentrations of bacterial origin may negatively affect mucus integrity by reducing its disulfide bonds (25) and can promote tumorigenesis through its genotoxic effects (2). H_2S may also synergize with nitric oxide to confer antibiotic resistance to bacteria and hence could lead to intestinal bacterial overgrowth of opportunistic pathogens during antimicrobial therapy, a hypothesis that requires further investigation (59). However, there has been a lack of consistency in the scientific literature on the toxic effects of H_2S on the intestinal mucosa and epithelial hyperproliferation (37).

In contrast, numerous observations are in support of the hypothesis that H_2S confers a broad range of health benefits. H_2S inhibits platelet aggregation, has vasodilating properties, and acts as an NATP channel opener in support of its potential

to treat cardiovascular disorders (65). These effects may also contribute, in part, to the benefits of H_2S in the context of a variety of other disorders, including intestinal inflammatory diseases. IBD has long been considered a risk factor for venous thromboembolism (23). In the gut, the integrity of the mucus layers also appears to be protected by endogenously produced H_2S (46). Moreover, while high H_2S concentrations may cause inflammation, low physiological H_2S levels exert potent anti-inflammatory benefits (57, 74). Others have reported that H_2S may suppress protective immunity in models of tuberculosis (51, 57). However, these studies did not identify the source of H_2S in their model. Additional research is needed to determine the contribution of H_2S produced by *Mycobacterium tuberculosis* in suppression of protective immunity reported in these studies. Indeed, a recent finding has demonstrated that *M. tuberculosis* produces copious quantities of H_2S, which in turn protects the microorganism against antimicrobial therapy (42).

It is now well accepted that H_2S generates cytoprotective effects, helps drive the resolution of tissue injury, and stabilizes the mucosal microbiome (19, 22, 46, 74). Tissue repair and wound healing result from the numerous well-described molecular and cellular effects of H_2S, including activation of sulfur-containing amino acid metabolism, inhibition of oxidative stress and inflammation, and stimulation of angiogenesis (19, 46, 52, 74). Indeed, H_2S has been characterized as a free radical scavenger, neutralizing reactive oxygen species, while concurrently enhancing the efficacy of endogenous antioxidant molecules (65). In turn, these effects contribute to resistance to oxidative stress and protect endothelial function, sustain blood flow, and preserve organ perfusion. Recent findings also demonstrate that a novel H_2S-releasing derivative linked to ketoprofen generates significantly greater analgesic benefits *versus* ketoprofen alone (11). These benefits are mediated, at least in part, by the release of endogenous anandamide–cannabinoid metabolites (11). Research data also revealed that the slow release of H_2S by such drugs significantly inhibits gastrointestinal damage caused by non-steroidal anti-inflammatory compounds, while retaining potent cyclooxygenase inhibitory activity (11, 73). Indeed, therapeutic H_2S-releasing compounds have been successfully tested in human clinical trials. In a recent Phase 2 human clinical trial, an H_2S-releasing naproxen derivative was shown to be gastric safe while producing significantly enhanced anti-inflammatory benefits in patients with arthritis *versus* naproxen alone (11).

A broad range of H_2S-based therapies are currently being developed worldwide. Dietary and recently developed therapeutic H_2S donors have been shown to yield a broad range of benefits, from the repair of microbiota dysbiosis to control of age-associated neurodegenerative disorders (15, 30, 44, 67, 74). H_2S signaling by gut microbes also yields cardioprotective effects (68). Taken together, the findings reported to date demonstrate that while H_2S is also known as a toxic gas, it yields significant health benefits. The toxic *versus* beneficial effects of H_2S remain controversial and appear to depend on the source and concentration of H_2S (5, 74).

Interestingly, homologs of the three enzymatic pathways involved in the endogenous production of H_2S in mammals (CBS, CSE, and 3MST) also exist in bacteria, which have the unique ability to also produce H_2S from L-serine metabolism (59, 74). Similar to what has been observed in mammals, bacterial H_2S may also scavenge metal ions and reactive oxygen species, as was recently established for *Pseudomonas aeruginosa* and *Escherichia coli* (40, 51, 59, 60). Whether or not these abilities qualify H_2S as a real gasotransmitter in bacteria remains a topic of controversy (76).

H_2S and the Gut Microbiome Biofilm

Gastrointestinal microbiota live in close interactions with themselves and with the outer mucus layer overlaying the host epithelium. They live as multispecies microbial communities known as biofilms, comprising adhering and free-swimming microbes (7, 48). These biofilms are typically coated with a complex extracellular matrix containing exopolysaccharides, proteins, RNA and DNA, and other organic materials. Recent findings have demonstrated that these microbiota biofilms are physically separated from the host intestinal tissues by secretion of thrombin from the epithelium (45). Indeed, microbial adhesion to and invasion through the host epithelium trigger potent inflammatory responses. In homeostasis, this appears to be prevented, at least in part, by the constitutive production of low concentrations of thrombin by the epithelium, which keeps the biofilm at bay, through proteolytic degradation of its matrix-associated proteins (45). In diseases where microbiota dysbiosis plays a central pathogenic role such as in IBD, epithelial thrombin is abnormally elevated (47). It remains to be shown whether such increased proteolytic activity during inflammation directly breaks down biofilm integrity to allow its microbial constituents to adhere to the epithelium. In turn, this loss of spatial segregation facilitates microbial translocation and disrupts intestinal barrier function, both well-established causes of various inflammatory disorders (34, 47).

When temporarily benign microbes or microbiota commensals become invasive and pathogenic, they are known as opportunistic pathogens or pathobionts (7, 27). While commensal microbiota antagonize the virulence of exogenous gut pathogens, exposure to enteropathogens may promote the local production of pathobionts. For example, in the presence of *Campylobacter jejuni*, the noninvasive *E. coli* strain activates its dormant virulence genes regulating fimbrial and flagellar expression and hence becomes an invasive proinflammatory pathobiont (28, 56). Similarly, upon exposure to the intestinal protozoan parasite, *Giardia duodenalis*, human commensal microbiota biofilms cause intestinal inflammation in germ-free mice and become lethal for the nematode *Caenorhabditis elegans*, while naïve biofilms remain innocuous in both models (4, 20). Pathobionts have been implicated in intestinal inflammation in humans. Attaching–invasive *E. coli* (AIEC) and *Enterococcus faecalis* pathobiont isolates are well-known contributors to the etiology of IBD (9, 41). These bacteria produce extracellular proteases and mucinases, lending further support to the hypothesis that they may contribute to disease by proteolytic fragmentation of gut microbiota biofilms, in addition to their known pathogenic effects on host tissues (9, 41, 53). In addition to this direct pathogenic effect, inoculation of pathobionts may synergize with commensals to exacerbate pathology, an effect that requires activation of Th17 lymphocytes from the adaptive immune system (22). Similarly, *H. pylori*-induced intestinal inflammation is driven, at least in part, by pathobiont-specific peripherally derived regulatory T cells (pT_{reg}) (78).

Hence, beyond the well-established changes in relative bacterial abundance, the physical, biological, and spatial

disruption of these biofilm communities, combined with the formation of pathobionts, plays a critical role in diseases associated with microbiota dysbiosis (3, 7, 9, 12, 22, 34, 41, 70). This concept is of key significance to the design of future therapeutic interventions targeting microbiota dysbiosis. In this context, endogenous production of H_2S and oral administration of H_2S donors have been shown to stabilize gut microbiota biofilm integrity and to prevent the formation of pathobionts, while concurrently inhibiting the development of acute inflammation (44, 46). The molecular structures and properties of the ever-increasing numbers of H_2S donors in experimental therapeutics have been the topics of recent reviews (62, 80). We invite readers interested in this area of research for drug development to visit these references.

Quorum-sensing molecules direct the social behavior of biofilm bacteria *via* intracellular messengers such as cyclic di-GMP, which coordinates the switch between biofilm and planktonic modes of growth (58, 69). More metagenomic and metatranscriptomic analyses are needed to better understand how commensal microbiota biofilms may release virulent planktonic pathobionts upon environmental stimulation. In the gut, luminal metabolites are key regulators of these phenomena and play central roles in niche selection and disease pathogenesis (21, 29, 38). Recent findings have revealed that intestinal iron may be key in the control of these biological traits.

It is well known that pathogenic bacteria have a high iron uptake capacity (61, 77). Similarly, AIEC overexpresses genes that regulate iron acquisition, including the *yersiniabactin* chu operon (13). Moreover, AIEC secretes cellulose in an iron-dependent manner to induce bacterial aggregation, which supports the hypothesis that iron may modulate biofilm formation (16). Anemia is a common extraintestinal complication in IBD patients, and dietary iron supplementation leads to disease exacerbation and significantly alters the gut microbiota *via* obscure mechanisms (35, 64). Thus, studies were undertaken in an attempt to determine whether the formation of pathobionts from microbiota biofilms in IBD may be regulated by iron.

Using a model of dinitrobenzene sulfonic acid (DNBS) colitis in rats, it was first observed that inflammation disrupts the mucus barrier and breaks down the microbiota biofilm into fragments that directly adhere to the epithelial surface, from which pathobionts invade the epithelium (46) (Fig. 2). These defects are similar to those reported in human patients with IBD (34, 41, 47). In animals with DNBS colitis, these disruptions were abolished when animals were given an H_2S donor, diallyl disulfide (DADS), orally twice daily for 5 days (46). Administration of DADS to control animals did not cause any macroscopic or microscopic damage to the colonic epithelium and did not lead to inflammation, further supporting that H_2S donors are not toxic (46). Inhibition of the pathogenic effects of colitis by 2,4,6-trinitrobenzenesulfonic acid included restoration of mucus integrity and dramatic anti-inflammatory benefits (44, 46). Subsequent studies then discovered that the invasiveness and proinflammatory effects of the pathobionts released from the microbiota biofilm were sensitive to iron chelators (44). Daily oral administration for 7 days of an H_2S donor (ATB-429; Antibe Therapeutics, Inc.) protected mice with DNBS colitis from mucus disruption, microbiota biofilm fragmentation and adherence to the epithelium, formation of invasive pathobionts, and inflammation (Fig. 2) (44, 46). Indeed, the dramatic attenuation of DNBS colitis by ATB-429 was associated with inhibition of pathobiont translocation to the liver (Fig. 3) and reduced levels of intracellular iron in fecal bacteria (Fig. 4). This was due, at least in part, to newly discovered iron-chelating properties of ATB-429 (Fig. 4) (44). $CSE^{-/-}$ mice lacking the CSE gene and hence incapable of producing adequate amounts of H_2S exhibited abnormalities in the mucus lining, disruption of microbiota biofilms, and severe inflammation, similar to what was seen in animals with DNBS colitis or in rats treated with a CSE inhibitor (46). The data obtained from rats and mice indicate that exogenous and endogenous H_2S protects the gastrointestinal mucosa from inflammatory injury, at least in part, through stabilizing effects on the intestinal microbiota (6, 46, 66).

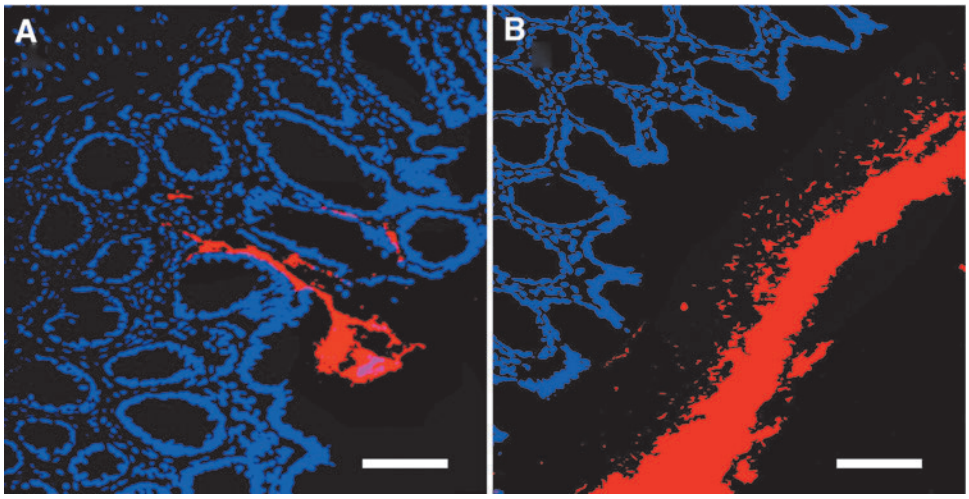

FIG. 2. H_2S donors maintain mucus barrier and microbiota biofilm integrity. Dysbiotic microbiota (*red*) in rats with DNBS colitis exhibit abnormal biofilm fragmentation and adhesion to the host intestinal tissues (nuclei stained in *blue*), coinciding with the formation and invasion of pathobionts through the epithelial lining **(A)**. Daily oral administration of an H_2S donor (ATB-429; Antibe Therapeutics, Inc.) completely abolishes these defects by maintaining microbiota integrity and the mucus barrier, illustrated as the unstained shadow between microbiota (*red*) and host tissues (*blue*), and preventing the formation of invasive pathobionts **(B)**. Bar = 50 μm [modified from the study by Buret *et al.* (7)]. DNBS, dinitrobenzene sulfonic acid.

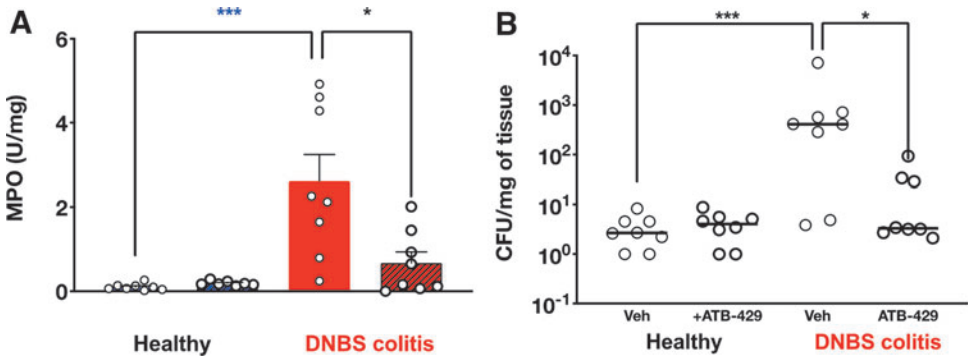

FIG. 3. Oral administration of the H_2S donor ATB-429 (50 mg/kg, twice daily, per os) reduces the severity of colitis and inhibits the translocation of commensal bacteria to the liver in C57Bl/6 mice with DNBS colitis, compared with mice given vehicle (veh) [modified from the study by Motta *et al.* (44)]. **(A)** Colitis (DNBS/veh) caused a significant granulocyte infiltration (indicated by an increase in MPO) compared with healthy mice (Healthy); treatment with ATB-429 (DNBS/ATB-429) significantly reduced MPO activity. **(B)** Colitis (DNBS/veh) caused significant microbial translocation to the liver (CFU counts per mL); treatment with ATB-429 (DNBS/ATB-429) reduced colitis-induced bacterial translocation; $n = 8$ mice per group. Kruskal–Wallis test, followed by Dunn's tests. $^*p < 0.05$; $^{***}p < 0.001$. For detailed methodology, see Motta *et al.* (44). MPO, myeloperoxidase activity.

Consistent with these findings, oral H_2S protects against gastrointestinal injury and promotes the resolution of inflammation and tissue injury caused by ethanol, ischemia–reperfusion, and nonsteroidal anti-inflammatory drugs (11, 71, 73, 74). In addition, as iron uptake is a key mechanism in conferring virulence to pathobionts dispersed from dysbiotic microbiota biofilms, we propose that this pathogenic mechanism may also be targeted with H_2S donors for therapeutic purposes (7). Recent observations correlate the metabolic response of the opportunistic nosocomial pathogen, *Acinetobacter baumannii*, to H_2S and reactive sulfur species with bacterial persulfide-sensing systems linked to biofilm formation, in further support of the importance of H_2S as a modulator of microbial biofilms (75). However, there are limited research data available to date on the effects of H_2S on the gut microbiota. More research is needed to characterize changes in microbiota metabolomics, transcriptomics, and proteomics in the context of exposure to either endogenous or exogenous H_2S.

Effects of the Diet

Plants contain H_2S where it plays important signaling roles, including in post-translational persulfidation, germination, root development, and fruit ripening, mostly through activation of antioxidant systems (10). It is not surprising therefore that diet alters H_2S accumulation in the human gut. Recent observations have established that exogenous H_2S played a central role in mediating the control of stress and

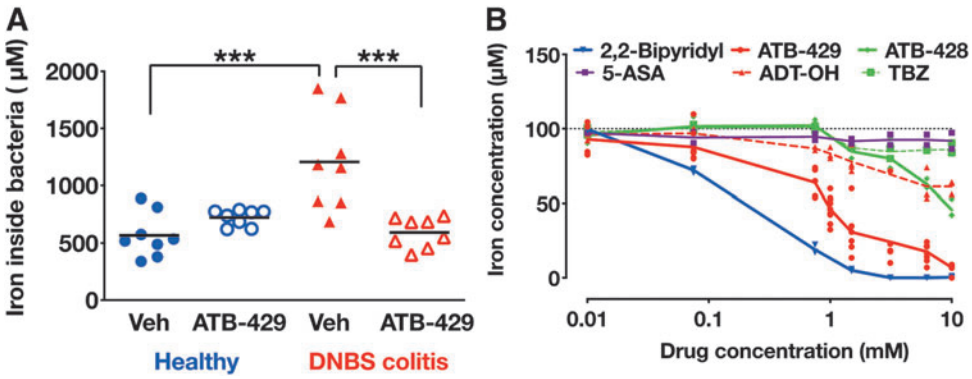

FIG. 4. Production of pathobionts in colitis coincides with elevated iron intake by commensal microbiota bacteria. Administration of an H_2S donor (ATB-429) reduces the intracellular iron content in fecal bacteria from mice with DNBS colitis. ATB-429 has iron-chelating properties [modified from Motta *et al.* (44)]. **(A)** Intracellular concentrations of iron in fecal microbiota (normalized to 1 g of feces) are significantly higher in microbiota from mice with colitis (DNBS) compared with those of control animals (vehicle, veh) and groups treated with ATB-429 (50 mg/kg, twice daily, per os); $n = 8$ mice per group. Kruskal–Wallis test, followed by Dunn's tests. $^{***}p < 0.001$. **(B)** H_2S-relasing compounds have iron-chelating properties, at least in part, in a drug-selective manner. H_2S-releasing ATB-429 (mesalamine linked to the donor ADT-OH), ATB-428 (to a lesser extent; mesalamine linked to the H_2S donor TBZ), and the positive control iron chelator 2,2-bipyridil, but not mesalamine (5-ASA) or the TBZ H_2S donor alone, have dramatic iron-chelating properties. Drugs were dissolved in bacterial media containing 100 μm of $FeCl_2$. After 24 h, the remaining concentration of free iron measured by ferrozine spectrophotometry was markedly reduced in a positive control iron chelator (2,2-bipyridil) and in ATB-429, ATB-428, and ADT-OH, but not in TBZ and mesalamine (5-ASA); $n = 3$ to 9 duplicates per concentration of drugs. For detailed methodology, see Motta *et al.* (44).

extension of life span resulting from caloric restriction (50, 73). Garlic, onion, cauliflower, broccoli, kale, cabbage, and leek are rich in organic polysulfides, which have long been associated with antimicrobial properties (15, 49). The volatile sulfur compounds in durian fruit are also known to react with glutathione to produce H_2S (36). Moreover, as L-cysteine is the key precursor of endogenous H_2S production in mammals, ingestion of this amino acid yields the physiological benefits of H_2S by enhancing its local production, which now can be measured using a novel gas-profiling technology (5, 79). For example, dietary cysteine intake has been associated with lower risk of stroke (33). Little is known of the direct effects of dietary H_2S or its precursors on gut microbiota.

In the gut, bile salts are conjugated with taurine and glycine before being hydrolyzed by bacterial bile salt hydrolase activity (24). Recent findings confirmed the interactions between H_2S, bile acid metabolism, bile salt hydrolase, enterohepatic circulation, and the gut microbiome. Using a model of non-steroidal anti-inflammatory drug (NSAID)-induced enteropathy in rats, these studies discovered that oral administration of the garlic H_2S donor, DADS, protected against mucosal injury through an interrelated modulation of bile physiology and the microbiota (6). DADS alone caused a significant decrease in several *Clostridiales* families and an increase in abundance of *Mucispirillum* (6). It had been shown previously that NSAIDs alter the gut microbiota and these changes, in turn, lead to increased concentrations of more cytotoxic secondary bile acids (39). Administration of DADs inhibited these cytotoxic abnormalities (6).

Other studies have reported that green plant monosaccharides such as sulfoquinovose were used as microbial nutrients as well as a source of sulfites to modify relative microbial abundance and activity in the human gut (8, 17). Anaerobic bacteria, for example, *Desulfovibrio* and *Bilophila* spp., use sulfoquinovose degradation intermediates (3-sulfolactate and 2,3-dihydroxypropane-1-sulfonate) for sulfite respiration to produce H_2S (17). This anaerobic pathway hence represents a critical component of H_2S production by intestinal microbiota from diet and may therefore be very significant to human health.

To what extent gut microbiota constituents are able to generate H_2S from dietary components, and/or respond to diet-derived H_2S, remains to be fully elucidated. Unraveling these mechanisms may bear great significance on how dietary approaches may be used to treat microbiota disruptions in disease. Together, the discoveries made to date reveal that endogenous and exogenous H_2S plays key roles in the direct modulation of microbiota–host interactions (Fig. 2). In the context of microbiota dysbiosis, H_2S appears to stabilize the microbiome biofilm, and this mechanism further contributes to resolution of inflammation and repair of tissue injury induced by this gasotransmitter (44, 46, 72).

Key Points

- Gut microbiota live as multispecies biofilms
- Mucus and epithelial thrombin maintain microbiota biofilms separated from epithelia
- Fragmentation and epithelial adherence of microbiota biofilms cause inflammation
- Commensal microbiota may become invasive pathobionts and cause inflammation
- Gastrointestinal H_2S is produced by bacteria or host cells or it comes from dietary sources
- High concentrations of bacterial H_2S are toxic to the host and low levels of host-derived or exogenous H_2S are therapeutic
- H_2S has potent cytoprotective and anti-inflammatory properties
- H_2S maintains mucus integrity
- H_2S inhibits pathogenic fragmentation of microbiota biofilms
- H_2S inhibits the production and invasiveness of pathobionts
- Diet-derived H_2S may shape microbiota in health and disease

Conclusions

While some reports indicate that high concentrations of bacterially derived H_2S in the gut may have toxic effects, it is becoming increasingly evident that low endogenous and exogenous doses of this gaseous mediator yield potent physiological benefits. In recent years, substantial evidence has been published on the therapeutic properties of various H_2S donors. These have been shown to generate anti-inflammatory and cytoprotective effects, promote angiogenesis, yield cardiovascular benefits, control age-associated neurodegenerative disorders, and act as potent analgesics. These properties are associated with strong cyclooxygenase inhibitory activity. Emerging data indicate that dietary H_2S may have at least some similar benefits, although much remains to be learnt on this topic. Adding to these effects, recent research has now demonstrated that H_2S is a potent stabilizer of the gastrointestinal mucus layers and microbiota biofilms. H_2S inhibits pathogenic fragmentation and tissue adherence of gut microbiota biofilms seen in disease and blocks the formation of pathobionts from the commensal microbiome. Novel H_2S donors (*e.g.*, ATB-429; Antibe Therapeutics, Inc.) disarm these newly released pathobionts by chelating environmental iron, which in turn prevents bacterial invasion and development of inflammation.

Future research needs to further characterize the mechanisms by which H_2S shapes the microbiome. In addition, in view of the role played by H_2S in tolerance to antibiotics, studies are warranted to determine how H_2S-based therapy targeting microbiota dysbiosis may affect antibiotic efficacy against enteropathogens. Finally, we need to improve our understanding of how immune pathways are affected by H_2S released from the gut microbiome. In view of the critical role played by microbiota dysbiosis in a broad range of disorders, the therapeutic potential of H_2S-induced microbiome biofilm stabilization warrants further investigation.

Authorship Confirmation Statement

All authors have contributed to this study and read, edited (where needed), and approved the article as submitted.

Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

3MST = 3-mercaptopyruvate sulfurtransferase
AIEC = attaching–invasive *E. coli*
CBS = cystathionine beta-synthase
CSE = cystathionine gamma-lyase
DADS = diallyl disulfide
DNBS = dinitrobenzene sulfonic acid
H_2S = hydrogen sulfide
IBD = inflammatory bowel disease
IECs = intestinal epithelial cells
MPO = myeloperoxidase activity
NSAID = non-steroidal anti-inflammatory drugs
pT_{reg} = peripherally derived regulatory T cells
SRB = sulfur-reducing bacteria