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Original Contribution

Detrimental effects for colonocytes of an increased exposure to luminal hydrogen sulfide: The adaptive response

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ABSTRACT

Protein fermentation by the gut microbiota releases in the large intestine lumen various amino-acid derived metabolites. Among them, hydrogen sulfide (H_2S) in excess has been suspected to be detrimental for colonic epithelium energy metabolism and DNA integrity. The first objective of this study was to evaluate in rats the epithelial response to an increased exposure to H_2S. Experiments from colonocyte incubation and intra-colonic instillation indicate that low millimolar concentrations of the sulfide donor NaHS reversibly inhibited colonocyte mitochondrial oxygen consumption and increased gene expression of hypoxia inducible factor 1α (*Hif-1α*) together with inflammation-related genes namely inducible nitric oxide synthase (*iNos*) and interleukin-6 (*Il-6*). Additionally, rat colonocyte H_2S detoxification capacity was severely impaired in the presence of nitric oxide. Based on the γH2AX ICW technique, NaHS did not induce DNA damage in colonocytes. Since H_2S is notably produced by the gut microbiota from sulfur containing amino acids, the second objective of the study was to investigate the effects of a high protein diet (HPD) on large intestine luminal sulfide content and on the expression of genes involved in H_2S detoxification in colonocytes. We found that HPD markedly increased H_2S content in the large intestine but the concomitant increase of the content mass maintained the luminal sulfide concentration. HPD also provoked an increase of sulfide quinone reductase (*Sqr*) gene expression in colonocytes, indicating an adaptive response to increased H_2S bacterial production. In conclusion, low millimolar NaHS concentration severely affects colonocyte respiration in association with increased expression of genes associated with intestinal inflammation. Although HPD increases the sulfide content of the large intestine, the colonic adaptive responses to this modification limit the epithelial exposure to this deleterious bacterial metabolite.

1. Introduction

Gut microbiota is now established as a key player in various aspects of health and disease [35]. Commensal bacteria affect their host by various mechanisms including the production of metabolites [42]. While carbohydrate fermentation is mainly considered beneficial for the host through the production of short chain fatty acids, protein fermentation gives rise to a wide variety of compounds, some of them being suspected to be detrimental for gut health when present at excessive concentrations [2,6,17]. Among them, hydrogen sulfide (H_2S) is primarily produced

Abbreviations: BAP, benzyo(a)pyrene; COX, cytochrome c oxidase; dsrA, dissimilatory sulfite reductase; ETHE1, dioxygenase; ETO, etoposide; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrasone; H_2S, hydrogen sulfide; HS^-, hemisulfide; HIF-1α, hypoxia inducible factor 1α; HPD, high protein diet; IBD, inflammatory bowel disease; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; KCN, potassium cyanide; MPO, myeloperoxidase; NO, nitric oxide; NPD, normal protein diet; SAA, sulfur containing amino acid; SOU, sulfide oxidation unit; SQR, sulfide quinone reductase; SRB, sulfate reducing bacteria; TST, thiosulfate sulfur transferase

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through fermentation of sulfur-containing amino acids (SAA) originating from undigested and partially-digested dietary and endogenous proteins or by reduction of inorganic sulfate by sulfate reducing bacteria (SRB) [7,33]. Sulfide concentrations in the luminal content in humans and rats have been reported to be in the millimolar range [32,37] with part of sulfide being present in bound form [26]. At the physiological colonic luminal pH, approximately one third of H_2S remains undissociated whereas the remaining two thirds is hydrosulfide anion (HS^-) [57]. In humans, colonic protein fermentation is increased in case of high protein diet (HPD) consumption and sulfide fecal excretion is increased after a meat-rich diet [20,38].

In the human colonic adenocarcinoma epithelial cell HT29 $Glc^{-/+}$ model, millimolar concentrations of NaHS (the sodium salt of H_2S which releases in aqueous medium HS^- and then H_2S) inhibit mitochondrial respiration through inhibition of the mitochondrial cytochrome c oxidase (COX) activity, and impair colonocyte butyrate and glutamine oxidation [31] these later substrates being preferred fuels for these cells [46]. At lower micromolar concentrations, H_2S is oxidized by the sulfide oxidation unit (SOU) with sulfide quinone reductase (SQR) representing the first and rate-limiting enzyme of this unit [9,40].

Both pro and anti-inflammatory properties of H_2S in intestinal mucosa have been reported, mostly depending on the concentration tested [33]. Indeed, low H_2S concentrations have been shown to participate in the resolution of colitis [19,55,56] while higher concentrations, likely originating from the luminal side, have been suspected to display pro-inflammatory effects on the large intestine mucosa [13,43]. At a given concentration of H_2S, the biological effects of this compound appear to depend on the cellular redox state and oxygen tension [53]. It has been shown by several studies (but not all) that fecal sulfide concentrations are higher and SRB more abundant in patients with ulcerative colitis than in control subjects (reviewed in [10]). Moreover, impaired H_2S detoxification in intestinal mucosa is associated with inflammatory bowel disease (IBD) [3,18]. In other studies, Attene-Ramos et al. [4,5], have shown *in vitro* that H_2S is able to damage DNA in intestinal epithelial cell lines. In addition, it has been demonstrated that H_2S produced endogenously is able to maintain colon cancer cellular bioenergetics supporting colonic tumor growth [23,52,54].

In this context, we first aimed to determine in rats, using an intracolonic instillation model, the effects of an increased NaHS luminal concentration on colonocyte inflammatory parameters, DNA integrity and mitochondrial respiration. Regarding this latter parameter, we determined the impact of nitric oxide ($NO^{\cdot}$) on the colonocyte capacity to detoxify H_2S. Then, we evaluated in rats the consequences of a HPD on sulfide content in large intestine and on the epithelial response in terms of detoxification metabolic pathway.

2. Material and methods

2.1. Animals and diets

The present protocols received written agreement from the local animal ethical committee (COMETHEA at Jouy-en-Josas, France, no. 12/143 and no. 12/090). Male Wistar rats (Harlan, Gannat, France) weighing 150 g (5–6 weeks) were fed for 1 week with a standard rodent diet containing 16% protein by weight.

2.1.1. Instillation study

Rats were starved for one night and then anesthetized with isoflurane and maintained on a heating plate (37 °C). A 1 mm catheter was introduced in the proximal colon. Colonic content was expulsed manually and then colon was flushed twice with 10 mL PBS at 37 °C. The catheter was linked to a pump that delivered drop-by-drop (1.5 mL/min) a phosphate-buffered saline (PBS) solution of NaHS (Sigma-Aldrich) used at 0.5 or 1.5 mM concentrations or PBS alone (control experiments) during 1 h at 37 °C (Fig. 1A). The whole colon was removed and the latest 1-cm segment was snap frozen in liquid nitrogen and stored at −80 °C up to myeloperoxidase (MPO) assay. Then, rats were sacrificed by the injection of a lethal dose of pentobarbital.

For colonocyte isolation, colon was flushed with a NaCl 9 g/L solution and then with a Ca^{2+} and Mg^{2+}-free Krebs Henseleit bicarbonate (pH7.4) buffer solution containing 10 mM HEPES, 5 mM DTT, and 2.5 g bovine serum albumin and equilibrated against a mixture of O_2 and CO_2 (19:1, vol/vol). Then, colon was perfused for 20 min at 37 °C with the same buffer containing 10 mM EDTA and then incubated in a buffer containing hyaluronidase (8 g/L) saturated with a mixture of O_2 and CO_2 containing (in mM) 120 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 Na_2SO_4, 10 HEPES, and 25 $NaHCO_3$ and enriched with 2.5 g/L bovine serum albumine (incubation medium). Colonocytes were maintained in Dulbelcco's

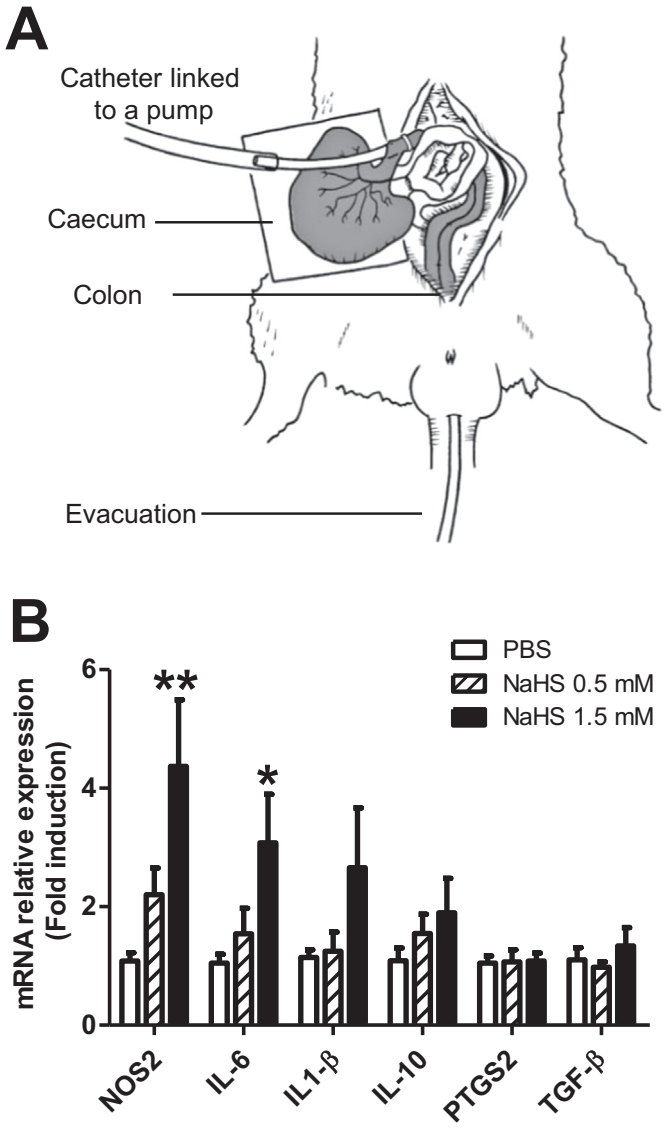

Fig. 1. Effects of intra-colonic instillation of NaHS on inflammation-related gene expression. (A) Experimental setting for 1 h colonic instillation in anesthetized rats. (B) Relative level of mRNA corresponding to inflammation-related genes in colonocytes of rats instilled with PBS without or with NaHS (0.5 or 1.5 mM) ($n=5$–11 rats/group). For each gene, mean relative mRNA expression in both NaHS-treated rat groups was compared to the mean value of control group (ANOVA, Bonferroni correction). Data are presented as mean values ± SEM. $^*p<0.05$, $^{**}p<0.01$.