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Gaseous metabolites as therapeutic targets in ulcerative colitis

Yao CK *et al.* Hydrogen sulphide and UC

Abstract

Diet therapies are currently under-utilised in optimising clinical outcomes for patients with active ulcerative colitis (UC). Furthermore, existing dietary therapies are framed by poorly defined mechanistic targets to warrant its success. There is good evidence to suggest that microbial production of gaseous metabolites, hydrogen sulfide (H₂S) and nitric oxide (NO) are implicated in the development of mucosal inflammation in UC. On a cellular level, exposure of the colonic epithelium to excessive concentrations of these gases are shown to promote functional defects described in UC. Hence, targeting bacterial production of these gases could provide an opportunity to formulate new dietary therapies in UC. Despite the paucity of evidence, there is epidemiological and clinical data to support the concept of reducing mucosal inflammation in UC *via* dietary strategies that reduce H₂S. Several dietary components, namely sulphur-containing amino acids and inorganic sulphur have been shown to be influential in enhancing colonic H₂S production. More recent data suggests increasing the supply of readily fermentable fibre as an effective strategy for H₂S reduction. Conversely, very little is known regarding how diet alters microbial production of NO. Hence, the current evidence suggest that a whole diet approach is needed. Finally, biomarkers for assessing changes in microbial gaseous metabolites in response to dietary interventions are very much required. In conclusion, this review identifies a great need for high quality randomised-controlled trials to demonstrate the efficacy of a sulphide-reducing dietary therapy for patients with active UC.

Key Words: Diet; Ulcerative colitis; Hydrogen sulfide; Nitric oxide; Sulphide-reducing diet

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Core Tip: There is room to develop efficacious dietary therapies in ulcerative colitis (UC) by targeting underlying pathogenic mechanisms. Emerging data indicates that dietary factors play a significant role in modulating two gaseous metabolites, hydrogen sulphide and nitric oxide, that affect the integrity of the colonic mucosal barrier in UC. These gases are produced by the colonic microbiota in response to sulphur-containing protein and to a lesser extent, inorganic sulphur (sulphates and sulphites), but suppressed by the presence of fermentable fibre. Preliminary work suggests that a multi-prong diet that targets reduction of these gases have therapeutic potential and further randomised-controlled trials are underway.

INTRODUCTION

Ulcerative colitis (UC) is characterised by chronic inflammation of the colonic epithelium as a result of an aberrant immune response to poorly understood initiating triggers^[1]. Diet is a well-recognised environmental factor in the development of UC^[1,2], but remains an under-utilised therapeutic tool amongst physicians and dietitians alike. Dietary management is currently directed at providing supportive symptomatic management. However, in the recent years, there has been a dogma shift towards harvesting dietary therapies with mechanistic targets for the induction of disease remission, as evidenced by the growing number of review articles in the area^[3-5].

Whilst most research have been focused on altered immune regulation in the early initiative events, there is now a good body of evidence generated over the last 20 years suggesting that UC is an epithelial disease^[6]. Metabolic defects in the colonic epithelium are central in its pathogenesis and may be responsible for mucosal barrier breakdown^[7]. In turn, microbial metabolites such as hydrogen sulfide (H₂S) and nitric oxide (NO) that are toxic at excessive concentrations, may further exert injurious effects on the epithelium^[8]. Diet is a major factor in colonic production of these metabolites. Hence, dietary strategies that minimise their production mechanistically may have therapeutic benefits in UC. This review aims to examine the evidence for H₂S and NO as causative agents in UC, the influence of diet on their colonic metabolism and to explore the

rationale as well as evidence to date for dietary strategies targeting these gaseous metabolites as a therapy in UC.

COLONIC PRODUCTION OF H₂S & NO

Luminal H₂S is derived solely from metabolic activities of the microbiota, namely from fermentation of sulfur-containing amino acids and dissimilatory sulfate reduction^[9]. Approximately 6-18 g/d of proteinaceous substrates are delivered to the colon for fermentation, the bulk of this originating from undigested dietary protein and a smaller proportion from endogenous protein secretions^[10]. A range of protein-fermenting microbes with the capacity to generate H₂S have been reported including *Escherichia coli*, *Clostridium spp.*, *Bacteroides spp.* and *Klebsiella pneumonia*^[10]. In contrast, the capacity to reduce sulfate within the microbiota appears to be limited. A smaller proportion of malabsorbed dietary inorganic sulfur (0.3-8 mmol/d)^[11] reach the colon as substrates for dissimilatory sulfate reduction. Sulfate- and sulfite-reducing bacteria such as *Desulfovibrio spp.* and *Bilophila wadsworthia* are highly specialised microbes with capacity for sulfate reduction^[11].

On the other hand, two major sources of luminal NO are known: (1) Mucosal production from arginine; or (2) Anaerobic bacterial denitrification which reduces nitrates to nitrites and to NO^[8]. To date, little work has been done to examine microbial populations capable of denitrification. Hence, the understanding of microbial pathways for gaseous production has important implications not only as potential therapeutic targets but has significant relevance for manipulation of dietary substrates.

ROLE AS LUMINAL TOXINS IN PATHOGENESIS OF UC

The most compelling argument for the colonic epithelium as the primary defect in UC has been derived from *ex vivo* studies showing diffuse structural and functional abnormalities in the absence of histological or endoscopic inflammation^[12-14]. A key functional defect identified is the impaired uptake and oxidation of butyrate by colonocytes for energy^[15,16]. As a result, the energy-starved colonic epithelium has

limited ability to perform other metabolic functions including the maintenance of barrier function. Furthermore, reduced structural integrity of the colonic mucus layer was reported by van der Post *et al*^[13]. This was characterised by a marked decrease in core mucus components in both inflamed and non-inflamed biopsy samples, with similar findings reported previously^[14]. Hence, the induction of mucosal inflammation may occur as a secondary response to the increased intestinal permeability^[6].

Several lines of observations support the involvement of luminal H₂S and NO in perpetuating functional defects of the colonic epithelium. These concepts are summarised in Figure 1. First, Levine *et al*^[17] showed that faecal release of H₂S was three-fold higher and more rapid in UC patients (both active and quiescent) compared to controls. Additionally, a greater relative abundance and activity of sulfate-reducing microbes, *Desulfovibrio*, has been documented in faecal or mucosal biopsy samples of patients with UC compared to non-UC controls^[18,19]. Gut dysbiosis may be the main pathogenic factor of UC, and the higher dominance of sulphate-reducing microbes may potentially contribute to the dysbiosis hypothesised in the pathogenesis of UC. No data currently exists of potential alterations to the abundance of protein-fermenting microbes in UC. Furthermore, in contrast to a healthy colonic epithelium where H₂S is effectively detoxified, enzymatic detoxification activity of H₂S have been shown to be significantly depressed in UC^[20]. Finally, elevated luminal H₂S concentrations are shown to be directly proportional to the severity of disease^[16,17], providing an evidence base for a pathogenic link with UC. Likewise, direct assessment of luminal NO using a rectal balloon in patients with active UC demonstrated markedly higher rectal NO levels in these patients compared to those with irritable bowel syndrome and healthy controls^[21].

Secondly, reduced carbohydrate fermentative ability, as was recently reported^[22], and decreased accessibility to short-chain fatty acids^[23] may have lead-on effects on altered sulfur metabolism. Insights gained by assessment of intestinal pH responses to dietary manipulation of fermentable fibres suggest that abnormalities in carbohydrate fermentative ability may be region specific^[24]. Reduced butyrate utilisation may increase luminal accumulation of H₂S as its regulatory role on detoxification pathways

are affected^[25]. On the other hand, fibre deprivation may act synergistically with H₂S to increase breakdown of the mucous layer^[26].

Thirdly, at excessive concentrations, continuous exposure of isolated colonocytes to combined H₂S and NO *in vitro* can produce extensive disruption of the epithelial barrier by interfering with cell membrane synthesis^[8], impeding butyrate oxidation and subsequently, cellular respiration, producing an energy-deficient state as described earlier. This theory was confirmed by Leung *et al*^[27] who induced a histological state that was similar to the pathology of UC in the colon of rats administered with sulfates (carrageenan). Furthermore, excessive H₂S and NO may exert other pathogenic effects, including direct immune effects and these are summarised in Figure 1.

Hence, restricting epithelial exposure to luminal H₂S and NO *via* reduced microbial production may hypothetically improve epithelial function and reduce mucosal inflammation in UC, a novel therapeutic strategy that was proposed two decades ago^[28] but has only achieved some progress in the last two years. Progress is hampered by difficulties in accurate measurements of luminal H₂S and NO to provide a biomarker for assessing the efficacy of interventions on these metabolites. These challenges are discussed further in the subsequent sections.

DIET AS PRIMARY STRATEGY FOR COLONIC H₂S & NO MANIPULATION

From discussions above, it can be hypothesised that a key strategy in reducing microbial H₂S and NO production is by reducing substrate availability. Food choice represents a rationale candidate for manipulation as substrate delivery to the colon is strongly influenced by dietary intake. Indeed, several lines of evidence exist supporting the efficacy of dietary manipulation on colonic H₂S production. In contrast, the influence of diet on the extent of bacterial denitrification has been inconsistently shown.

First, acute dietary studies in healthy controls changing from low to a high animal protein diet consistently raised faecal H₂S levels^[29,30]. Magee *et al*^[29] reported this increase in H₂S levels to be linear with increasing intake of red meat (from 0 to 600 g/d). In another study, a four-day animal-based diet specifically increased a sulfite-reducing

species, *Bilophila wadsworthia*, while a plant-based diet reduced this cluster^[31]. Similarly, the animal-based diet significantly increased sulfide reductases needed for H₂S production^[31]. Another source of inorganic sulfur in the diet occurs naturally in the form of glucosinolates in the Brassica vegetables family. However, a two-week diet high in brassica was associated with a reduction in the abundance of sulphate-reducing bacteria in a randomized crossover study with ten healthy adults^[32], which seems to indicate that natural inorganic sulfur is not a determining factor in H₂S production associated with sulfate-reducing bacteria. Thirdly, whilst assessment of sulfate-reducing bacteria may be useful, it does not provide a comprehensive picture of functional alterations in microbial H₂S metabolism *in vivo*. Preliminary insights were gained with the use of a gas-sensing technology incorporating real-time, accurate measurements of H₂S to enable further understanding of the extent of dietary influence on microbial H₂S production^[33]. A comparison between faecal slurries spiked with cysteine, a sulfur-containing amino acid, and sodium sulfate showed marked differences in faecal H₂S generation, with cysteine vigorously stimulating H₂S over sulfate. This finding indicates that protein fermentation may be a major pathway for H₂S production than dissimilatory sulfate reduction. Furthermore, faecal H₂S was effectively reduced by readily fermentable fibres, resistant starch and fructo-oligosaccharides, both of which are prebiotics, and even in the presence of excessive faecal H₂S production using cysteine^[33]. The likely mechanism for H₂S suppression by fermentable fibre in the presence of cysteine is the shift from protein to carbohydrate fermentation as microbes preferentially ferment fibre than protein^[10]. Suppression of H₂S has been reported by another study where a 1.5-fold increase in total dietary fibre that accompanied the reduction in animal protein had a negative impact on H₂S production^[30] and in a second study, the addition of resistant starch to a high meat diet reduced markers of protein fermentation including H₂S^[34]. Both inulin and fructo-oligosaccharides, well-established prebiotics were also shown to reduce H₂S levels in pigs^[35].

In addition, one of the strategies targeting the microbiota is probiotics with specific probiotic strains shown to be effective in inducing remission in active UC^[36]. However,

its properties on the gut microbiota warrants further investigation, particularly with regards to the influence of different probiotic strains on H₂S production. On the other hand, a promising probiotic treatment for UC is recent development of a 'smart probiotic' where *E. coli* Nissle 1917 was genetically engineered to detect colonic NO and would theoretically be able to release biologic therapy at the site of elevated colonic NO^[37]. This engineered probiotic had previously been shown to have positive impact on the intestinal barrier function, and were able to reduce inflammation in dextran sulfate sodium -induced colitis mice model^[38]. Prebiotics are another key player in microbiome manipulation that have been suggested to have a positive effect on the microbiome. Their mechanisms in modulating microbial H₂S have already been discussed earlier. However, randomized controlled trials (RCTs) in UC patients that evaluated the efficacy of prebiotic supplementation alone demonstrated limited weak effect^[39] which indicates that a multi-prong approach, not just prebiotic supplementation, is required to achieve clinical effects.

Secondly, there is some evidence from epidemiological studies that provide clues for the influence of dietary sulfur-containing protein, sulfates and sulfites on the clinical course of UC. One study reported a correlation between a high protein intake and increased risk of developing UC^[40] whilst only one study has shown an association between a high intake of sulfur amino acids and sulfate with a three-fold greater risk of disease relapse^[41]. Subsequently, the potential clinical efficacy of a sulfur-restricted diet was first described from a small open-label study in eight UC patients. The low sulfur diet combined with stable salazopyrin therapy was associated with histological and clinical improvement^[9]. Changes in colonic H₂S production was unfortunately, not measured as a mechanism for efficacy but the promise of this dietary approach warrant further investigation in a controlled trial.

Furthermore, there has been growing interest in the role of carrageenan, a sulphated polysaccharide food additive that escapes digestion in the small intestine almost intact and is fermented to release sulphates^[42], which is then metabolised to produce H₂S. In 2017, a randomized controlled (RCT) trial by Bhattacharyya *et al*^[43] assessed the role of

carrageenan, a sulphated polysaccharide food additive, in maintaining relapse in 14 UC patients in remission. Following a year of no-carrageenan diet, relapse rates appeared to be higher in the five patients receiving 200 mg carrageenan, a dose slightly below average intakes of carrageenan in the US diet, than those who received placebo capsules. Unfortunately, significant recruitment issues impacted on the sample size of the study, making it difficult to ascertain whether this was a real clinical effect.

Finally, the major sources of nitrites in the diet are food preservatives in cured and processed meat, while the major source of dietary nitrates is vegetables^[44]. Thus far, the effect of different sources of nitrite and nitrate in the diet on microbial NO production are unknown and should be further investigated in clinical studies.

TRANSLATING PROPOSED DIETARY STRATEGY INTO CLINICAL APPLICATION

Several dietary strategies can therefore be implied for future clinical application from studies thus far. First, a multi-prong intervention targeting dietary substrates with H₂S-modulating abilities, expanding on Roediger^[9]'s earlier work, is warranted in active UC patients. This approach should consider reducing intake of sulfur-containing protein such as methionine, cysteine and taurine, and added sources of inorganic sulfur to reduce excessive/chronic H₂S exposure to the colonic epithelium. Major sources of these foods are listed in Table 1. Inorganic sulfur exist as food additives in several forms, sulfur dioxide (E220), sulfites (E221-E227) and as a sulphated polysaccharide, carrageenan (E407). In Australia and Europe, food labelling requirements mandate only the labelling of added sulphites (in amounts > 10 mg/kg) in food product without specifying the amount used. Inorganic sulfur intake by foods and beverages has been showed to be six-fold ¹higher in the western diet in comparison to a typical African rural diet^[45,46]. However, food composition tables on sulfur-containing protein, inorganic sulfur and carrageenan are far from complete to adequately assess habitual intake of UC patients, to ensure successful design of the dietary therapy. More importantly, there are grounds that increasing a combination of fibre, rather than restricting total fibre,

maybe an efficacious strategy for H₂S suppression whilst improving nutrient delivery to the colonic epithelium in UC. Resistant starch and fructo-oligosaccharides, whilst efficacious, are fermented in the proximal colon^[10]. Hence, a strategy that will carry the fermentation of these fibres across the entire colon by combining with a minimally fermentable fibre is required.

Indeed, tolerability and the potential clinical effects of a dietary approach incorporating strategies discussed above have already been evaluated. In an open-label dietary advice study, Day *et al*^[47] reported excellent tolerability of dietary strategy called the 4-strategies to a sulphide-reducing (4-SURE) diet by patients with mild to moderately active UC. This was despite the 38% increase in dietary fibre and the four-fold increase in resistant starch intake by these patients. Food-related quality of life also increased markedly. Whilst it was impractical to assess colonic H₂S in this study, markers of protein fermentation, namely faecal branched chain fatty acids were used as a surrogate. The significant reduction in faecal branched to short-chain fatty acid ratio following the 4-SURE study indicated that protein fermentation being the major pathway for luminal H₂S production was reduced. Whilst the study did not intend to primarily assess clinical end-points due to the uncontrolled study design, there were indicators for the diet to positively affect clinical outcomes and mucosal healing. data supported by a significant reduction in faecal calprotectin. A second dietary approach, called the Ulcerative Colitis Exclusion Diet (UCED), also incorporated a similar exclusion of decreasing intake of total protein, sulphur-containing amino acids, food additives along with additional restrictions of animal and saturated fat, haeme, whilst increasing intake of tryptophan, pectin and resistant starch^[48]. In a RCT comparing a combination of UCED with faecal transplant, faecal transplant or diet alone, Sarbagili Shabat *et al*^[48] observed that clinical response and endoscopic remission were the greatest for the UCED diet. Furthermore, the promising outcomes of the UCED was supported by an earlier open-label study in paediatric patients with mild to moderate active UC on stable maintenance therapy, where the diet treatment showed that

patients had a significant decrease in sulfur-containing amino acids consumption as well as a significant increase in total fiber consumption^[49].

Whilst these proposed dietary approaches are not quite ready for clinical application until RCTs have been performed (currently underway) to replicate the promising findings, it does suggest that patients with active inflammation do tolerate a certain increase in high fibre foods and builds on the suggestion to minimise intake of processed foods. Moreover, the limitations of the available reported clinical trials targeting reduction of H₂S production as a treatment strategy for UC (Table 2) suggest the need for larger, high quality dietary studies incorporating gut microbiome composition and function assessment including changes in microbial H₂S metabolism.

BIOMARKERS FOR ASSESSING RESPONSE OF DIETARY THERAPY

It is key that a biomarker for assessing diet response is incorporated early on after dietary therapy is administered as a way of assessing whether the diet is achieving its intended mechanistic effect. An example of this is the reduction in breath hydrogen production after introduction of a diet low in fermentable carbohydrates as a biomarker of intervention success^[50]. However, in the case of dietary approaches targeting colonic H₂S and NO, there are difficulties with accessing reliable measurement techniques for these volatile gases, particularly with *ex vivo* measurements often requiring freshly passed faecal samples^[17,33], which introduces practical issues for trial patients. Currently, measurements for H₂S mainly involve faecal sulphide, urinary sulphate or breath H₂S^[51]. Sensitivity of these measurements are impacted by its adsorption or susceptibility to oxidation, yielding low concentrations^[51]. In contrast, the only reported assessment of luminal NO has been using direct sampling (*via* a tonometric balloon) and measurement *via* a rapid-response chemiluminescence technique^[21]. While the method has good sensitivity, it is unknown whether this biomarker is directly responsive to alterations in dietary nitrate and nitrite intake. Finally, there is potential for direct intestinal gas sampling, such as the gas-sensing capsule^[52], but these do not yet measure H₂S or NO. In the absence of reliable direct measurements, indirect

assessments could target markers of protein fermentation for H₂S, quantification of sulphate- or sulphite-reducing bacteria which are dependent on availability of proteinaceous substrates for growth^[53], and have capacities for denitrification and sulphate-reduction^[54]. Hence, an effective biomarker for monitoring the success of sulphide- and NO-reducing dietary approaches remains elusive and is very much needed to support the development of the proposed dietary therapies. Therefore, as in most studies, assessment of dietary response is primarily assessed by different questionnaires such as dietary intake questionnaires, food-related quality of life or health-related quality of life questionnaire. Combined biomarker measurements with assessment by questionnaires can be the ideal tool for estimating the effect of specific dietary exposure.

CONCLUSION

Microbial H₂S and NO metabolites have causative roles in the pathogenesis of UC *via* their damaging effects on the colonic epithelium. Modulation of their production within the colonic lumen in order to reduce colonic epithelial exposure to these luminal stressors presents an attractive therapeutic target that has yet to be adequately explored. The current evidence suggests that dietary manipulation is likely to be an effective strategy to modify colonic H₂S production whereas little is known regarding dietary modulation of NO. It is also clear that sulfur-containing amino acids are major substrates that promote H₂S production over inorganic sulphur but data has emerged suggesting that increasing fermentable fibre is highly efficacious in reducing H₂S production. These findings have been utilised to inform the design of multi-prong dietary approaches which have yielded promising therapeutic efficacy in mild to moderate active UC. However, key to advancing the success of this research is the urgent need for better technology to accurately assess luminal concentrations of these volatile gases. Finally, before implementation into dietary practice can be pursued, further investigations into their efficacy on altering disease activity using robust dietary

trial designs (which are currently underway), expansion of food composition data and mechanisms of H₂S reduction are highly warranted.

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SIMILARITY INDEX

PRIMARY SOURCES

1

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