Enhanced Synthesis and Diminished Degradation of Hydrogen Sulfide in Experimental Colitis: A Site-Specific, Pro-Resolution Mechanism

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Abstract

Hydrogen sulfide (H₂S) is produced throughout the gastrointestinal tract, and it contributes to maintenance of mucosal integrity, resolution of inflammation, and repair of damaged tissue. H₂S synthesis is elevated in inflamed and damaged colonic tissue, but the enzymatic sources of that synthesis are not completely understood. In the present study, the contributions of three enzymatic pathways to colonic H₂S synthesis were determined, with tissues taken from healthy rats and rats with colitis. The ability of the colonic tissue to inactivate H₂S was also determined. Colonic tissue from rats with hapten-induced colitis produced significantly more H₂S than tissue from healthy controls. The largest source of the H₂S synthesis was the pathway involving cysteine amino transferase and 3-mercaptopyruvate sulfurtransferase (an α-ketoglutarate-dependent pathway). Elevated H₂S synthesis occurred specifically at sites of mucosal ulceration, and was not related to the extent of granulocyte infiltration into the tissue. Inactivation of H₂S by colonic tissue occurred rapidly, and was significantly reduced at sites of mucosal ulceration. This correlated with a marked decrease in the expression of sulfide quinone reductase in these regions. Together, the increased production and decreased inactivation of H₂S at sites of mucosal ulceration would result in higher H₂S levels at these sites, which promotes resolution of inflammation and repair of damaged tissue.

Introduction

Hydrogen sulfide is produced in virtually every organ system in the body [1,2] and can modulate a variety of physiological processes, including vasodilation [3], neurotransmission [4], nociception [5,6] and inflammation [7–9]. Based on these observations, a number of H₂S-releasing therapeutic agents are in development for a wide range of disorders [8,10–17]. The importance of H₂S in the gastrointestinal tract is highlighted by its triphosphate, particularly in circumstances of reduced oxygen metabolism, driving production of adenosine from healthy controls. The largest source of the H₂S synthesis was the pathway involving cysteine amino transferase and 3-mercaptopyruvate sulfurtransferase (an α-ketoglutarate-dependent pathway). Elevated H₂S synthesis occurred specifically at sites of mucosal ulceration, and was not related to the extent of granulocyte infiltration into the tissue. Inactivation of H₂S by colonic tissue occurred rapidly, and was significantly reduced at sites of mucosal ulceration. This correlated with a marked decrease in the expression of sulfide quinone reductase in these regions. Together, the increased production and decreased inactivation of H₂S at sites of mucosal ulceration would result in higher H₂S levels at these sites, which promotes resolution of inflammation and repair of damaged tissue.

The majority of H₂S production in mammalian tissue is enzymatically regulated, with the pyridoxal-5'-phosphate (P5P)-dependent enzymes cystathionine-beta-synthase (CBS) and cystathionine-gamma-lyase (CSE) being the most extensively studied [1]. During a bout of hapten-induced colitis in rats, the capacity of colonic tissue to produce H₂S is substantially increased, in parallel with the degree of mucosal inflammation [25]. The H₂S produced in this setting exerts significant beneficial effects in terms of reducing inflammation and enhancing healing of the ulcerated tissue [25]. Based on pharmacological studies (i.e., using inhibitors of CBS and CSE), CBS appeared to account for the majority of the observed increase in colonic H₂S synthesis during colitis [25]. However, administration of inhibitors of both CBS and CSE did not completely abolish colonic H₂S production, raising the possibility that there may be other sources of H₂S synthesis in the colon [25]. We previously reported that enteric bacteria did not account for a significant portion of what we have measured as colonic tissue H₂S synthesis [36].

A third enzymatic pathway for H₂S synthesis, which does not require P5P as a co-factor, was identified in the brain of CBS-deficient mice by Shibuya et al. [37] (Figure 1). Cysteine aminotransferase (CAT), which requires α-ketoglutarate as a co-factor, converts L-cysteine into 3-mercaptopyruvate, which can then be converted by mercaptopyruvate sulfurtransferase (3MST) and promote healing [26].

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to H₂S and pyruvate [37,38]. This pathway has also been shown to contribute to H₂S synthesis in the vascular endothelium of the thoracic aorta [37,38].

In the present study, we examined the contribution of the CAT-3MST pathway and the two P5P-dependent pathways to H₂S synthesis in the healthy and inflamed colon. We also attempted to determine the relative contributions of the ulcerated versus non-ulcerated colonic mucosa to H₂S synthesis, and we examined the capacity of these tissues to inactivate H₂S. Our results demonstrate that there is a site-selective enhancement of H₂S synthesis, as well as decreased H₂S inactivation, in the regions of ulceration. Such changes are consistent with an important role of this gasotransmitter in repair of injury and resolution of inflammation.

Methods and Materials

Animals

Male, Wistar rats (from Charles River Breeding Farms, St-Constant, QC, Canada) were housed in plastic cages and maintained under controlled temperature (20°C), humidity (60%–70%), and light cycle (12 h:12 h light-dark). The rats were fed standard laboratory chow and water ad libitum. All experimental protocols were approved by the Animal Research Ethics Board at McMaster University, and adhered to the guidelines established by the Canadian Council on Animal Care.

Mice with a targeted disruption of the CSE gene [39] and from age-matched [16 weeks], wild type littermates on the C57BL/6j X 129SvEv background were housed in the animal care facility at Lakehead University (experimental protocols were approved by the Animal Care Committee of Lakehead University). Heterozygous CBS deficient (CBS+/−) mice on the C57BL/6 background were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and bred and maintained in the animal care facility at McMaster University. Homozygous CBS deficient mice (CBS−/−) display significant growth retardation and rarely survive past 5 weeks of age, therefore CBS+/- mice were used [40]. Age-matched (20 weeks), wild type (CBS ++/) littermates were used as controls.

Induction of colitis

Colitis was induced in conventionally housed rats using dinitrobenzene sulfonic acid (DNBS) [41], a modified version of the original trinitrobenzene sulfonic acid model of colitis [42]. Rats were given 30 mg DNBS intracolonically in 0.5 mL of 50% ethanol. Groups of rats (n=4) were euthanized 6 h to 28 days after DNBS administration for determination of H₂S synthesis. Colonic inflammation was assessed by the measurement of myeloperoxidase (MPO) activity, using a method modified [43] from that described by Bradley et al. [44]. MPO is an enzyme found primarily in the azurophilic granules of neutrophils, and has been used extensively as a quantitative index of granulocyte infiltration.

Separation of colonic mucosa and muscularis

After the rats were euthanized, the colons were excised, opened along the mesenteric border, washed thoroughly to remove fecal material and immediately placed in ice-cold potassium phosphate buffer, pH 8.0 (12% w/v). Sections of the distal colon were visualized under a dissecting microscope and pinned along the edges of the sections with care taken to minimize damage to the tissue. The border between the mucosa and smooth muscle layer was identified, then these two layers were gently separated using forceps, as described by Lowie et al. [45], and immediately snap-frozen in liquid nitrogen (for measurements of H₂S synthesis) or fixed in neutral-buffered formalin (for histology). In rats that had received DNBS, the mucosal and smooth muscle layers were separated in the same manner, then sections were cut at the border between the ulcer and the immediately adjacent non-ulcerated tissue, yielding separate mucosal and smooth muscle sections from both ulcerated and non-ulcerated areas. The tissues that were fixed in formalin were processed and stained with hematoxylin & eosin for microscopic examination, to confirm separation of the mucosal layer from the smooth muscle.

Measurement of H₂S synthesis

The capacity of tissue to produce H₂S was measured from homogenized tissue in the presence of exogenous substrate and/or inhibitors using a modified version of a previously described zinc-trapping assay [14,46]. Production of H₂S via the CAT-3MST pathway was determined using the substrate α-ketoglutarate (α-KG; 100 μM unless otherwise stated) and the competitive CAT inhibitor L-aspartate, and O-carboxymethyl-hydroxylamine hemihydrochloride (CHH; an inhibitor of aminotransferases, including CAT). Addition of the substrate, L-cysteine (10 mM), was necessary for detection of H₂S synthesis (P5P-dependent or -independent) by colonic tissue. H₂S synthesis via CSE and CBS required the presence of P5P (2 mM unless otherwise stated), while that via CAT/3MST required α-KG.

Measurement of H₂S inactivation

The ability of colonic tissue to inactivate H₂S, such as through metabolism and/or sequestration, was measured using a modified version of the above-mentioned zinc-trapping assay. Instead of including L-cysteine as a substrate for H₂S synthesis, vials containing either tissue homogenates or buffer were ‘spiked’ with 33 μL of 30 μM NaHS, the same H₂S-releasing compound that is used to generate the standard curve for the H₂S assay. The samples were then incubated for 5–90 min in a 37°C shaking water bath, after which trichloroacetic acid (TCA; 50%; 0.4 mL) was injected into the reaction mixture through the serum cap to halt the reaction. The vials were then transferred to a 50°C shaking water bath for 60 min to allow for the trapping of evolved H₂S by the zinc acetate. The greater the ability of a tissue to...
metabolize/sequester H₂S, the less H₂S would be trapped in the zinc acetate and subsequently detected in the reaction with FeCl₃ and N,N-dimethyl-p-phenyline-diamine sulfate salt, which results in methylene blue formation. To determine the recovery of H₂S in this assay in the absence of enzymatic activity, samples were treated in the same way, except that the TCA was added prior to addition of the NaHS. In this case, the amount of H₂S recovered 30 min later was ~80% of that recovered if the NaHS was added to vials not containing any tissue. In addition, in some experiments, potassium cyanide (1 mM) was added to the tissue samples immediately prior to the addition of NaHS to abolish mitochondrial activity and 30 min later the effects on recovery of H₂S were determined.

Expression of H₂S producing and catabolizing enzymes

Western blot analysis was used to determine colonic expression of CBS, CSE, CAT, 3MST, and sulfide quinone reductase (SQR) in samples from rats with colitis and healthy control rats. Colonic tissue was processed and blots were prepared as previously described [27]. Proteins were separated on 4-20% gradient polyacrylamide gels. Rabbit polyclonal anti-CSE (1:200), anti-CBS (1:800), anti-CAT (1:800), anti-3MST (1:200), and anti-SQR antibodies (1:500) were used. Enzyme expression was visualized using a secondary anti-rabbit IgG antibody conjugated to horseradish peroxidase (1:1000) and an enhanced chemiluminescence detection kit on a Chemi-doc gel imaging system (Bio-Rad). The intensity of the bands was determined and analyzed using ImageLab 2.0 software (Bio-Rad). The expression of each enzyme was normalized to the expression of β-actin.

Figure 2. H₂S synthesis by healthy colonic tissue occurs via multiple pathways. Panel A: In the absence of pyridoxal-5'-phosphate (PSP), α-ketoglutarate increased the production of H₂S in by colonic tissue in a concentration-dependent manner (**p<0.01 vs. the group with no α-ketoglutarate added). Panel B: Maximal production of H₂S from L-cysteine by the healthy colon was observed in the presence of 3 mM PSP or 300 μM α-ketoglutarate. The maximal production of H₂S in the healthy colon in the presence of α-ketoglutarate was markedly higher than the maximal production of H₂S in the absence of PSP. Panel C: At a concentration of 1 mM, L-aspartate significantly inhibited H₂S synthesis by healthy colonic tissue. Panel D: CHH (O-carboxymethyl-hydroxylamine hemihydrochloride) concentration-dependently inhibited α-ketoglutarate-dependent colonic H₂S synthesis. The reactions shown in Panels C and D were carried out in the absence of pyridoxal-5'-phosphate and presence of α-ketoglutarate (100 μM). In the absence of both PSP and α-ketoglutarate there was no detectable H₂S production. Each bar represents the mean ± SEM of 4–6 rats (*p<0.05, **p<0.01, ***p<0.001 vs. the control group). doi:10.1371/journal.pone.0071962.g002
Materials

Isoflurane was obtained from Abbott Laboratories (Montreal, QC, Canada). 4–20% gradient polyacrylamide gels were purchased from Bio-Rad Laboratories (Mississauga, ON, Canada). Anti-CSE, anti-CAT, and anti-SQR primary antibodies were obtained from Proteintech (Chicago, IL, USA). Anti-CBS and anti-3MST primary antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). The secondary anti-rabbit IgG antibody conjugated to horseradish peroxidase was purchased from Invitrogen (Camarillo, CA, USA). The enhanced chemiluminescence detection kit was obtained from GE Healthcare (Baie d’Urfe, QC, Canada). All other reagents were purchased from Sigma-Aldrich Co. (Oakville, ON, Canada).

Statistical analysis

All data are expressed as the mean ± SEM. Groups of data were compared to one another using a one-way analysis of variance and the Neuman-Keuls test. An associated probability of less than 5% was considered significant.

Results

Healthy colonic tissue produces H2S via the CAT-3MST pathway

Colonic H2S synthesis via the CBS/CSE pathways versus the CAT-3MST pathway could be delineated through inclusion or exclusion of the co-factors P5P and α-KG (Figure 1). Healthy rat colonic tissue produced H2S in the absence of P5P and the presence of α-KG, consistent with synthesis via the CAT-3MST pathway (Figure 2A). The amounts of H2S produced increased with the concentration of α-KG.

To determine the relative contributions of the CBS/CSE versus the CAT-3MST pathways to colonic H2S synthesis in rats, we determined the optimal concentrations of co-factors (P5P and α-KG, respectively) for each pathway, by performing concentration-
H2S is produced via CAT-3MST in inflamed colon

A pilot time-course study demonstrated that the greatest granulocyte infiltration of the colon occurred at 3 days after DNBS administration (MPO activity in DNBS-treated rats averaged 22.2 ± 4.3 U/mg, versus 4.6 ± 0.5 U/mg in healthy controls; p < 0.05). Colonic H2S synthesis was significantly elevated in samples from rats with colitis, both through the P5P-dependent pathways and the CAT/3MST pathway (Figure 4A). As in the case for colonic tissue from healthy rats, there was substantially greater H2S synthesis from the CAT/3MST pathway in tissue from rats with colitis than there was from the P5P-dependent pathways. The two inhibitors of CAT (CHH and L-aspartate) each substantially reduced α-KG-dependent H2S synthesis by inflamed colonic tissue (Figure 4B).

Ulcerated mucosa is the major site of elevated H2S production

H2S synthesis via CAT-3MST from the mucosa and muscularis layers of the colonic tissue from healthy rats was comparable (Figure 5A), as was the case for H2S synthesis via P5P-dependent pathways (Figure 5B). However, the mucosa from sites of ulceration produced significantly more H2S via the CAT-3MST pathway (Figure 5A) and the P5P-dependent pathways (Figure 5B). H2S synthesis from samples of the muscularis layer were relatively low, though a significant increase in synthesis via the P5P-dependent pathways was observed when the tissue was from a region underlying a site of ulceration (Figure 5B). H2S synthesis from mucosal or muscularis samples from rats with colitis, but taken from sites that were not ulcerated, was similar to that from samples taken from healthy controls. However, the elevated synthesis of H2S from tissues taken from sites of ulceration did not appear to be related to inflammation (e.g., granulocyte infiltration) at those sites. MPO activity, a marker of granulocyte infiltration, was comparable in samples of mucosa from sites of ulceration versus non-ulcerated sites (21.5 ± 0.5 U/mg versus 17.8 ± 2.0 U/mg, respectively; ns), but significantly elevated versus healthy controls (5.1 ± 0.5 U/mg; p < 0.05). The same was the case for samples of muscularis from sites of ulceration versus non-ulcerated sites (10.9 ± 2.9 U/mg vs. 8.6 ± 3.2 U/mg, respectively; ns), in contrast to the significant elevation of MPO versus healthy controls (0.5 ± 0.2 U/mg; p < 0.05).

Increased expression of H2S-producing enzymes during colitis

Consistent with the predominance of CSE as a source of P5P-dependent H2S synthesis, expression of this enzyme was markedly up-regulated in inflamed colonic tissue as compared to tissue from healthy controls (Fig. 6A). Likewise, Western blots confirmed the presence of CAT (data not shown) and 3MST in the healthy and inflamed colon of rats (Fig. 6B), with a substantial increase in 3MST expression in inflamed colonic tissue (Fig. 6B). Interestingly, the increased expression of 3MST and CSE during colitis occurred at sites of mucosal ulceration, but not in non-ulcerated...
tissue from rats with colitis or in tissue from healthy controls (Fig. 6C and D).

H₂S degradation is markedly reduced at sites of ulceration

Both the healthy and inflamed colon rapidly inactivated H₂S in vitro (Figure 7A), but there was significantly less inactivation of H₂S in samples from rats with colitis than in healthy controls. Approximately 20% of the H₂S that was added to the samples could not be recovered, and presumably was bound to tissue. Indeed, pretreatment of the tissue samples with potassium cyanide (1 mM) increased the recovery of H₂S after 30 min of incubation substantially (to 56.7±5.6% for tissue from healthy rats, and to 63.6±4.6% for tissue from rats with colitis). This suggests that the majority of the decrease in recovery of H₂S from the tissue samples was due to metabolic inactivation.

Inactivation of H₂S was substantially reduced in mucosal samples taken from sites of ulceration than that from adjacent, non-ulcerated tissue and to tissue from healthy controls (***p<0.001 and *p<0.05, respectively). Each bar represents the mean ± SEM of 3-5 rats.
ulceration when compared to immediately adjacent, non-ulcerated sites or to healthy controls (Figure 7C). No differences in inactivation of H₂S were observed for muscularis samples from healthy rats or rats with colitis, and there were no differences in H₂S recovery between muscularis samples taken from sites of ulcerated versus non-ulcerated sites (data not shown).

Discussion

H₂S is produced throughout the GI tract [47,48] and contributes to many digestive functions [49], including epithelial secretion [20–22], smooth muscle contraction [18,19] and mucosal defense [14,23–26,50]. H₂S also promotes resolution of inflammation and repair of injury in the gastrointestinal tract and elsewhere [7,14,25,26,51]. In the present study we have demonstrated that colonic H₂S synthesis does not occur solely through P5P-dependent pathways (CSE and CBS). Rather, a P5P-independent, α-KG-dependent pathway via the enzymes CAT and 3MST is the major source of H₂S synthesis in both the healthy and inflamed colon. We also demonstrated that in colitis the ulcerated mucosa is the major site of both P5P-dependent and α-KG-dependent H₂S synthesis and that this synthesis is not influenced by the extent of granulocyte infiltration into the tissue. Furthermore, inactivation of H₂S occurs rapidly in colonic tissue (in the mucosa much more quickly than in muscle), but at a significantly lower rate in ulcerated colonic tissue than in tissue immediately adjacent to ulcers or in healthy colonic tissue. Moreover, increased expression of H₂S-producing enzymes and decreased expression of the key H₂S inactivating enzyme (SQR) were observed specifically at sites of mucosal ulceration, but not in mucosa immediately adjacent to ulcers or in the healthy mucosa. One explanation for the decrease in expression of SQR at sites of ulceration is that it is just a consequence of the tissue destruction at those sites. However, at these same sites we observed significant up-regulation of 3MST, which like SQR, is primarily localized to mitochondria.

Our observation that H₂S synthesis was selectively up-regulated at sites of ulceration is consistent with an important role of this mediator in driving tissue repair [25,27,52]. We previously observed a marked up-regulation of CSE and CBS expression and a corresponding increase in H₂S synthesis at the margins of gastric ulcers, which is the key area of re-epithelialization and angiogenesis that drive ulcer healing [27]. Administration of inhibitors of H₂S synthesis resulted in delayed ulcer healing, while administration of H₂S donors accelerated ulcer healing [27]. The healing of ulcers in the colon has similarly been shown to be enhanced by H₂S donors [10,25,28,29]. It is well recognized that enzymes producing other mediators that promote ulcer healing, such as cyclooxygenase-2 and endothelial nitric oxide synthase, are also up-regulated at the margins of ulcers [53,54]. The trigger(s) for the elevated H₂S synthesis at sites of ulceration are not yet

Figure 7. Catabolism of H₂S by healthy and ulcerated colonic tissue. Panel A: Catabolism of H₂S was significantly reduced in tissue from rats with colitis as compared tissue from healthy controls (p<0.05, **p<0.01). Each point represents the mean ± SEM of 5–10 samples. Panel B: Catabolism of H₂S was significantly reduced in mucosal tissue from sites of ulceration as compared to mucosal tissue from healthy controls (**p<0.001) and as compared to mucosal tissue from adjacent, non-ulcerated sites in rats with colitis (p<0.05). Recovery of H₂S was measured 30 min after addition of NaHS to the tissue samples. Panel C: Expression of sulfide quinone reductase (SQR) was significantly reduced (*p<0.05) at sites of ulceration as compared to adjacent, non-ulcerated mucosa and to tissue from healthy controls. Each bar represents the mean ± SEM of 5 rats.
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known, but one possibility is that local release of vascular endothelial growth factor could play an important role. Vascular endothelial growth factor is an important signal for ulcer healing in the gastrointestinal tract and has been shown to promote angiogenesis, a crucial step in ulcer healing [53], in part through induction of CSE and elevation of H2S synthesis [52]. Bacteria-derived signals may also be involved in triggering up-regulation of colonic H2S synthesis when there are breaches of the epithelial barrier.

The levels of H2S at any given site is dependent upon rates of synthesis and of catabolism. Thus, the reduced rates of inactivation of H2S that we observed at sites of ulceration would contribute to producing elevated local levels of H2S, further contributing to healing. In healthy colonic tissue, H2S is rapidly degraded, mainly by oxidation to thiosulfate, and primarily through the enzymes SQR and rhodanese (thiosulfate transferase) [30,51,56–58]. SQR and rhodanese are highly expressed in the healthy colonic epithelium [30,51,56,59]. In vitro, colonic epithelial cells are capable of oxidizing H2S up to concentrations of 50 μM, via the mitochondrial sulfide oxidizing enzymes (59). The reduced inactivation of H2S at sites of ulceration may be attributable to the observed, marked reduction of SQR expression specifically at sites of ulceration in the colon. Linden et al. [58] recently suggested that these efficient catabolic pathways act to keep resting sites of ulceration in the colon. The resulting much lower levels of H2S would contribute to the on-demand mechanisms underlying this observation remains unknown.

Our observation that most of the H2S produced by colonic tissue is derived via a z-KG-dependent pathway was surprising.

Previous studies have focused on the enzymes CSE and CBS as important sources of H2S synthesis both in healthy and inflamed colonic tissue [25,28,40]. We previously reported that CBS was the major enzymatic source of colonic H2S, but this was based largely on our observation that CHH, an inhibitor of CBS, caused a dramatic exacerbation of colitis in rats, that was more profound than was seen with inhibitors of CSE. However, in the present study, using CSE−/− and CBS+/− mice, we found that CBS was the major source of P5P-dependent H2S synthesis in the healthy colon. Moreover, while CHH has been widely used as an inhibitor of CBS and has been shown to suppress P5P-dependent H2S synthesis [23,48,63], it also inhibits aminotransferases [62,64], and therefore can block the synthesis of H2S that occurs via the CAT-3MST pathway. This was confirmed in the present study. A recent study by Asimakopoulou et al. [65] tested the selectivity of several commonly used putative inhibitors of H2S-producing enzymes and concluded that no selective inhibitors of CBS are currently available.

In summary, the CAT-3MST enzymes represent the primary pathway for H2S synthesis in the healthy and inflamed colon. Marked increases in colonic H2S synthesis, via both P5P-dependent and z-KG-dependent pathways, occur specifically at sites of ulceration. The signals responsible for triggering this up-regulation of H2S synthesis are not yet clear. A marked reduction in tissue inactivation of H2S also occurs selectively at sites of ulceration. The resulting higher local levels of H2S in the microenvironment of the ulcer would act to promote rapid restoration of epithelial barrier integrity. These findings may have important implications with respect to improving treatments for chronic ulcerative conditions of the gastrointestinal tract, including Crohn’s disease and ulcerative colitis.

Author Contributions

Conceived and designed the experiments: KLF JGPF RW JLW. Performed the experiments: KLF. Analyzed the data: KLF JGPF RW JLW. Contributed reagents/materials/analysis tools: RW JLW. Wrote the paper: KLF JGPF RW JLW.

References

1. Kimura H (2011) Hydrogen sulfide: its production, release and functions. Amino Acids 41: 113–121.
2. Wang R (2012) Physiological implications of hydrogen sulfide: A whiff exploration that blossomed. Physiol Rev 92: 79–108.
3. Zhao W, Zhang J, Lu Y, Wang R (2001) The vasorelaxant effect of H2S as a novel endogenous gaseous KATP channel opener. EMBO J 20: 6008–6016.
4. Abe K, Kimura H (1996) The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci 16: 1066–1071.
5. Distriuti E, Sediari L, Mencarelli A, Renga B, Orlandi S, et al. (2006) Evidence that hydrogen sulfide exerts antiinflammatory effects in the gastrointestinal tract by activating KATP channels. J Pharmacol Exp Ther 316: 325–335.
6. Elakidi-Valentinis E, Santos K, Camargo EA, Denadai-Sousa A, Teixeira SA, et al. (2010) Differential effects of COX2 and endogenous hydrogen sulfide in carrageenan-induced knee joint synovitis in the rat. Br J Pharmacol 159: 1459–1474.
7. Duffon N, Natividad J, Verdu EF, Wallace JL (2012) Hydrogen sulfide and resolution of acute inflammation: A comparative study utilizing a novel fluorescent probe. Sci Rep 2: 499.
8. Wallace JL, Caliendo G, Santagada V, Cirino G, Fiorucci S (2007) Gastrointestinal safety and anti-inflammatory effects of a hydrogen sulfide-releasing dicyclofenac derivative in the rat. Gastroenterology 132: 261–271.
9. Zanardo RC, Beancalene V, Distriuti E, Fiorucci S, Cirino G, et al. (2006) Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. FASEB J 20: 2118–2120.
10. Fiorucci S, Orlandi S, Mencarelli A, Caliendo G, Santagada V, et al. (2007) Enhanced activity of a hydrogen sulfide-releasing derivative of mesalamine (ATB-429) in a mouse model of colitis. Br J Pharmacol 150: 996–1002.
11. Li L, Sahin-Telles M, Tan CH, Whiteman M, Moore PK (2009) GYY4137, a novel hydrogen sulfide-releasing molecule, protects against endotoxin shock in the rat. Free Radic Biol Med 47: 103–113.
12. Li L, Whiteman M, Guan Y, Neo KL, Chong Y, et al. (2008) Characterisation of a novel, water soluble hydrogen sulfide releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. Circulation 117: 2351–2360.
13. Szabo C (2007) Hydrogen sulphide and its therapeutic potential. Nat Rev Drug Discov 6: 917–935.
14. Wallace JL, Caliendo G, Santagada V, Cirino G (2010) Markedly reduced toxicity of a hydrogen sulfide-releasing derivative of naproxen (ATB-346). Br J Pharmacol 159: 1296–1314.
15. Wallace JL (2007) Hydrogen sulfide-releasing anti-inflammatory drugs. Trends Pharmacol Sci 28: 501–505.
16. Chattopadhayay M, Kodela R, Nash N, Dastagirzada YM, Velasquez-Martinez CA, et al. (2012) Hydrogen sulfide-releasing NSAIDs inhibit the growth of human cancer cells: a general property and evidence of a tissue type-independent effect. Biochem Pharmacol 83: 715–722.
17. Caliendo G, Cirino G, Santagada V, Wallace JL (2010) Synthesis and biological effects of hydrogen sulfide (H2S): development of H2S-releasing drugs as pharmaceuticals. J Med Chem 53: 6275–6286.
18. Hosoki R, Matsaki N, Kimura H (1997) The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem Biophys Res Commun 237: 527–531.
19. Teague B, Aisedu S, Moore PK (2002) The smooth muscle relaxant effect of hydrogen sulfide in vitro: evidence for a physiological role to control intestinal contractility. Br J Pharmacol 137: 139–145.
20. Iee F, Takakusa H, Hayashi S, Takahashi K, Koyama M, et al. (2011) Stimulation of duodenal HCO3− secretion by hydrogen sulfide in rats: relation to gastroduodenal, nitric oxide and sensory neurons. Acta Physiol 201: 117–126.
21. Pouokam E, Diener M (2011) Mechanisms of actions of hydrogen sulphide on the microvasculature. Br J Pharmacol 159: 1236–1246.
22. Schicho R, Krueger D, Zeller F (2006) Hydrogen sulfide is a novel prosecretory neuromodulator in the guinea-pig and human colon. Gastroenterology 131: 1542–1552.
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23. Fiorucci S, Antonelli T, Distruiti E, Rizzo G, Mancarelli A, et al. (2005) Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. Gastroenterology 129: 1210–1224.

24. Wallace JL (2010) Physiological and pathophysiological roles of hydrogen sulfide in the gastrointestinal tract. Annu Rev Redox Signal 12: 1125–1133.

25. Wallace JL, Yong L, Mc��ght W, DICay M, Martin GR (2009) Endogenous and exogenous hydrogen sulfide promotes resolution of colitis in rats. Gastroenterology 137: 569–578.

26. Wallace JL, Ferraz JGP, Muscara MN (2012) Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury. Antioxid Redox Signal 17: 58–67.

27. Wallace JL, DICay M, Mc身ght W, Martin GR (2007) Hydrogen sulfide enhances ulcer healing in rats. FASEB J 21: 4070–4076.

28. Hirata J, Naito Y, Takagi T, Mizumura K, Suzuki T, et al. (2011) Endogenous hydrogen sulfide is an anti-inflammatory molecule in dextran sodium sulfate-induced colitis in mice. Dig Dis Sci 56: 1379–1386.

29. Matsunami M, Kirishi S, Okai T, Kagahata A (2012) Hydrogen sulfide-induced colonic mucosal cytoprotection involves T-type calcium channel-dependent neuronal excitation in rats. J Physiol Pharmacol 63: 61–18.

30. Goubern M, Andriamihaja M, Niel J, Blachier F, Bossu F (2007) Sulfide, the first inorganic substrate for human cells. FASEB J 21: 1699–1706.

31. Blachier F, Davia M, Minou M, Benetini P, Atanasii C, et al. (2010) Luminal sulfide and large intestine mucosa: friend or foe? Anmico Acids 39: 335–347, 2010.

32. Theissen U, Hoffmeister M, Grieshaber M, Wallace JL (2003) Single eubacterial origin of eukaryotic sulfate:quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during anoxic and sulfideic times. Mol Biol Evol 20: 1564–1574.

33. Erlod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, et al. (2007) Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. Proc Natl Acad Sci U S A 104: 15560–15565.

34. Fu M, Zhang W, Wu L, Yang G, Li H, et al. (2012) Hydrogen sulfide (H2S) metabolism in mitochondria and its regulatory role in energy production. Proc Natl Acad Sci U S A 109: 2943–2948.

35. Kimura Y, Goto Y, Kimura H (2010) Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. Antioxid Redox Signal 12: 1–13.

36. Flannigan KL, McCoy KD, and Wallace JL (2011) Eukaryotic and prokaryotic contributions to colonic hydrogen sulfide synthesis. Am J Physiol Gastrointest Liver Physiol 301: G188–G193.

37. Shibuya N, Ikemi Y, Kimura Y, Nagahara H, Kimura H (2009) Vascular endothelial expression 3-mercaptopyruvate sulfurtransferase and production of hydrogen sulfide in murine peripheral tissues but not in the CNS. Br J Pharmacol 165: 2178–2190.

38. Szabo S, Vincze A, Sandor Z, Judas M, Gombos Z, et al. (1998) Vascular production in colon cancer cells. Antioxid Redox Signal 12: 1101–1109.

39. Linden DR, Furne J, Stoltz GJ, Abdel-Rehim MS, Levitt MD, et al. (2008) Production and exogenous hydrogen sulfide promotes resolution of colitis in rats. Dig Liver Dis 42: 103–109.

40. Fiorucci S, Distruiti E, Cirino G, Wallace JL (2006) The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. Gastroenterology 131: 259–271.

41. Martin GR, Mc身ght W, DICay MS, Coffin CS, Ferraz JG, et al. (2010) Hydrogen sulfide synthesis in the rat and mouse gastrointestinal tract. Dig Liver Dis 42: 103–109.

42. Picton R, Ego Madeira, Ferraz JGP, Muscara MN (2012) Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury. Antioxid Redox Signal 17: 1–10.

43. Picton R, Ego MG, Merril GA, Langman MJ, Singh S (2002) Macular protection against sulfide: importance of the enzyme rhodanese. Gut 50: 201–205.

44. Szabo S, Vincze A, Sandor Z, Judas M, Gombos Z, et al. (1998) Vascular production in colon cancer cells. Antioxid Redox Signal 12: 1101–1109.

45. Linden DR, Furne J, Stoltz GJ, Abdel-Rehim MS, Levitt MD, et al. (2012) Detoxification of H2S by differentiated colonic epithelial cells: implications of the sulfide oxidizing unit and of the cell respiratory capacity. Antioxid Redox Signal 17: 1–10.

46. Picton R, Ego MG, Merril GA, Langman MJ, Singh S (2002) Macular protection against sulfide: importance of the enzyme rhodanese. Gut 50: 201–205.