

Perturbing Transsulfuration Pathway and Sulfur Recycling, A New Direction Towards Therapeutic Intervention of Inflammatory Bowel Disease

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ABSTRACT

Combating inflammatory bowel disease (IBD) is becoming a growing challenge to the global health management. Our bowel can be envisioned as a big vessel reactor where millions of reactions occur at each moment, every day to meet the physiological demand of our body for quality control of our life. This includes the orchestration of several steps which ultimately funnel down to the intestinal system to drives the end point of the digestion process. Intestinal sulfur recycling by transsulfuration pathway can modulates the integrity of our bowel symptoms. Limiting the sulfur amino acid metabolism and mitigating hydrogen sulfide (H₂S) generation can help to stabilize the colonic biochemistry with monitoring of metabolites for subsequent health risk assessment. Regulating protein sulphydration in colonic tissue could play detrimental roles in the modulation of several contributing factors related to IBD.

KEYWORDS

Transsulfuration pathway; Hydrogen sulphide; Sulphydration; Inflammatory bowel disease; Biomarkers; Therapy.

INTRODUCTION

BACKGROUND

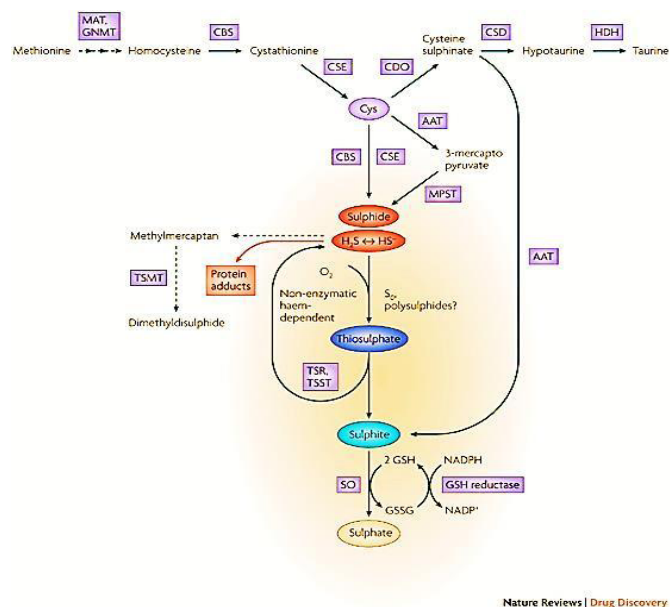
Factors Related to IBD Pathogenesis Connecting Transsulfuration Homeostasis:

Environment: Our bowel is challenged daily with a load of foods, primarily sourced from the environment. Many food components contain sulfides, polysulfide compounds, which ultimately breakdown to generate gaseous H₂S in situ [1, 2]. Hydrogen sulfide can interact with metabolic cytochrome c oxidase and carbonic anhydrase, by chelating with metal-binding porphyrin cofactor [3]. Evolutionarily, H₂S has been viewed as an important prebiotic element [4]. In nature, H₂S concentration increases in the deep ocean, oceanic volcanoes and mountain spring while the oxygen concentration decreases. Importantly, hydrogen sulfide can bypass the cellular oxygen demand and modulate energy production [5]. Thus, H₂S can sense the hypoxia related changes inside the cell [6].

Micro biome: There are many micro bacteria that can produce H₂S [7]. Some of these bacteria exist in the gut mucosal system of our gastrointestinal tract [8, 9]. Examples of these bacteria include sulfate reducing bacteria (SRB), thiobacillus, desulfobacter among others. Some of this microbiota could play a potent role during GI infection and inflammation, and thus could be one of the leading causes of IBD [8, 10-13]. Therefore, H₂S producing colonic microbiota in the GI tract can also modulate the mucosal immune system, and the IBD state, by producing H₂S or sulfur metabolites [8, 11, 13].

Immune Response: Immune surveillance in the GI tract plays a determining role in the progression of IBD [9, 14]. Our mucosal immune checkpoint does its best by T cell activation and adaptive immunities, like dendritic cell, and macrophage activation to nullify the toxins components that enter in our bowl during food intake [15]. It stimulates the cytokine (TNF- α , IFN- γ) and chemokine production by the processing of the

MHC class I & II peptides [10, 11]. H₂S can trigger T cell activation [16]. Mammalian tissue contains enzymes, Cystathionine beta synthase (CBS) and Cystathionine gamma lyase (CSE) that can endogenously produce H₂S (Figure 1) [3]. H₂S has been implicated in many inflammatory disease conditions, such as pancreatitis, sepsis, and joint and pulmonary inflammation [3, 17-19].



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Figure 1: Enzymatic pathways of H₂S 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-synthase (CBS) catalyses the production of cystathionine by transferring serine to homocysteine. Cystathionine-lyase (CSE), a pyridoxal 5'-phosphate-dependent enzyme, subsequently converts cystathionine to cysteine (Cys). CSE catalyses a α -disulphide elimination reaction that results in the production of pyruvate, NH₄⁺ and thio cysteine. Thiol cysteine may react with cysteine or other thiols to form hydrogen sulphide (H₂S). 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 H₂S 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 sulphiny 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. H₂S can also yield protein adducts, and can be converted to methylmercaptan and dimethylsulphide by thiol-S-methyltransferase (TSMT). Non-enzymatic oxidation of sulphide can also yield the generation of polysulphides and elemental sulphur

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Genome: Given the recent understanding on how several genomic alterations play a key role in the pathophysiological phenotype of many diseases, more targeted therapy can be achieved in IBD. The metabolic burden could trigger unfolded protein response (UPR) and thus (endoplasmic reticulum) ER stress to the colonic tissue. This event could lead to the upregulation (EIF2A, ATF6, IRE1, and PERK) and down regulation of several genes [10, 20]. H₂S can also cause ER stress by protein sulfhydrylation of protein tyrosine phosphatase 1B (PTP1B), a newly identified post translational modification [21]. Additionally, homocysteine, a component of sulfur amino acid metabolism, is also known to trigger ER stress [22]. Homocysteine is found to be high in fistula patients and possibly modulates methionine dependent genomic alteration [23, 24]. H₂S mediated sulfhydrylation of transcription factor (NF- κ B) leads to the induction of anti-apoptotic genes [25, 26]. H₂S can even inhibit the phenyl isothiocyanate (cancer preventive) mediated apoptosis of colon cancer [27]. Recent reports also show that CBS plays a pivotal role in the chemo resistance of various cancers [28, 29]. This raises the possibility that the antimicrobial agents that initially cure the gut mucosal inflammation during IBD, might develop resistance, possibly by sulfur amino acid metabolite.

Maturation of IBD by Transsulfuration and Sulfide Homeostasis:

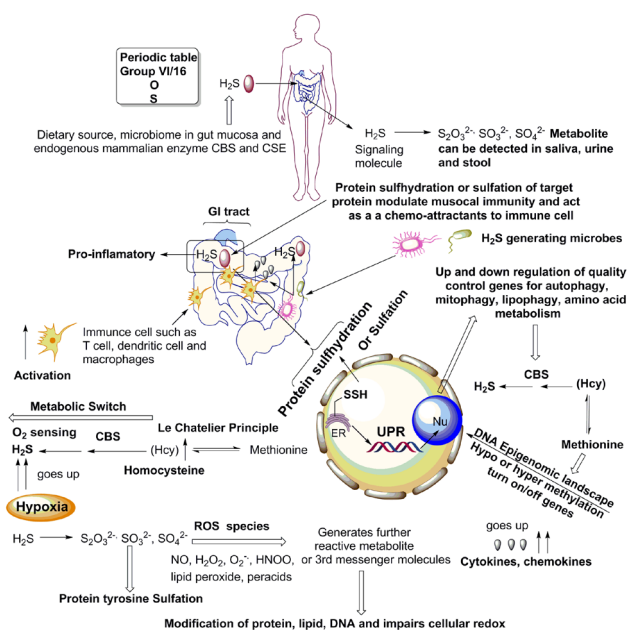
Hydrogen sulfide can activate the macrophages and T cell to produce the pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) [16, 19]. The gut mucosal bacteria can also produce harmful chemokine and cytokines after activation [8]. Thus, it implicates that over the progression of IBD, H₂S acts as a pro-inflammatory molecule (scheme 1) [30]. At advanced stages of the disease, endogenous formation of H₂S could be augmented due to increase of CBS transcriptome, as observed in colon cancer [31]. In pathological condition, excess sulfide could be further pumped out by gut mucosal bacteria to promote H₂S production [13, 32, 33]. H₂S can induce ER stress by NF- κ B activation and protein sulfhydrylation during reductive stress to adjust the redox homeostasis (scheme 1) [25, 26]. Hydrogen sulfide modified NF- κ B leads to the anti-apoptotic effect of the cell by protein sulfhydrylation. The protein sulfhydrylation by H₂S might impact many important cellular events, such as autophagy, lipophagy and other quality control genomic loci, including H₂S synthesizing genes (CBS) [20, 34]. Additionally, the upregulation of homocysteine can cause ER stress and alteration of sterol-triglyceride synthesizing genes [22]. H₂S mediated sulfhydrylation and sulfation of targets proteins possibly

cause the chemo attraction of immune cells [35]. Beside H_2S , it's metabolites (thiosulfate, sulfite, and sulfate), could also modify protein (tyrosine sulfation) and initiate the immune cell infiltration to trigger inflammation [35, 36]. However, sulfhydration or sulfation guided prolonged infiltration of immune cell to the colonic tissue, could direct the IBD towards an auto immune disorder. Cellular hypoxia can also facilitate the mucosal colonic damage by altering oxygen gradient and metabolic junctions, thus trigger inflammation to the bowl.37 Importantly, It has been reported that hydrogen sulfide can bypass the metabolic oxygen demand and modulate energy production at mitochondria [5, 29, 31]. Being a pre-biotic element and same family member of periodic table (group VI/16), it is possible that nature has evolutionarily preserved the sulfide generation either to mimic the oxygen demand during cellular hypoxia (scheme 1). Therefore, under hypoxic condition, the cellular environment can shift towards augmented production of H_2S and H_2S producing enzymes CBS to aggravate the severity of IBD. In addition, the microbiota can produce H_2S to overcome mucosal barrier in the microenvironment leading to IBD progression. Suppressing H_2S production could prevent excess sulfhydration mediated post-translational modification of protein. Inhibition of CBS could also impact on post genomic modulation of DNA methylation landscape (scheme 1) during IBD (such as hypo or hyper methylation) as observed in other chronic liver disease [38, 39]. An imbalance in sulfur homeostasis could be pivotal in the advancement of IDB. Hence, CBS inhibition can diminish the H_2S synthesis and many other sulfur metabolite related alternation of cell signaling events, thereby preventing the aggressive onset of IBD.

Role of hydrogen sulfide and its metabolite, protein sulfhydration and endogenous H_2S producing machinery (CBS/CSE) in advancement of IBD and possible and non-invasive bio-marker for health risk assessment for IBD. Upregulation of transsulfuration and hydrogen sulfide generation can cause excess protein sulfhydration that can causes several cascades of events such as immune modulation, oxygen sensing in hypoxic tissue, ER stress as UPR and alteration of pro-inflammatory cytokine-chemokine level. Imbalanced in transsulfuration can also change the epigenome by pushing the equilibrium toward the augmented production of methionine. Generation of reactive sulfur species by combination with other reactive oxygen species can modify the DNA, protein and lipid to impair cellular redox.

Technological Advances to Monitor Transsulfuration for IBD Assessment:

Hydrogen sulfide is a highly water and lipid soluble gaseous compound. In case of H_2S poisoning, H_2S and its metabolite $S_2O_3^{2-}$ can be found in high concentration in urine and blood/serum, and treatment of hydroxocobalamin can be used for detoxification [40, 41]. H_2S can be detected in urine and stool [42]. Commercially developed analytical electrodes can measure H_2S in various biological and preclinical samples such as blood, serum, and urine at low concentration in the order of 100 nM [43]. Besides, there are several other method such as HPLC, colorimetry, amperometry, voltammetry, titrimetric, fluorometry, turbid metric, led acetate trap analysis exits that is routinely used. Specially, the measurement of H_2S metabolite (thiosulfate, sulfate and sulfite), as used previously by forensic department during sulfide poisoning [40, 44-46]. Given recent advances in imaging, H_2S can be imaged in living mammals to locate the actuate state of inflammation in accordance with sulfide production [47]. A dye Sulfidefluor-5 probe (SF5) that specifically lit up by H_2S synthesis in vivo, has been used to image the mice during zymosan mediated inflammation triggered by immune filtration (Figure 2) [47]. In addition to this study, a chemo luminescent dye Spiro Adamantane 1,2-dioxetane has been recently reported that can lit up by H_2S generation, both *in vitro* and *in vivo* [48]. This dye can image H_2S production both in solution (buffer) as well as in mice when treated with Na_2S (in situ H_2S generator), compared to a vehicle control. This dye can be further used for the evaluation of several pre-clinical animal model of IBD to confirm whether the phenotypic onset of IDB is related to the endogenous production of hydrogen sulfide or not. This will further implicate the physiological role of H_2S during IBD progression, and thereby the regulation of sulfur amino acid metabolism for the development of next generation therapeutics for IBD management. Besides imaging, bacteriology profile of the sulfur regulating bacteria (such as sulfate reducing bacteria (SRB), thiobacillus, desulfobacter, etc.) in feces and urine sample could be analyzed as the early prognostic marker of IBD [42, 49-51]. The micro biome culture for H_2S and its me-



Scheme 1: H_2S act as proinflammatory cytokine inducer, immune cell activator, trigger unfolded protein response (UPR) by ER stress, protein sulfhydration/protein sulfation and causes metabolic switch during hypoxia. This can cause the up and down regulation of many genes and DNA epigenome methylation, later compromising immune system and pro-activation of hydrogen sulfide producing microbiota at the progressive stage of IBD.

Scheme 1: Novel treatment strategy for IBD management by targeting CBS;

tabolites in the urine and feces samples might tell us the severity of the IBD [13, 42].

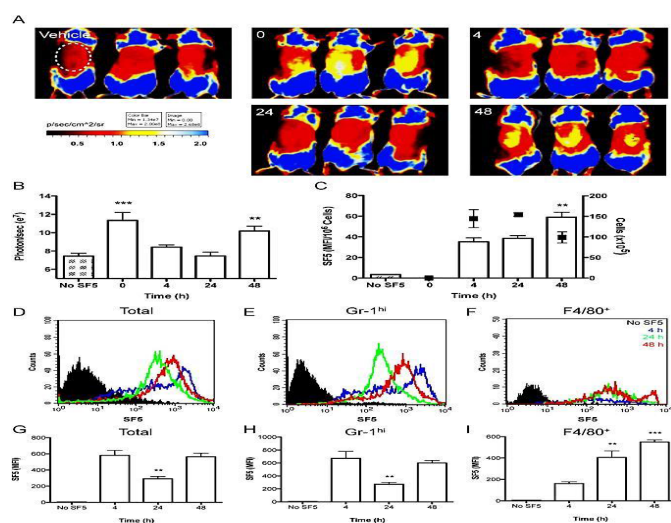


Figure 2: Acute inflammation impairs both tissue and cellular hydrogen sulfide synthesis. Tissue and cellular incorporation of SF5 probe was measured over 48 h in a model of zymosan-induced air pouch inflammation; data are representative of two experiments done in triplicate. Vehicle or SF5 (100 μ l; 10 μ M) were injected into the pouch 30 min prior to whole mouse imaging with a IVIS Spectrum (Caliper Life Sciences) during a 48 h time course (A). Fluorescence emission was measured within a region of interest (ROI; white dotted line) encapsulating the air pouch expressed as photons/sec over the time course (B). SF5 fluorescence intensity in inflammatory exudates was analyzed by spectrofluorometry (C) and expressed as a ratio of infiltrating cell number ($n = 3$). Total cell numbers are overlaid (black points; right Y-axis). Reprinted with permission from Ref (47). Neil. Dufton, Jane. Natividad, Elena F. Verdu, and John L. Wallace. Hydrogen sulfide and resolution of acute inflammation: A comparative study utilizing a novel fluorescent probe, *Scientific Report*, 2012, 2, 499. Copyright© 2016 Nature Publishing Group (npg).

CONCLUSION

Regulation of transsulfuration pathway by inhibiting the cystathionine beta synthase (CBS) enzyme could be a potential target for the treatment of IBD. Targeted therapy by using small molecule inhibitors and gene silencing of CBS could be helpful to prevent the severe onset of IBD. Sulfur amino acid metabolite (hydrogen sulfide, thiosulfate, sulfite, sulfate and homocysteine) in the saliva, as well as in urine, feces could give us predictive warnings for the non-invasive health risk assessment of IBD (scheme 1). Several methods can be adopted for the targeted drug delivery of small molecule inhibitors and gene silencer (siRNA) specific to CBS enzyme. For examples, siRNA or anti-sense oligonucleotide for CBS gene can be used with nano liposomal formulation [29]. Similarly, other formulation like PLGA-PEG microsphere can be used to formulate the CBS siRNA or small molecule inhibitor of CBS. Given only few numbers of CBS specific inhibitors exists, it will be important to develop library of compounds by high throughput screening to come up with a potent and selective CBS specific

inhibitors. In addition to these, many other polymer capsule strategies that are heavily used in the pharmaceutical industry can be adopted to prepare small molecule oral drugs formulations for the prevention of IBD. Selective inhibition of CBS with differential regulation of hydrogen sulfide activity remains an open challenge to scientific community for future management of IBD.

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