# The vasodilatory mechanism of nitric oxide and hydrogen sulfide in the human mesenteric artery in patients with colorectal cancer

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**Abstract.** Recent studies have focused on the role of gasotransmitters in cancer progression and prevention. Therefore, the current study was designed to explore the vasodilator activity of NO and $H_2S$ in the human mesenteric arteries of patients with colorectal cancer (CRC) via the activation of $K^+$ channels. A total of two sets of experiments were established for the current investigation. Blood samples from patients with CRC were obtained to detect serum levels of endocan and malondialdehyde (MDA). The role of $K^+$ channels in mediating the vasodilation of the human mesenteric artery in response to sodium nitroprusside (SNP) and sodium disulfide ($Na_2S$) was assessed. The level of serum endocan was indicated to be decreased in patients with CRC compared with healthy individuals, while the level of serum MDA remained unaltered between groups. The arterial rings pre-contracted with norepinephrine were first relaxed by the cumulative addition of increasing concentrations of either SNP (30 nM-30 $\mu$M) or (1-6 mM). Maximal relaxation rates were then calculated at 15 min intervals for 60 min. Pre-incubation of arterial rings for 20 min with individual $K^+$ channel blockers was indicated to significantly reduce SNP- and $Na_2S$-induced relaxation at different time points. Pre-treatment of L-nitro-arginine methyl ester did not alter vasodilation that was induced by $Na_2S$. Furthermore, vasodilation of the CRC mesenteric artery was not altered by the synergistic application of SNP and $Na_2S$, while pre-incubation of arterial rings with D,L-propargylglycine significantly enhanced vasodilation induced by SNP. These results indicated that endothelial dysfunction and oxidative stress do not serve roles in the pathogenesis of CRC. The dilatory mechanisms of NO and $H_2S$ in mesenteric arteries of patients with CRC were $K^+$ channel- and time-dependent, and the activity of cystathionine γ-lyase enzyme inhibited the ability of exogenous NO in vasodilation processes.

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## Introduction

Globally, colorectal cancer (CRC) is the third most frequently detected neoplasm and the second leading cause of cancer-associated mortality, with >1.8 million new CRC cases and 881,000 deaths being reported globally in 2018, with ~1 death per 10 confirmed cases (1,2). While the incidence of CRC in Middle Eastern countries is low (3), there is a higher annual incidence rate of CRC in the Kurdistan region of Iraq with an estimated ~38-61.7 cases/100,000 people from 2006 to 2014. CRC is the fourth most widespread cancer in males and females residing in this province, causing ~8.6% mortality of the total annual fatalities in this region (4). There is strong evidence that CRC is the most common type of adenocarcinoma in this region (5).

Commonly endogenous gasotransmitters that are used are NO, CO and $H_2S$ (6). These small molecules of gas, which have limited concentrations and particular functions, are endogenously and enzymatically generated (7). NO is produced from L-arginine by NO synthase (8,9), while $H_2S$ is synthesized from L-cysteine by both cystathionine β-synthase and cystathionine γ-lyase (CSE), or mercaptopyruvate sulfurtransferase (10,11). The aforementioned gaseous molecules modulate different biological pathways and functions such as SMC relaxation, at physiologically relevant concentrations (6) by opening a number of membrane ion channels (12). NO is an essential regulator of angiogenesis (13) and vasorelaxation via the activation of guanylate cyclase and the production of cyclic guanidine monophosphate (cGMP) (14). cGMP increases endothelial $Ca^{+2}$ concentration and contributes to the opening of localized $Ca^{+2}$-dependent $K^+$ channels ($K_{Ca}$) (15). In contrast, $H_2S$ exerts its effects on vasodilation (16) and angiogenesis (17) via the direct activation of $K_{ATP}$ channels (18).

NO and $H_2S$ also act synergistically or antagonistically to stimulate their downstream pathways, ranging from biosynthesis to their signaling cascade within target cells (19). NO and $H_2S$ are mutually dependent on regulating vasodilation, angiogenesis (20) and endothelial homeostasis (21). The role of these gases in cancer remains unclear, as both exhibit tumor promotion and anti-tumor properties (22). Furthermore, NO and $H_2S$ have been indicated to modulate a variety of cancer

cell functions, including proliferation, invasion, metastasis and tumor angiogenesis (23). Additionally, the enzymes responsible for NO and $H_2S$ production are upregulated in CRC cells and endogenously produce low-to-mid concentrations of $H_2S$ or NO to support cell proliferation, while exogenous delivery of $H_2S$ or NO suppresses the division of colon cancer cells (24).

Intra-tumor blood vessels are vital for tumor growth, metastasis, cancer treatment (25) and the acquisition of differential reactivity by functional mature blood vessels in the tumor micro-environment, representing an appropriate target for anti-tumor therapeutic agents (26). Cancerous cells exhibit accelerated metabolism and, therefore, demand high reactive oxygen species (ROS) concentrations to maintain high proliferation rates (27). The high level of ROS damages and/or destroys cells by oxidizing proteins, lipids and nucleic acids (28). These observations clarify that oxidative stress and cancer are closely associated (29). Several researchers are investigating the consequences of ROS and endothelial dysfunction in cancer (30-32). Based on literature reviews, to the best of our knowledge, no studies appear to have presented an association between oxidative stress, endothelial functions and vascular reactivity of NO and $H_2S$ in patients with CRC. Therefore, the current study aimed to assess endocan as an endothelial functional marker and malondialdehyde (MDA) as an oxidative stress marker in patients with CRC. Additionally, the present study investigated the probable mechanisms responsible for NO- and $H_2S$-induced vasodilation in the human mesenteric artery of patients with CRC. To elucidate these mechanisms, the possible roles of different $K^+$ channels in the vasodilation response produced by NO and $H_2S$ were explored.

## Materials and methods

*Patients*. The current study was a case-control study. For the first experiment, patients were recruited at two hospitals in Erbil, Iraq: The Oncology Department of Rizgary and Nanakaly Hospitals. Venous blood samples were taken from 44 patients (male, 24; female, 20) with different stages of CRC. Additionally, 40 healthy volunteers (male, 22; female, 18) of similar ages were recruited randomly in Erbil city as control samples. For the second experiment, colorectal tumour specimens were obtained from patients with CRC undergoing partial colectomy at Consultancy Medical City and Welfare private hospitals in Erbil. In both experiments, patients were recruited between August to November 2016 and patient's median age was 55 years old (ranged between 35-70). Patients with underlying immunodeficiency disorder or immunodeficiency state and individuals who had other co-morbid health problems which could introduce heterogeneity to the sample, such as additional acquired brain injury, arthritis, chronic obstructive pulmonary disease, asthma, diabetes mellitus, ankylosing spondylitis, connective tissue diseases and other inflammatory diseases were excluded.

The blood samples were obtained by phlebotomy under an aseptic technique. Blood was placed into a clot activator tube for serum separation. The sera were then separated under centrifugation at 448 x g for 5 min at 37˚C. Patients with an underlying immunodeficiency disorder or state of immunodeficiency, and individuals who presented with other co-morbid health problems which could introduce heterogeneity to the sample (including arthritis, asthma, diabetes mellitus, hypertension and other inflammatory diseases) were excluded.

*Determination of endocan*. The concentration of endocan was determined using the Human ESM1 ELISA kit (cat. no. E-EL-H1557; Elabscience, Inc.) by the Sandwich-ELISA method (33). The micro ELISA plate was pre-coated with antibodies specific to endocan. Standards or samples were added to the suitable micro ELISA plate wells and combined with the endocan-specific antibodies (Elabscience, Inc.). A biotinylated detection antibody, part of the aforementioned kit, specific for endocan and avidin-horseradish peroxidase (HRP)-conjugate and substrate were then added to each well. Only the wells that contained endocan, biotinylated detection antibodies and avidin-HRP conjugate appeared blue in color and the reaction was stopped with a 1N $H_2SO_4$ solution and the color turned yellow. The absorbance was measured spectrophotometrically (ELISA reader; Biotek) at a wavelength of 450 nm.

*Determination of serum MDA*. MDA was determined according to the Ohkawa method (34). The procedure started by thiobarbituric acid (TBA) preparation, in which 0.66 g TBA was dissolved in 100 ml of 0.05 M of NaOH with simple heating at 45˚C. Trichloroacetic acetic acid (TCA) was then prepared by dissolving 17.5 g TCA in 100 ml of distilled water. TCA2 was prepared by dissolving 70 g of TCA in 100 ml of distilled water. Finally, 150 ml of either control or CRC patient's serum was added, 1 ml of TCA1 was mixed for 2 min and placed in a boiling water bath for 15 min and 1 ml of TCA2 was added and incubated for 20 min at 37˚C. The solution was centrifuged for 5 min at 448 x g. The supernatant was read (Unico SpectroQuest SQ2800 UV-Visible Spectrophotometer; Unico) at 532 nm.

*Myographical recording*

*Vessel collection and preparation*. Human mesenteric arteries were collected during surgery from patients with CRC undergoing partial colectomy. The arteries supplying blood to the tumors were dissected surgically and placed into beakers containing cold modified Krebs solution (Sigma-Aldrich; Merck KGaA) (Fig. 1) and aerated with 95% $O_2$ and 5% $CO_2$. The excess tissue and fat were removed in the laboratory and the arteries were cut into rings (length, ~3-4 mm).

*Recording of isometric tension*. The procedure by Furchgott and Zawadzki (35) with certain modifications in the Krebs solution's concentration was followed to study the vasodilator activity of the isolated mesenteric arteries. The arterial rings were propped by two stainless steel clamps. The first clamp was attached to a hook at the underside of the organ bath and the second was connected to the force transducer through a thread to record the isometric tension of the mesenteric arteries. Data were recorded using LabChart 7.1 data acquisition software (ADInstruments, Inc.). The propped arterial rings were immersed in modified Krebs solution (NaCl 5.10 gm/l, $NaHCO_3$ 1.94 gm/l, $MgSO_4$ 0.686 gm/l, KCl 2.24 gm/l, $KH_2PO_4$ 0.15 gm/l, $CaCl_2$ 0.277 gm/l and $C_6H_{12}O_6$ 2 gm/l) and contained in a 10 ml organ chamber. Krebs

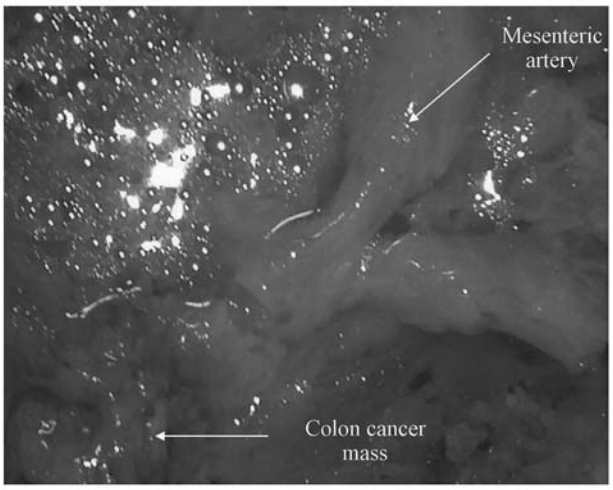


Figure 1. Mesenteric artery feeding a colon tumor.

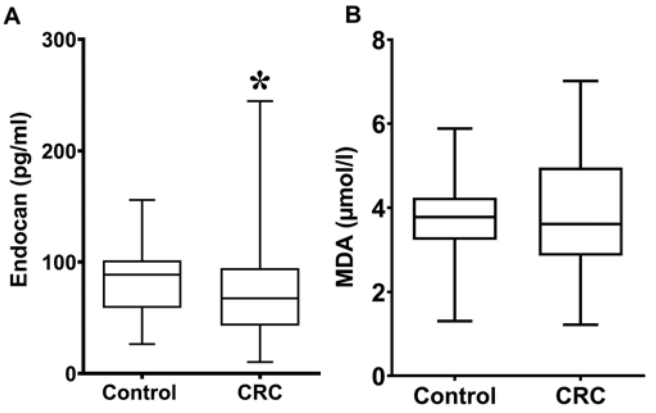


Figure 2. Comparison between (A) endocan and (B) MDA level in patients with CRC and healthy controls. Endocan was significantly decreased in patients with CRC compared with the healthy control group, while there were no significant differences MDA levels between groups. *P<0.05 vs. control. MDA, malondialdehyde; CRC, colorectal cancer.

solution was maintained at a pH 7.4 and was constantly aerated with 95% $O_2$ 5% $CO_2$ at 37˚C (panLab; Harvard Apparatus).

The mesenteric arterial rings were tensed to a stable basal strain of 4 gm prior to being left to be equilibrated for 2 h. The Krebs solution was replaced at 15-20 min intervals in the bath chamber until it reached stability. Experimental substances were added to the bath chambers, according to protocol. The arteries were incubated at 37˚C with channel blockers for 20 min prior to pre-contraction with norepinephrine (NE; 1 $\mu$M; Sigma-Aldrich; Merck KGaA). Following this, relaxation occurred by bolus dose application of sodium nitroprusside (SNP; Sigma-Aldrich; Merck KGaA) or sodium sulphide ($Na_2S$; Hangzhou J&H Chemical Co.).

*Experimental protocol*. The arterial rings, which were pre-contracted with NE, were first relaxed by the cumulative addition of either SNP (30 nM-30 $\mu$M) or $Na_2S$ (1-6 mM). Based on these initial experiments, the relative half-inhibitory concentration ($IC_{50}$) of SNP (2.3 $\mu$M) or $Na_2S$ (2.4 mM) was used to retest the ability to relax pre-contracted rings in three separate sets of experiments. In the first experiment, when the NE-induced contraction reached the uppermost value, SNP (2.3 $\mu$M) or $Na_2S$ (2.4 mM) was added and left for 60 min, and the maximal relaxation rate (%) was calculated four times at each 15 min interval (n=8). Following this, the role of $K^+$ channels in the progress of SNP and $Na_2S$ mediated relaxation were tested via incubation at 37˚C of the arterial rings for 20 min using tetraethylammonium (TEA; 1 mM), glibenclamide (GLIB; 0.1 $\mu$M), barium chloride (BaCl2; 1 mM) and 4-aminopyridine (4-AP; 1 mM) (all supplied from Hangzhou J&H Chemical Co.). In the second experiment, the role of endogenous NO and $H_2S$ were tested by the pre-incubation of arterial rings with endothelial NO and CSE antagonists, L-nitro-arginine methyl ester (L-NAME; $3x10^{-4}$ M) or D,L-propargylglycine (PAG; 10 mM) (Sigma-Aldrich; Merck KGaA), respectively for 20 min prior to applying SNP (n=8). Finally, to examine whether the combination of $H_2S$ and NO potentiates or inhibits vasorelaxation when the NE-induced contraction reached the highest value, SNP and $Na_2S$ were added simultaneously and left for 60 min (n=8).

*Statistical analysis*. Comparisons between patients with CRC and healthy individuals were performed using a Mann-Whitney test, and values were presented as median and quartiles. Statistical analysis of myographical data was performed using a two-way ANOVA followed by Dunnett post-hoc test. Maximum relaxation responses were calculated as a percentage of the contraction produced by NE and expressed as the mean ± standard error of the mean. The tension created by NE was defined as 0% relaxation, and the baseline tension prior to the addition of NE was determined as 100% relaxation.

The graphs, calculations and statistical analyses were performed using GraphPad Prism software (version 6.0; GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

## Results

*Serum endocan and MDA concentrations*. Serum endocan concentration was significantly lower in patients with CRC (67.56; 43.04-94.28) compared with healthy individuals (88.68; 59-101.3; Fig. 2A). There were no significant differences in MDA concentration between patients with CRC (3.62; 2.86-4.96) and healthy individuals (3.78; 3.23-4.24; Fig. 2B).

*Measurement of $IC_{50}$*. SNP concentrations ranging from 30 nM-30 $\mu$M induced a relaxant effect on CRC mesenteric arteries following pre-contraction with NE (1 $\mu$M) with an $IC_{50}$ value of 2.42±0.16 $\mu$M (CI 95%, 1.18-4.95 $\mu$M). The percentage of relaxation was 80.74±7.256%. $Na_2S$ at concentrations from 1-6 mM had a relaxant effect on mesenteric arteries of CRC pre-contracted with NE. The calculated $IC_{50}$ value was 3.54±1.07 mM (CI 95%, 1.4-5.68 mM) and the percentage of relaxation was 84.43±22.05%. The concentration-response curve for the effect of SNP and $Na_2S$ against NE-mediated contractions are presented in Fig. 3A and B, respectively.

*The role of $K^+$ channels in the NO-induced relaxation*. Pre-incubation of mesenteric arteries with either GLIB (0.1 $\mu$M; n=6) or TEA (1 mM; n=6) exhibited a significant reduction of net SNP-induced vasorelaxation in mesenteric

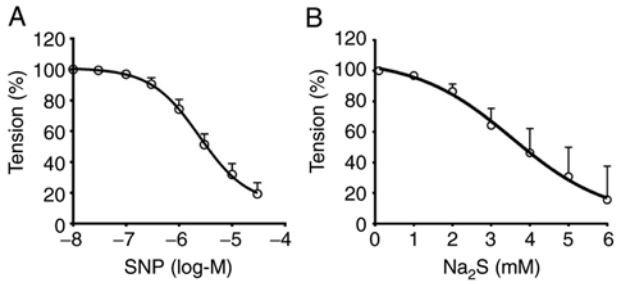


Figure 3. Cumulative dose-response curve for the vasorelaxant effects of (A) SNP (30 nM-30 $\mu$M) and (B) $Na_2S$ (1-6 mM) on norepinephrine (1 $\mu$M)-induced contraction in the mesenteric arteries of patients with colorectal cancer. SNP, sodium nitroprusside; $Na_2S$, sodium sulfide.

arteries at all-time points (Fig. 4). In contrast, vasorelaxation reduction by $BaCl_2$ (1 mM; n=6) and 4-AP (1 mM; n=6) was significant for 15-45 min; however, reduction was not significant at 60 min compared with the SNP treatment group.

*The role of $K^+$ channels in the $H_2S$-induced relaxation*. The impairment of $Na_2S$-inducing relaxation in mesenteric arteries was significantly sustained at time points 30, 45 and 60 mins following 4-AP incubation (n=6; Fig. 5). GLIB (n=6) and $BaCl_2$ (n=6) reduced vasorelaxation responses produced by $Na_2S$ only at time point 30 min. In contrast, TEA failed to ameliorate the vasorelaxation response of $Na_2S$.

*Interaction effects of SNP and $Na_2S$*. The combination of SNP and $Na_2S$ did not significantly alter the relaxation responses at any time point compared with the relaxation induced by the application of SNP or $Na_2S$ alone (Fig. 6A). Additionally, pre-incubation of the arterial rings with L-NAME (n=6) did not significantly change the extent of $Na_2S$-induced relaxation at any time point compared with the NasS treatment group (Fig. 6B). However, treating the mesenteric arterial rings with PAG (n=6) significantly increased vasorelaxation induced by the SNP at all-time points (Fig. 6C).

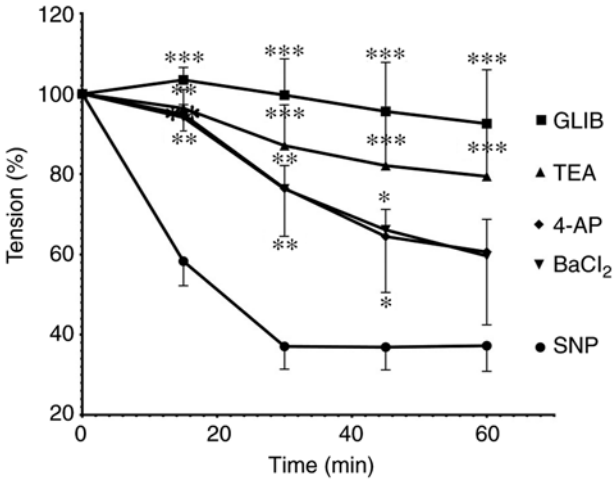


Figure 4. Time-dependent change of relaxation responses to SNP in mesenteric arteries preincubated with GLIB (10 $\mu$M), TEA (1 mM), 4-AP (1 mM) and $BaCl_2$ (1 mM). SNP-induced vasorelaxation was significantly inhibited by GLIB, TEA, 4-AP and $BaCl_2$ pretreatment. $^*$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001 vs. the SNP treatment group. SNP, sodium nitroprusside; GLIB, glibenclamide; TEA, tetraethylammonium; 4-AP, 4-aminopyridine; $BaCl_2$, barium chloride.

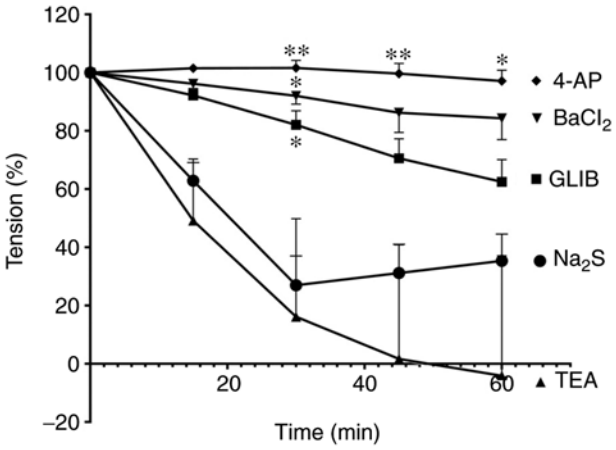


Figure 5. Time-dependent change of relaxation responses to $Na_2S$ in mesenteric arteries preincubated with 4-AP (1 mM), $BaCl_2$ (1 mM), GLIB (10 $\mu$M) and TEA (1 mM). TEA had no significant effect on $Na_2S$-induced time-dependent arterial relaxation. $Na_2S$-induced vasorelaxation was significantly inhibited by 4-AP, $BaCl_2$ and GLIB pretreatment. $^*$P<0.05 and $^{**}$P<0.01 vs. the $Na_2S$ treatment group. $Na_2S$, sodium sulfide; 4-AP, 4-aminopyridine; $BaCl_2$, barium chloride; GLIB, glibenclamide; TEA, tetraethylammonium.

## Discussion

The current study revealed that the endothelium cells of patients with CRC were functioning normally since the levels of serum endocan, which is an endothelial cell marker, were significantly decreased compared with the controls (36). In this regard (37-39), the reduction of endocan may be associated with chemotherapy, VEGF receptor-2 kinase inhibitor treatment or the downregulation of endocan expression, indicating that the expression of endocan is associated with the development and differentiation of CRC (36). Furthermore, it has been demonstrated that endocan is associated with colon tumor size, depth of invasion, lymph node metastasis, distant metastasis and Dukes' staging (40). In addition to endocan, there has been a growing interest in MDA as a marker of oxidative stress in the progression of cancer (41). The present investigation demonstrated that there were no changes in the serum MDA levels in patients with CRC compared with controls. This is in contrast to a previous study that reported a considerable elevation in serum MDA levels in patients with CRC (42). Subramanyam *et al* (43) reported that chemotherapeutics normalized oxidative stress in patients with CRC. Collectively, these results indicated that the arteries in patients with CRC were intact and that their endothelia were functioning properly.

Furthermore, the current study demonstrated that SNP markedly relaxed mesenteric arteries in patients with CRC. A previous study by (44) recorded 69% relaxation in the mesenteric arteries in experimental rats compared with the control group, while a relaxation of 103% was recorded in a study investigating human arm veins (45). To investigate the mechanism of SNP-induced relaxation, the role of $K^+$ channels was investigated in the mesenteric arteries of patients with CRC.

The results of the present study revealed that $K^+$ channels exhibited a significant role of SNP-induced relaxation in

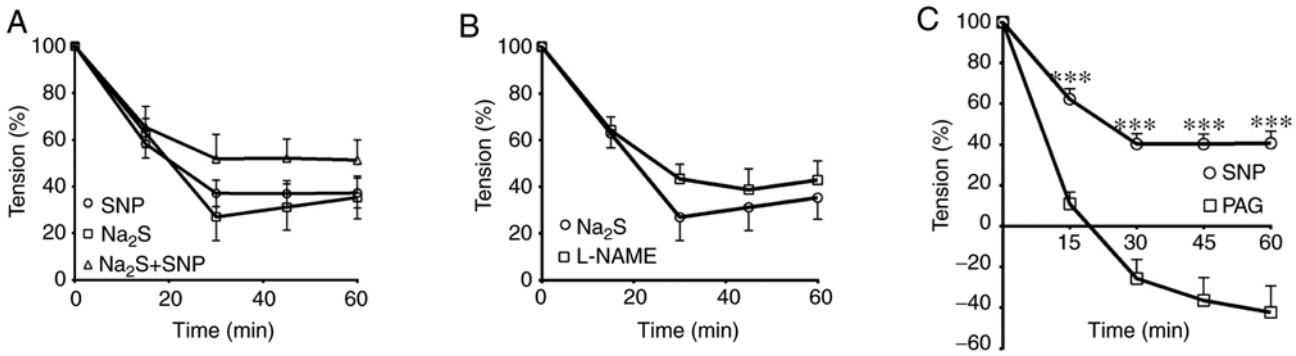


Figure 6. Combined effect of SNP and $Na_2S$ on the time-dependent relaxation responses of the mesenteric arteries precontracted with norepinephrine. (A) Combination of SNP and $Na_2S$ did not significantly alter time-dependent relaxation compared with SNP or $Na_2S$ alone. (B) L-NAME ($3x10^{-4}$ M) did not have a significant effect on $Na_2S$-induced time-dependent relaxation. (C) SNP-induced relaxation was significantly increased by PAG (10 mM) pretreatment. ***P<0.001 vs. the SNP treatment group. SNP, sodium nitroprusside; $Na_2S$, sodium sulfide; L-NAME, L-nitro-arginine methyl ester; PAG, D, L-propargylglycine.

mesenteric arteries following pre-treatment with TEA, GLIB, $BaCl_2$ or 4-AP. All of these significantly inhibited vasodilation. A previous study demonstrated that NO activated several $K^+$ channels of the small muscle cells (SMCs) of mesenteric and cerebral arteries in rats and rabbits, including ATP-sensitive $K^+$ channels, and induced membrane hyperpolarization by lowering $[Ca^{2+}]_i$ levels via the inhibition of $Ca^{2+}$ influx or $Ca^{2+}$ release from intracellular storage (46). Furthermore, NO hyperpolarized arterial SMCs via the activation of both $K_V$ and $K_{Ca}$ channels on vascular SMCs (VSMCs) in the rat superior mesenteric, coronary, cerebral and large arteries (47-49) through a cGMP-dependent mechanism, subsequently inhibiting the depolarization of the evoked membrane and upsurge in $[Ca^{2+}]_i$ (50). The data obtained are compatible with earlier findings, where it was observed that NO regulated VSMC $K_{IR}$ currents (51). In contrast, Hempelmann *et al* (52) reported that neither 4-AP nor $BaCl_2$ modulated NO-induced relaxation in the rat basilar artery. This conclusion indicated that NO may exert vasodilation, possibly by opening different $K^+$ channels. Consequently, it can be concluded that $K^+$ channels serves a crucial role in the vasodilation mechanism of NO.

To the best of our knowledge, in terms of the influence of $Na_2S$ on arterial relaxation, the current study was the first to observe the potency of $Na_2S$ in relaxing the mesenteric arteries of patients with CRC. The present study demonstrated that this relaxation was dependent on the activation of $K_{ATP}$ and $K_V$ channels. The importance of $K_{ATP}$ channel activation has been observed in the mesenteric arteries of the human colon (53), rat arterial smooth muscle (54) and human mammary arteries (55). The latter mechanism occurred either through the hyperpolarization of SMC membranes, which may close voltage-gated $Ca^{2+}$ channels (56), or through channel protein sulfhydration (16). On the other hand (53), concluded that $H_2S$ relaxed pre-contracted human mesenteric arterial rings in a concentration-dependent assay. Similarly, $H_2S$ induced vasorelaxation in rat aortas, which was diminished by KCNQ-type $K_V$ channel blockage (57).

In contrast, it has previously been shown that $K_{ATP}$ channels do not mediate $H_2S$-induced relaxation in the guinea-pig ileum or the trout urinary bladder (58). Previous studies have reported that $K_{IR}$ channels weakly participate in the relaxation of mesenteric arteries in patients CRC and that the mechanism of relaxation in rat aortas was mainly mediated by the stimulation of $K_{IR}$ channels and subsequent $K_{IR}$-dependent hyperpolarization from endothelium to the SMCs (59-61).

However, $H_2S$ was demonstrated to activate $BK_{Ca}$ (62), $IK_{Ca}$ and $SK_{Ca}$ channels in endothelial cells (16) and $BK_{Ca}$ channels in SMCs of mesenteric arteries (62) and cerebral arterioles (63). The results of the present study reported that TEA did not alter the vasodilation of mesenteric arteries induced by $Na_2S$, indicating that $K_{Ca}$ may not be a considerable factor for $H_2S$-induced vasorelaxation. Similar results were observed by Tang *et al* (64), who noted that different $K_{Ca}$ channel blockers were ineffective in the vascular impact of $H_2S$. Contrary to the current results, the maximum relaxation of VSMC in rat (16,54,59) and human mammary (55) arteries induced by sodium hydrogen sulfide was significantly attenuated by $K_{Ca}$ channel blockers. Whereas both $H_2S$ and NO are vasorelaxant factors with dissimilar mechanisms of action when applied in combination (54), the results of the current study reported that the combination of SNP and $Na_2S$ donors did not significantly alter maximum relaxation compared with the administration of SNP or $Na_2S$ alone. The generation of a novel molecule (perhaps nitrosothiol) via the combination of $H_2S$ and NO does not relax blood vessels *in vitro* or *in vivo* (65). Consequently, the formation of this unique molecule most likely signifies an approach to biological inactivation or possibly sequestration of released NO (66). In contrast, rat aortic relaxation was prolonged when the gas donors were combined (67). This synergistic action may be due to the production of S-nitrosothiol (HSNO) and nitroxyl (HNO) as the result of a chemical reaction between $H_2S$ and nitrite (68), which releases NO and polysulfides, and relaxes VSMCs through soluble guanylyl cyclase activation (69).

Simultaneously, pre-incubation of L-NAME did not alter $Na_2S$-induced relaxation. Similar results have been reported by (70,71). These authors demonstrated that L-NAME did not modify the $Na_2S$-induced relaxation in isolated porcine irides. This conclusion indicated that endogenous NO does not have an impact on the vasoactivity of the $H_2S$ donor. In contrast, pre-incubation of the arterial rings of patients with CRC with PAG did increase the relaxation activity induced by the NO donor. The justification for this reaction is associated with the activity of endogenous $H_2S$ in inhibiting the action of NO. In a

similar manner, SNP-induced vasorelaxation in rat aortas and human internal mammary arteries was diminished by a low concentration of $H_2S$ through the suppression of NO action or inhibition of NO synthase (56,64).

In 2015, Kashfi *et al* (72) investigated the effect of a NO- and $H_2S$-releasing hybrid on the growth properties of various (HCT116 and NCM356) CRC cell lines. Additionally, in 2017, Oláh *et al* (24) explored the expression of NO- and $H_2S$-generating enzymes in primary CRC tissues and the HCT116 CRC cell line. A limitation of the current study is that only the vasodilatory activity of NO and $H_2S$ donors in the human mesenteric artery of patients with CRC was investigated. Therefore, future studies should focus on assessing the molecular signaling pathways in CRC tissues and cell lines to provide a more comprehensive model of the expression patterns of NO and $H_2S$ enzymes.

In conclusion, low endocan and normal MDA levels in patients with CRC revealed that endothelial dysfunction and oxidative stress were not involved in the pathogenesis of CRC. Furthermore, the mechanism of NO and $H_2S$-induced mesenteric artery vasodilation was time- and $K^+$ channel-dependent, as NO dilates mesenteric arteries via the activation of $K_{ATP}$, $K_{Ca}$, $K_{IR}$ and $K_V$ channels, while the vasodilation activity of $H_2S$ is due to the modulation of $K_{ATP}$ and $K_V$ channels. Additionally, NO and $H_2S$ interacted at the enzyme level and the activity of the CSE enzyme inhibited the ability of exogenous NO in the vasodilatation process.

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**Availability of data and materials**

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

**Authors' contributions**

AYH performed the experiments and co-wrote the manuscript. IMM designed the experiments and co-wrote the paper. ASS designed the experiments, analyzed data and co-wrote the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The present study was authorized and approved by the Human Ethics Committee of Salahaddin University-Erbil. Patients provided written informed consent.

**Patient consent for publication**

All patients provided written informed consent for the publication of data in the current study.

**Competing interests**

The authors declare that they have no competing interests.

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