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Research Article
Effect of Hydrogen Sulfide on Ileum and Colon Motility in Experimental Model of Peritonitis in Rats

[1]Evren Tuncer and [2]Şahin Yildirim

[1]Department of Pharmacology, Faculty of Medicine, Atılım University, Ankara, Türkiye
[2]Department of Pharmacology, Faculty of Medicine, Cumhuriyet University, Sivas, Türkiye

Abstract

Background and Objective: Studies investigating the association of H_2S and nitric oxide signaling with intestinal motility in peritonitis are limited. The objective of this study was to explore how H_2S influences the motility of the ileum and colon within an experimental peritonitis model. **Materials and Methods:** Three groups with 6 Wistar albino rats in each were used: Group 1 (Control), Group 2 (Peritonitis) and Group 3 (Sham). The experiment involved inducing contractions using either carbachol or electrical field stimulation (EFS) and relaxation responses are recorded upon the addition of L-cysteine, DL-PAG or NaHS, irrespective of whether L-NAME was added or not. Shapiro-Wilk Francia test, Levene test and Independent -Samples-t tests were used for the statistical analysis. **Results:** After induction with EFS or after pre-contraction with carbachol, relaxation rate (%) with NaHS was higher than relaxation rates (%) with L-cysteine and DL-PAG in the colon for all groups. The relaxation with NaHS was similar in all ileum groups after induction with EFS ($p = 0.073$), but in Group 2, responses were higher ($p = 0.003$ and $p<0.001$). The L-NAME did reveal a significant change in the presence of NaHS after pre-contraction either with carbachol or EFS in the colon for all groups ($p<0.05$). **Conclusion:** The NaHS induced significantly greater relaxation in the colon and it was more effective in the Peritonitis groups. The addition of L-NAME did result in a critical response with NaHS, after pre-contraction with carbachol in the colon Peritonitis group. The well-established fact is that a strong interplay exists between the NO and H_2S signaling pathways.

Key words: H_2S, colon, ileum, motility, relaxation, peritonitis

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Corresponding Author: Evren Tuncer, Department of Pharmacology, Faculty of Medicine, Atılım University, Ankara, Türkiye

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hydrogen Sulfide (H_2S) is a water-soluble gaso-transmitter which travels through cell membranes by its lipophilic nature. The H_2S has a role in normal physiology in mammals and exerts toxicity at high concentrations[1-3]. The production of H_2S involves the metabolic breakdown of the sulfur-containing amino acid cysteine and requires principally the presence of cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE)[1]. The colon may be exposed to H_2S due to its production by luminal microbiota and may also synthesize H_2S by expressing both enzymes[4,5].

The H_2S was shown to lead to smooth muscle relaxation and to modulate inflammation in the colon[1]. Nevertheless, H_2S was demonstrated to inhibit the spontaneous and cholinergic-stimulated contraction of ileum in rats[6-10].

Peritonitis is characterized as inflammation affecting the serosal membrane that lines the abdominal cavity and the internal abdominal organs[11]. Several studies have been reported in the context of the association between endogenous or exogenous H_2S and peritoneal inflammation[12,13]. The synthesis of endogenous H_2S was found to increase in cecal ligation and puncture-induced peritonitis in a study[14]. Besides, H_2S administration was shown to reduce peritoneal inflammation in experimental models of peritoneal dialysis or zymosan-induced peritonitis[13,15].

Peritonitis can cause ileus through a combination of inflammation, bacterial toxins and the formation of fibrinous adhesions[16]. Bacterial lipopolysaccharide (LPS) can contribute to ileus by triggering an inflammatory response, potentially leading to reduced muscle contractility[17,18].

To date, no research has explored the relationship between Hydrogen Sulfide (H_2S), nitric oxide synthetase (NOS) and the motility of the colon and ileum simultaneously in the presence of peritonitis. The objective was to determine the impact of hydrogen sulfide on the motility of the colon and ileum and the interaction between H_2S and NOS within an experimental peritonitis model in rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Medical School of Sivas Cumhuriyet University, Türkiye between August 10, 2021 and September 05, 2021 and between 09.00-18.00 hrs.

Study design and experimental animals: Eighteen male Wistar albino rats in total, aged 14-16 weeks, with an average weight ranging from 250 to 300 g were used for this study. Wistar albino rats were taken from Experimental Animal Laboratory at Sivas Cumhuriyet University, Türkiye. The rats were kept in ventilated rooms under required conditions, maintaining a constant temperature of 22±2°C, a 12 hrs light/dark cycle and a level of humidity that was 50±5%. The rats had ready access to both water and food, except for a 12 hrs food restriction period prior to surgical procedures to optimize tissue performance and obtain the most suitable preparations from the intestines.

Study groups: In total, 18 Wistar albino rats were categorized as follows:

- **Group 1 (Control group, n = 6):** Only one laparotomy procedure was applied concurrently with Group 2 and 3 as the 2nd surgical procedure consisting colon and ileum excisions. Peritonitis was not induced in Group 1
- **Group 2 (Peritonitis group, n = 6):** First laparotomy was applied and peritonitis was induced. The second laparotomy was applied for both colon and ileum excisions
- **Group 3 (Sham group, n = 6):** First laparotomy was applied, but peritonitis was not induced. The second laparotomy was applied for both colon and ileum excisions

Surgical procedure: The rats received a subcutaneous pre-anesthetic medication of 3 mg/kg xylazine (Bayer, Germany), followed by 90 mg/kg ketamine administered subcutaneously for anesthesia (Pfizer, USA). After shaving abdominal hair, antiseptic cleansing was carried out with 10% povidone iodine (Ataman Chemicals, Türkiye). Laparotomy was conducted after a paramedian skin incision of 3 cm was applied.

Peritonitis was induced by cecal constriction and perforation by yellow-tip injector needle (20-Gauge) at 4 points in Group 2. A diagnosis of peritonitis and sepsis was made according to physical signs such as fever, color changes, adhesions in the peritoneum and intraabdominal organs and through biochemical measurements.

The peritoneum, abdominal wall and skin were closed after the 1st surgical procedure in Groups 2 and 3. If peritonitis was confirmed, a second surgical procedure was performed at 24 hrs in Group 2. In Group 3, peritonitis was not induced and a second surgical procedure was performed at 24 hrs after the 1st laparotomy. Both the colon and ileum were excised during the second surgical procedure in Groups 2 and 3. In Group 1, only 1 laparotomy procedure was performed and the colon and ileum were excised during the procedure.

Using the cecum as a reference point, sections of the ascending colon and ileum were removed, each measuring 4 cm in length for every rat.

Table 1: Pro-interleukin 1β and tumor necrosis factor-α levels in the study groups

Groups	Pro-interleukin 1β (pg/mL)	Tumor necrosis factor-α (ng/mL)
Group 1: Control (n = 6)	59.24±7.73	57.39±6.51
Group 2: Peritonitis (n = 6)	82.90±12.03[a,b]	80.77±6.61[a,b]
Group 3: Sham (n = 6)	58.09±8.47	59.49±8.10

[a]p<0.01, when comparing Control group and [b]p<0.01 when comparing Sham group

Biochemical analysis: Blood samples were collected from 3 groups simultaneously after the 1st surgical procedure performed in Groups 2 and 3. To monitor peritonitis, pro-interleukin 1β and tumor necrosis factor-α were measured in blood samples with commercially available ELISA (Enzyme-Linked Immunosorbent Assay) kits (Table 1). The C-Reactive Protein (CRP) was measured by quantitative immunoturbidimetric assay (COBAS CRP4).

Sacrification protocol: Sacrification was done after colon and ileum excision in the 2nd surgical procedure in Group 2 and 3 and after the 1st surgical procedure in Group 1 and simultaneously in the 3 groups.

Isolated organ bath protocol: The extracted ileum and colon preparations were isolated from fecal and fat layers and divided into preparations of 3×4 mm in diameter. These preparations were kept in Krebs solution during the study (NaCl 115.48 mmol/L, KCl 4.61 mmol/L, $CaCl_2$ 2.5 mmol/L, $MgSO_4$ 1.16 mmol/L, $NaHCO_3$ 21.9 mmol/L, NaH_2PO_4 1.14 mmol/L and glucose 10.09 mmol/L). An isolated organ bath filled with Krebs solution was allowed to stabilize at a temperature of 37°C while being continuously supplied with a gas mixture of 95% O_2 and 5% CO_2, regulated by a MAY heating circulator. On each occasion, two ileum and two colon preparations were suspended in individual isolated organ baths. Tissues were prepared by wash-out 4 equal times per hour until the application of drugs or electrical field stimulation. A 1 g preload was applied to the tissues suspended in the organ baths. If the tonus was disrupted during wash-out, it was restored to 1 g.

Ethical consideration: The ethical guidelines established by the Experimental Animals Ethics Committee of Sivas Cumhuriyet University Medical School were adhered to, with approval granted on 10.10.2019 under the reference number 316. All experimental procedures were adhered to the regulations set forth by the local ethics committee for experimental animals.

Experiment protocol

Electrical field stimulation: Regarding each rat, a single ileum and a single colon preparation were hung using ring wires in 10 mL isolated organ baths and attached to a Grass isometric force transducer (FT 03, Grass Instruments, Quincy, Massachusetts, USA). Smooth muscle isometric contractions and relaxations were noted through these transducers using a Grass recording system (79 E, Grass Instruments, Quincy, Massachusetts, USA). Electrical field stimulation-mediated contraction responses were obtained, for 5 sec with 120 sec intervals, at a voltage of 10 and 1 millisec parameters and at a frequency of 2, 4, 8, 16 and 32 Hz. A contraction response occurring at 16 Hz was considered as a submaximal contraction. To reduce the influence of prostaglandins and the sympathetic nervous system on this contraction response, we introduced guanethidine at a concentration of 10^{-6} M (from Merck, Sigma Life Sciences, Germany), which acts as an inhibitor of noradrenaline secretion from sympathetic nerve terminals. Additionally, indomethacin at a concentration of 10^{-5} M (from Merck, Sigma ALDRICH, Germany), a cyclooxygenase inhibitor, was introduced into the organ baths 15 min prior to the electrical field stimulation procedure.

Relaxation responses: For each experiment, colon and ileum strips prepared for the Control, Peritonitis and Sham groups were either pre-contracted with carbachol (Merck, Sigma Aldrich, Germany) at a dose of 10^{-6} M or induced with EFS.

After a plateau or stabilized contractile response was achieved in the ileum and colon preparations pre-contracted with carbachol or induced by electrical field stimulation (EFS), NaHS (H_2S donor; Cayman Chemical, USA) at a dose of 10^{-7} to 3.10^{-3} M, L-cysteine (H_2S precursor; Merck, Sigma Life Sciences, Germany) at a dose of 10^{-6} to 3.10^{-3} M or DL-propargylglycine (DL-PAG, an inhibitor of CSE; Merck, Sigma Life Sciences, Germany) at a dose of 10^{-6} to 3.10^{-3} M were added to observe the relaxation responses of each molecule following washout periods. In order to eliminate the influence of nitric oxide, L-NAME (Merck, Sigma Life Sciences, Germany) was included in the organ baths (10^{-4} M), 20 min prior to the administration of these compounds.

The experiment was repeated for each ileum and colon group via pre-contraction with carbachol or induction with EFS and relaxation responses were observed with the addition of NaHS, DL-PAG or L-cysteine either with the addition of L-NAME or not.

Statistical analysis: The SPSS 25.0 software (IBM Corporation, Armonk, New York, United States) was used to analyze the data. The normal distribution was assessed using the Shapiro-Wilk Francia test and the variance homogeneity was assessed using the Levene test. The comparison of the Emax and pD2 variables in the ileum and colon strips was conducted using the Independent-Samples-t test, along with Bootstrap results.

For comparing the Emax and pD2 variables among the Control, Peritonitis and Sham groups, the One-Way ANOVA (Robust Test: Brown-Forsythe) was applied and complemented with bootstrap results. Subsequent *post hoc* analyses were carried out using Tukey's HSD and Games-Howell tests. In the Table 2 and 3, quantitative variables are given as Mean±Standard Deviation. A confidence level of 95% was chosen with $p<0.05$.

Data obtained from ELISA was analysed using the one-way ANOVA (*post hoc* Tukey's HSD) (Table 1).

RESULTS

Proinflammatory cytokines: The Peritonitis group exhibited elevated levels of CRP, pro-interleukin 1β and tumor necrosis factor-α in comparison to the other groups ($p<0.01$). The measurement of CRP levels was conducted via the COBAS CRP4 method. The ELISA was used to determine pro-interleukin 1β and tumor necrosis factor-α levels for all groups (Table 1).

Relaxation responses for ileum strips: Following the contractions induced by EFS, the relaxation response rate with L-cysteine (%) was found to be highest in Group 3 and the lowest in Group 2, whether L-NAME was added or not ($p<0.001$). After pre-contractions with carbachol, L-cysteine-mediated relaxation response rate (%) was observed to be greater in Group 3 ($p<0.001$) and lowest in Group 2, with or without L-NAME (Table 2). The presence of L-NAME, when combined with L-cysteine and administered via EFS or carbachol, did not demonstrate a significant effect in the Control and Sham groups as indicated in Table 3 ($p>0.05$). However, it did elicit a noteworthy response in EFS-induced contractions when comparing the ileum and colon specimens within the Peritonitis groups ($p = 0.003$, Table 3).

Following EFS-induced contractions, the relaxation response rate with DL-PAG (%) in Group 3 was observed to be 9.8±0.9 and 10.8±1.1%, regardless of the presence of L-NAME. When DL-PAG was introduced after the contraction triggered by EFS, pD2 was elevated in Group 1 as compared to both Group 2 and Group 3, with or without L-NAME (Table 2). Any significant effect due to L-NAME in the presence of DL-PAG either with EFS or carbachol in the Sham groups was not observed ($p>0.05$) (Table 3).

After pre-contraction with carbachol, the relaxation rate with NaHS (%) was observed as 72.3±8.9% in the ileum Group 2, which was higher compared to that in Group 1 and Group 3 ($p = 0.003$ and $p<0.001$, respectively); however, when L-NAME was present, there were no discernible differences among the groups (Table 2). Whether with EFS or carbachol, relaxation (%) with NaHS was higher than relaxation (%) with L-cysteine or relaxation (%) with DL-PAG both in the ileum and colon for all groups ($p<0.05$, Table 3).

The pD2 values were similar among the groups, besides those mentioned above (Table 2).

Relaxation responses of ileum samples for all groups were demonstrated in Fig. 1.

Relaxation responses for colon strips: Following EFS induction, the percentage of L-cysteine-mediated relaxation in the colon of Group 2 was not as high compared to that in the ileum, whether L-NAME was present or not ($p = 0.003$ and $p<0.001$, respectively, Table 3). Conversely, after pre-contraction with carbachol, the addition of L-cysteine resulted in similar levels of relaxation in all groups, regardless of the presence of L-NAME, (Table 3). The L-NAME did reveal a significant effect in the presence of L-cysteine whether with EFS or carbachol among all colon groups and the relaxation responses were lower in the Peritonitis groups ($p<0.05$, Table 2).

Following EFS induction, the percentage of DL-PAG-mediated relaxation was observed to be greater in Group 3 compared to Group 1 or 2, with or without L-NAME (Table 2, $p<0.001$). However, after pre-contraction with carbachol and the subsequent addition of DL-PAG, the percentage of relaxation was minimally observed in Group 1, whether L-NAME was present or not (Table 2). When comparing the colon and ileum specimens within just the Sham groups, with or without L-NAME, relaxation levels were similar (Table 3). The L-NAME did reveal a significant effect in the presence of DL-PAG whether with EFS or carbachol in all colon groups ($p<0.05$, Table 2).

Following EFS induction and the addition of NaHS, the relaxation rate was remarkable in Group 2, with or without L-NAME. When NaHS was introduced in the presence of L-NAME after EFS induction, pD2 was lower in Group 2 or 3 compared to Group 1. After pre-contraction with carbachol and the addition of NaHS, relaxation rates were similar across all groups. However, the relaxation response was more prominently reversed in Group 2 when compared to Group 1 under the presence of L-NAME ($p = 0.022$, Table 2).

Table 2: Relaxation responses in ileum and colon strips among control, peritonitis and Sham groups

Relation reponses	Group 1 Control (n = 6) Mean±SD	Group 2 Peritonitis (n = 6) Mean±SD	Group 3 Sham (n = 6) Mean±SD	p-value	Pairwise comparison (p-value) Group 1 vs Group 2	Group 1 vs Group 3	Group 2 vs Group 3
Emax (relaxation (%))-ileum							
EFS: L-Cysteine	19.1±0.6	16.3±0.3	22.2±2.3	<0.001	0.008	0.004	<0.001
EFS: L-NAME+L-Cysteine	17.3±0.7	14.5±0.8	21.8±1.8	<0.001	0.003	<0.001	<0.001
L-Cysteine	17.4±1.2	17.2±1.6	22.7±1.4	<0.001	0.967	<0.001	<0.001
L-NAME+L-Cysteine	15.3±1.1	15.2±1.1	21.6±2.0	<0.001	0.992	<0.001	<0.001
EFS: DL-PAG	2.7±0.2	7.7±0.5	10.8±1.1	<0.001	<0.001	0.012	<0.001
EFS: L-NAME+DL-PAG	1.8±0.3	6.6±1.1	9.8±0.9	<0.001	<0.001	<0.001	<0.001
DL-PAG	5.2±0.9	8.9±2.3	11.0±1.2	<0.001	0.003	<0.001	0.088
L-NAME+DL-PAG	4.4±0.5	7.3±2.6	9.9±8.8	0.232	ns	ns	ns
EFS: NaHS	62.7±2.2	70.8±9.6	62.1±6.2	0.073	ns	ns	ns
EFS: L-NAME+NaHS	38.4±1.9	30.1±7.6	35.0±3.4	0.034	0.027	0.474	0.230
NaHS	58.2±2.3	72.3±8.9	56.2±4.8	<0.001	0.003	0.833	<0.001
L-NAME+NaHS	56.1±2.8	53.4±6.5	53.9±5.2	0.627	0.635	0.737	0.984
Emax (relaxation (%))-colon							
EFS: L-Cysteine	20.6±1.6	12.6±1.5	23.0±2.9	<0.001	<0.001	0.151	<0.001
EFS: L-NAME+L-Cysteine	18.1±2.6	10.8±2.2	22.6±1.8	<0.001	<0.001	0.008	<0.001
L-Cysteine	18.6±3.1	17.3±1.9	22.8±2.4	0.005	0.651	0.028	0.005
L-NAME+L-Cysteine	15.7±3.8	14.8±3.6	21.9±1.9	0.031	0.880	0.012	0.044
EFS: DL-PAG	3.6±0.6	4.1±2.5	11.1±0.8	<0.001	0.844	<0.001	<0.001
EFS: L-NAME+DL-PAG	2.7±0.9	3.5±0.8	10.8±0.7	<0.001	0.229	<0.001	<0.001
DL-PAG	2.3±0.4	3.5±0.6	10.2±0.9	<0.001	0.018	0.021	<0.001
L-NAME+DL-PAG	1.9±0.2	2.7±0.7	9.2±0.7	<0.001	0.075	0.049	0.007
EFS: NaHS	84.7±6.9	95.6±6.9	80.7±10.2	0.017	0.084	0.679	0.017
EFS: L-NAME+NaHS	41.2±5.4	50.4±5.2	40.5±4.1	0.006	0.015	0.967	0.009
NaHS	85.9±8.6	96.1±3.6	88.5±7.8	0.060	ns	ns	ns
L-NAME+NaHS	25.3±3.8	20.7±1.3	22.6±2.2	0.028	0.022	0.213	0.446
pD2-Ileum							
EFS: L-Cysteine	5.54±0.8	5.34±0.3	5.58±0.1	0.676	ns	ns	ns
EFS: L-NAME+L-Cysteine	5.45±0.1	5.75±0.4	5.67±0.6	0.459	ns	ns	ns
L-Cysteine	5.64±0.12	5.34±0.9	5.36±0.5	0.663	ns	ns	ns
L-NAME+L-Cysteine	5.44±0.21	5.41±0.12	5.37±0.8	0.969	ns	ns	ns
EFS: DL-PAG	6.01±0.18	5.21±0.13	5.31±0.7	0.010	0.013	0.030	0.923
EFS: L-NAME+DL-PAG	5.88±0.11	5.58±0.10	5.38±0.4	0.011	0.121	0.008	0.363
DL-PAG	5.25±1.1	5.75±1.0	5.65±0.9	0.666	ns	ns	ns
L-NAME+DL-PAG	5.65±0.86	5.25±0.45	5.32±0.4	0.491	ns	ns	ns
EFS: NaHS	5.21±0.56	5.26±0.55	5.16±0.8	0.965	ns	ns	ns
EFS: L-NAME+NaHS	5.16±0.47	5.64±0.34	5.54±0.6	0.224	ns	ns	ns
NaHS	5.78±0.75	5.34±0.67	5.44±0.7	0.542	ns	ns	ns
L-NAME+NaHS	6.05±0.89	6.01±0.45	5.78±0.4	0.723	ns	ns	ns
pD2-Colon							
EFS: L-Cysteine	5.14±0.4	5.85±0.6	5.18±0.8	0.118	ns	ns	ns
EFS: L-NAME+L-Cysteine	5.25±0.3	5.45±0.8	5.21±0.4	0.721	ns	ns	ns
L-Cysteine	5.14±0.6	5.36±0.4	5.45±0.6	0.605	ns	ns	ns
L-NAME+L-Cysteine	5.36±0.9	5.21±0.09	5.62±0.7	0.566	ns	ns	ns
EFS: DL-PAG	5.12±0.6	5.45±0.08	5.11±0.4	0.307	ns	ns	ns
EFS: L-NAME+DL-PAG	5.76±0.8	5.38±0.12	5.43±0.6	0.486	ns	ns	ns
DL-PAG	5.23±1.2	5.59±1.1	5.62±0.4	0.744	ns	ns	ns
L-NAME+DL-PAG	5.12±0.34	5.12±0.54	5.23±0.9	0.942	ns	ns	ns
EFS: NaHS	5.56±0.33	5.13±0.34	5.22±0.5	0.177	ns	ns	ns
EFS: L-NAME+NaHS	5.98±0.37	5.46±0.14	5.11±0.3	<0.001	0.017	<0.001	0.120
NaHS	5.34±0.87	5.21±0.76	5.36±0.7	0.937	ns	ns	ns
L-NAME+NaHS	5.01±0.23	6.36±0.45	5.41±0.6	<0.001	<0.001	0.306	0.007

One-way ANOVA (Robust Statistic: Brown-Forsythe)-(Method: Bootstrap), *post hoc* Test: Tukey's HSD-Games Howell and SD: Standard Deviation

Upon adding NaHS after L-NAME was added to the colon specimens following the pre-contraction with carbachol, pD2 was lower in Group 1 or 3 than in Group 2 ($p<0.001$ and $p = 0.007$, respectively, Table 2). Notably, NaHS elicited a significant alteration with L-NAME after both EFS induction and pre-contraction with carbachol in the colon's Control, Peritonitis and Sham groups ($p = 0.006$, $p = 0.028$, respectively; Table 2). Either after induction with EFS or after pre-contraction with carbachol, relaxation rate (%) with NaHS was higher than relaxation rate (%) with L-cysteine and relaxation rate (%) with DL-PAG in the colon Control, Peritonitis and Sham groups.

Table 3: Comparison of relaxation responses between ileum and colon strips in Control, Peritonitis and Sham groups

	Emax (relaxation (%))			pD2		
Groups	Ileum Mean±SD	Colon Mean±SD	p value	Ileum Mean±SD	Colon Mean±SD	p-value
Group 1: Control (n = 6)						
EFS: L-Cysteine	19.1±0.6	20.6±1.6	0.057	5.54±0.8	5.14±0.4	0.299
EFS: L-NAME+L-Cysteine	17.3±0.7	18.1±2.6	0.483	5.45±0.1	5.25±0.3	0.152
L-Cysteine	17.4±1.2	18.6±3.1	0.397	5.64±0.12	5.14±0.6	0.073
L-NAME+L-Cysteine	15.3±1.1	15.7±3.8	0.809	5.44±0.21	5.36±0.9	0.836
EFS: DL-PAG	2.7±0.2	3.6±0.6	0.006	6.01±0.18	5.12±0.6	0.006
EFS: L-NAME+DL-PAG	1.8±0.3	2.7±0.9	0.042	5.88±0.11	5.76±0.8	0.723
DL-PAG	5.2±0.9	2.3±0.4	<0.001	5.25±1.1	5.23±1.2	0.977
L-NAME+DL-PAG	4.4±0.5	1.9±0.2	<0.001	5.65±0.86	5.12±0.34	0.191
EFS: NaHS	62.7±2.2	84.7±6.9	<0.001	5.21±0.56	5.56±0.33	0.217
EFS: L-NAME+NaHS	38.4±1.9	41.2±5.4	0.259	5.16±0.47	5.98±0.37	0.007
NaHS	58.2±2.3	85.9±8.6	<0.001	5.78±0.75	5.34±0.87	0.370
L-NAME+NaHS	56.1±2.8	25.3±3.8	<0.001	6.05±0.89	5.01±0.23	0.020
Group 2: Peritonitis (n = 6)						
EFS: L-Cysteine	16.3±0.3	12.6±1.5	<0.001	5.34±0.3	5.85±0.6	0.092
EFS: L-NAME+L-Cysteine	14.5±0.8	10.8±2.2	0.003	5.75±0.4	5.45±0.8	0.430
L-Cysteine	17.2±1.6	17.3±1.9	0.923	5.34±0.9	5.36±0.4	0.961
L-NAME+L-Cysteine	15.2±1.1	14.8±3.6	0.800	5.41±0.12	5.21±0.09	0.008
EFS: DL-PAG	7.7±0.5	4.1±2.5	0.006	5.21±0.13	5.45±0.08	0.003
EFS: L-NAME+DL-PAG	6.6±1.1	3.5±0.8	<0.001	5.58±0.10	5.38±0.12	0.011
DL-PAG	8.9±2.3	3.5±0.6	<0.001	5.75±1.0	5.59±1.1	0.797
L-NAME+DL-PAG	7.3±2.6	2.7±0.7	0.002	5.25±0.45	5.12±0.54	0.660
EFS: NaHS	70.8±9.6	95.6±6.9	<0.001	5.26±0.55	5.13±0.34	0.633
EFS: L-NAME+NaHS	30.1±7.6	50.4±5.2	<0.001	5.64±0.34	5.46±0.14	0.258
NaHS	72.3±8.9	96.1±3.6	<0.001	5.34±0.67	5.21±0.76	0.760
L-NAME+NaHS	53.4±6.5	20.7±1.3	<0.001	6.01±0.45	6.36±0.45	0.208
Group 3: Sham (n = 6)						
EFS: L-Cysteine	22.2±2.3	23.0±2.9	0.608	5.58±0.1	5.18±0.8	0.252
EFS: L-NAME+L-Cysteine	21.8±1.8	22.6±1.8	0.459	5.67±0.6	5.21±0.4	0.149
L-Cysteine	22.7±1.4	22.8±2.4	0.931	5.36±0.5	5.45±0.6	0.783
L-NAME+L-Cysteine	21.6±2.0	21.9±1.9	0.795	5.37±0.8	5.62±0.7	0.577
EFS: DL-PAG	10.8±1.1	11.1±0.8	0.601	5.31±0.7	5.11±0.4	0.557
EFS: L-NAME+DL-PAG	9.8±0.9	10.8±0.7	0.057	5.38±0.4	5.43±0.6	0.869
DL-PAG	11.0±1.2	10.2±0.9	0.221	5.65±0.9	5.62±0.4	0.942
L-NAME+DL-PAG	9.9±8.8	9.2±0.7	0.850	5.32±0.4	5.23±0.9	0.827
EFS: NaHS	62.1±6.2	80.7±10.2	0.003	5.16±0.8	5.22±0.5	0.879
EFS: L-NAME+NaHS	35.0±3.4	40.5±4.1	0.030	5.54±0.6	5.11±0.3	0.147
NaHS	56.2±4.8	88.5±7.8	<0.001	5.44±0.7	5.36±0.7	0.847
L-NAME+NaHS	53.9±5.2	22.6±2.2	<0.001	5.78±0.4	5.41±0.6	0.237

Independent t-test (Bootstrap) and SD: Standard Deviation

When NaHS was introduced after pre-contraction with carbachol, L-NAME did not give rise to a significant alteration in the ileum ($p>0.05$, Table 2). However, it did produce a notable effect in the colon of the Peritonitis group when compared with the ileum ($p<0.001$, Table 3).

Relaxation responses of colon samples for all groups are demonstrated in Fig. 1.

Comparison of Emax and pD2 between the ileum and colon strips: When L-cysteine was introduced, either with or without L-NAME, following EFS induction, Emax was greater in the ileum than in the colon for Group 2. With the addition of L-cysteine together with L-NAME after pre-contraction with carbachol, pD2 in the ileum was higher than in the colon for Group 2 ($p = 0.008$) (Table 3).

After induction with EFS, DL-PAG either with L-NAME or not, Emax values correlating with the colon was higher for Group 1, the ileum for Group 2 and it was similar both in the ileum and colon for Group 3. Without L-NAME, when DL-PAG was applied after EFS induction, pD2 was greater in the ileum for Group 1 but higher in the colon for Group 2. However, during the EFS induction process, at the time L-NAME was combined with DL-PAG, pD2 was dominant in the ileum for Group 2. When DL-PAG was added, either with or without L-NAME, following pre-contraction with carbachol, Emax exhibited an increase in the ileum for both Group 1 and 2

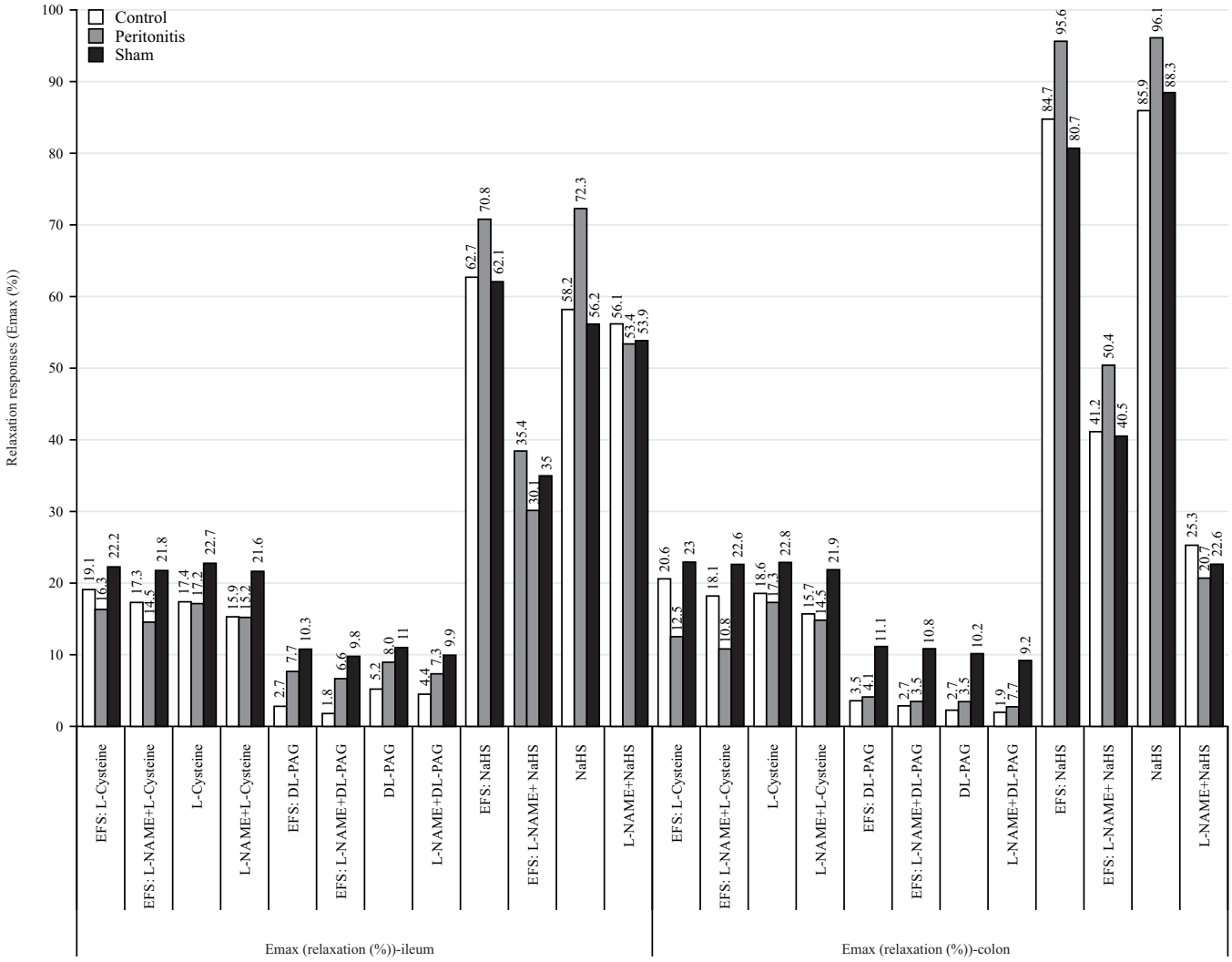

Fig. 1: Emax (%) (relaxation responses) of the Control, Peritonitis and Sham groups after the administration of compounds

(Table 3). In the absence of L-NAME, after EFS induction, NaHS led to a higher Emax in the colon for Group 1, 2 and 3 ($p<0.05$, Table 3). When NaHS was introduced after L-NAME, following EFS induction, pD2 was found to be higher in the colon for Group 1 and Emax showed a significant increase in the colon for Group 2 and 3. With the addition of NaHS without L-NAME after pre-contraction with carbachol, Emax showed an increase in the colon for Group 1, 2 and 3. With the introduction of NaHS to L-NAME after pre-contraction with carbachol, Emax and pD2 levels were higher in ileum for Group 1. For Group 2 and 3, Emax was also higher in the ileum when compared to the colon examples (Table 3). The NaHS did reveal a more significant change in the colon following the addition of L-NAME after pre-contraction with carbachol. ($p<0.028$, Table 2).

DISCUSSION

Results showed that the relaxation response after L-cysteine treatment was lower in the Peritonitis group by comparison with the Sham group. The NaHS provided a higher relaxation response compared to that with L-cysteine. A higher NaHS-induced relaxation response in the ileum after pre-contraction with carbachol was observed in the Peritonitis group when set against that in the other cohorts. Either for pre-contraction with carbachol or induction with EFS, NaHS provided a greater relaxation response in the colon than in the ileum for all groups. Relaxation responses after DL-PAG were higher in the Sham groups for both the ileum and colon. With L-NAME, there was a decrease in the relaxation response when NaHS was introduced, but it had no impact on relaxation

when L-cysteine or DL-PAG was added. For ileum strips induced with EFS, there was a higher potency of DL-PAG in the Control groups as against the colon strips. For the Peritonitis group after induction with EFS, the potency of DL-PAG was higher in the colon than in the ileum examples. The NaHS did have a higher potency for the Peritonitis group as against the Control and Sham groups in the presence of L-NAME regarding the colon strips precontracted with carbachol.

In a study, lipopolysaccharide-induced endotoxic shock was shown to be associated with an increased plasma level of H_2S and nitrate/nitrite in mouse and it led to an increased synthesis of H_2S after the addition of L-cysteine[19]. In a previous study, lipopolysaccharide stimulation was shown to decrease H_2S synthesis[13]. They also demonstrated a predominance of neutrophils, a decrease in macrophage count and a reduction in H_2S synthesis at the 4th hr following LPS stimulation. As the inflammation was resolved (24-48 hrs of LPS stimulation), macrophage count and H_2S synthesis were shown to increase. Measurements were made 24 hrs following peritonitis induction while the H_2S level was expected to increase. However, the relaxation response after L-cysteine was not as high as that in the Peritonitis group when set against that in the Sham group. It could be suggested that surgical stress, rather than an infectious insult, might impact the synthesis of endogenous H_2S. Exceptional findings regarding a change in H_2S synthesis after sepsis or peritonitis induction were reported. In one study of an experimental cecal ligation-peritonitis model, plasma H_2S concentration was shown to increase 4-8 hrs after peritonitis and liver CSE activity and H_2S levels returned to normal limits at the 8th hr[14]. In a severe acute pancreatitis model of rats, H_2S was shown to increase in the intestine and to inhibit intestinal motility, but promote the inflammation[20]. Current study provides corroborating evidence consistent with the acute pancreatitis model mentioned, suggesting increased relaxation rates in the Peritonitis groups, likely attributed to the elevated H_2S levels. The relaxation response was found higher in the Sham group after treatment with DL-PAG. The lower level of inflammation and reduced neutrophil migration observed in the Sham group, when set against that observed in the Peritonitis group, could be linked to the lesser inhibition of CSE. This, in turn, could result in a higher level of endogenous H_2S synthesis in the Sham groups. For ileum strips, the relaxation response observed after DL-PAG was greater in the peritonitis examples when compared to the response seen in the Control groups. This outcome could be attributed to a substantial inhibition of CSE in the Control groups and a heightened production of endogenous H_2S synthesis in the Peritonitis groups, likely due to increased CSE efficiency in response to inflammation. Consequently, the blockade by DL-PAG may have been reduced. In the Peritonitis group, the relaxation response occurred after DL-PAG treatment was higher in the ileum than in the colon, which might indicate a higher level of CSE blockage in the colon. The L-NAME did not induce a significant alteration in the relaxation response when treated with DL-PAG after pre-contraction with carbachol within the ileum groups. However, it had a notable impact on all the other groups both in the colon and ileum. This may be related to the interaction between NO and the production of endogenous H_2S production due to CSE and other alternative pathways.

The contractile effect of NaHS was seen to be facilitated by TRPV1 channels[21-23]. At higher concentrations than that which is necessary to diminish spontaneous motility, NaHS might also inhibit neural mediated tachykinergic and cholinergic contractions in the human colon[24]. As per the previous results, a significantly higher relaxation response was observed with NaHS when compared to that with L-cysteine in our study. The NaHS is known as a fast-release H_2S donor and its effect does not necessitate CSE activity, which might be affected by sepsis. The relaxation responses observed with NaHS were higher in the Peritonitis groups and this may be related to the increased production of endogenous H_2S due to inflammation. Conflicting results concerning this relationship have been documented before[25]. The fast-release H_2S donor, NaHS, can elevate inflammatory markers, in contrast to the anti-inflammatory effects of the slow-release H_2S donor, GYY4137[26]. In one study, NaHS was observed to have no effect on proinflammatory cytokines at low concentrations, but it did enhance the cytokine levels at higher concentrations[26]. Similar to the contractile response observed following the administration of NaHS, it appears that the proinflammatory effect of NaHS is also dependent on its concentration. In rats with peritoneal inflammation induced by peritoneal dialysis fluids, NaHS was demonstrated to reduce the expression of ED-1 and MCP-1, which represent monocyte/macrophage cells. This finding supports the anti-inflammatory properties of H_2S[15]. The NaHS was shown to increase bacterial ingestion capacity and the chemotaxis of macrophages and to interfere with the ability of neutrophils, neutrophil infiltration and to induce neutrophil apoptosis[13,26,27]. The L-NAME did exert dramatic decreases in relaxation responses in both the ileum and colon strips treated with NaHS, but not with L-cysteine or DL-PAG. This might be due to the fact that NaHS is a H_2S donor and stimulated NOS more than L-cysteine, which is a H_2S precursor dependent on CSE. It appears that the reduction in relaxation responses following NaHS was more pronounced in the colon-peritonitis group when precontraction with carbachol was applied, particularly with L-NAME. It might be a result of the presence of higher endogenous H_2S levels in the colon-peritonitis groups and their interaction with NO synthase enzymes in a dose-dependent manner.

Free H_2S levels were shown to comprise 8% of the total H_2S in the human colon and the remainder was shown to bind to fecal components resulting in it being inactive[28]. The CBS and CSE are present in the myenteric plexus neurons in the colon and the colon mucosa has the capacity to convert L-cysteine into H_2S[4,5]. The H_2S has been demonstrated to have the ability to neutralize reactive species and superoxide dismutase, N-acetylcysteine and glutathione[1]. Laboratory experiments have suggested that H_2S can suppress motor patterns in both the human colon and jejunum examples in a manner that is dependent on its concentration[29]. In rats, it was shown to be K-channel dependent, reproducible by pinacidil and reversible by NOS inhibitor[1]. The H_2S's relaxant effect on the colon has also been demonstrated to impede the L-type calcium channel, indicating it as a potential mechanism[30]. In a rat model, it was demonstrated that NaHS could suppress spontaneous phasic contractions in both the circular and longitudinal muscle layers and this effect was not contingent on neural activity. However, it may be linked to guanylyl cyclase, SKCa and KATP channels[31].

In the vascular system, NO stimulates the rapid generation of H_2S. Furthermore, it enhances CSE activity and governs H_2S production by promoting the overexpression of CSE in the endothelium[32]. The inhibition of NO synthase, which leads to the suppression of NO release, causes a reduction in the inhibitory responses of H_2S in vascular smooth muscle[33]. We obtained similar outcomes in the intestinal system organs when assessing the impact of L-NAME. After demonstrating a significant relaxation response with NaHS both in the ileum and colon, it was more pronounced in the colon groups when L-NAME was not present. Particularly within the vascular system, H_2S may directly engage with the enzyme eNOS through sulfhydration of essential cysteines. This interaction aims to enhance the production of NO from eNOS while reducing the formation of superoxide[34]. By the same logic, during intestinal ischemia, the aim is to improve intestinal perfusion via vasodilation of mesenteric vessels through pathways related to the NO-H_2S interaction[35]. An option worth considering for new experiments is the inclusion of endothelial nitric oxide synthase (eNOS) knockout (eNOSKO) pups along with the administration of NO donors. This would allow for the investigation of the interaction between NO and H_2S.

The NaHS provided similar relaxation responses but a significant response was obtained between the peritonitis and Sham groups in the colon after the EFS induction. This might suggest that the relaxation effect of NaHS is possibly conveyed post-junctionally, rather than being neural in nature.

Relaxation responses were showed regarding the treatments with L-cysteine, DL-PAG and NaHS in both ileum and colon strips after precontraction either with carbachol or induction with EFS in an experimental model consisting of Control, Peritonitis and Sham groups. We could not measure plasma or intraluminal free H_2S levels, nor the association between NaHS levels and inflammatory cytokines levels. In our study, ileum and colon strips were analyzed separately in the laboratory, which meant that it was not possible to measure the systemic inflammatory response. It was also not possible to measure spontaneous contractions in our study. There are several enzymes for the production of endogen H_2S synthesis in humans. In our study, we specifically inhibited CSE, but it is worth noting that cystathionine beta-synthase (CBS), 3-mercaptopyruvate sulfurtransferase (3-MST), AD-amino acid oxidase (DAO) and the alternate potential enzymes could also be targeted to suppress the alternative endogenous pathways for H_2S synthesis as the next step.

CONCLUSION

The NaHS provided a more pronounced relaxation response when compared to L-cysteine and a greater relaxation response in the colon than in the ileum. The addition of L-NAME did decrease the relaxation responses in the presence of NaHS. The reduction in relaxation responses following NaHS was notably more pronounced in the colon in the presence of L-NAME. The interaction between eNOS and hydrogen sulfide may enable therapeutic agents to be used in gastrointestinal system disorders. Therapeutic investigations regarding H_2S might be initiated in colon pathologies due to their high response rates. For inflammatory bowel diseases or acute/chronic pancreatitis, NaHS and other H_2S donors combined with NO enhancing agents could be alternatives to the routine treatment algorithms.

SIGNIFICANCE STATEMENT

The aim of this study is to guide the further studies regarding the emerging treatment options for peritonitis. Endogen H_2S has a critical role on the contractility of intestines throughout the peritonitis phase. Administration of exogen H_2S and L-NAME demonstrated that NO has significant roles on the motility during the inflammation process. The findings show that there is a substantial interaction between NO and H_2S pathways in terms of relaxation and the reversal of relaxation during peritonitis which is a serious clinical condition that requires novel therapies. The treatments of gastrointestinal system disorders are going to be reshapened via taken into consideration of this strong interplay between endogen gaseous neurotransmitters.

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