Original Research

Colonic hydrogen sulfide produces portal hypertension and systemic hypotension in rats

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Impact statement

Accumulating evidence suggests that gut-derived molecules affect the control of the circulatory system. Mechanisms controlling liver circulation have been profoundly studied; however, the effects of gut bacteria-derived molecules on portal blood pressure have not been established. In the colon, hydrogen sulfide is produced by intestinal tissues and gut sulfur bacteria. We found that colon-administered hydrogen sulfide increases portal blood pressure while decreasing the systemic arterial blood pressure. The hemodynamic response to hydrogen sulfide was more pronounced in cirrhotic rats which showed reduced hydrogen sulfide liver metabolism, i.e. lower expression of rhodanese, an enzyme converting hydrogen sulfide to sulfate. We propose that colon-derived hydrogen sulfide may affect the regulation of portal and arterial blood pressures and may be involved in portal hypertension.

Abstract

Hydrogen sulfide, a toxic gas, at low concentrations is also a biological mediator in animals. In the colon, hydrogen sulfide is produced by intestinal tissues and gut sulfur bacteria. Gut-derived molecules undergo liver metabolism. Portal hypertension is one of the most common complications contributing to the high mortality in liver cirrhosis. We hypothesized that the colon-derived hydrogen sulfide may affect portal blood pressure. Sprague-Dawley rats were maintained either on tap water (controls) or on water solution of thioacetamide to produce liver cirrhosis (CRH-R). Hemodynamics were measured after administration of either saline or Na_2S, a hydrogen sulfide donor, into (1) the colon, (2) the portal vein, or (3) the femoral vein. Expression of enzymes involved in hydrogen sulfide metabolism was measured by RT-PCR. CRH-R showed a significantly higher portal blood pressure but a lower arterial blood pressure than controls. Saline did not affect hemodynamic parameters. In controls, intracolonic hydrogen sulfide decreased arterial blood pressure and portal blood flow but increased portal blood pressure. Similarly, hydrogen sulfide administered into the portal vein decreased arterial blood pressure but increased portal blood pressure. In contrast, hydrogen sulfide administered into the systemic vein decreased both arterial and portal blood pressures. CRH-R showed significantly greater responses to hydrogen sulfide than controls. CRH-R had a significantly higher liver concentration of hydrogen sulfide but lower expression of rhodanese, an enzyme converting hydrogen sulfide to sulfate. In conclusion, colon-administered hydrogen sulfide increases portal blood pressure while decreasing the systemic arterial blood pressure. The response to hydrogen sulfide is more pronounced in cirrhotic rats which show reduced hydrogen sulfide liver metabolism. Therefore, colon-derived hydrogen sulfide may be involved in the regulation of portal blood pressure, and may contribute to portal hypertension.

Keywords: Hydrogen sulfide, gut-derived mediators, arterial blood pressure, portal blood pressure, liver

***Experimental Biology and Medicine* 2018; 243: 96–106. DOI: 10.1177/1535370217741869**

Introduction

Hydrogen sulfide (H_2S) has been known as a toxic gas in medical literature for three centuries. Namely, in 1713, in his book entitled *De Morbis Articum Diatriba*, Bernardino Ramazzini described the toxic effects of the gas in victims of the occupational exposure.[1] However, accumulating evidence suggests that H_2S is also an important biological mediator in the circulatory system.[2,3] Thus far, the research has been focused mainly on the cardiovascular effects of H_2S produced enzymatically by vasculature, the kidneys, and the heart. Interestingly, recent evidence indicates that the circulatory system may also be influenced by

colon-derived H_2S.[4,5] In fact, colon may be the largest pool of H_2S in mammals. This is because H_2S and its derivatives are produced by both intestinal tissue and gut sulfur bacteria.[6,7]

Blood collected from intestines transports numerous nutrients and gut bacteria products via the portal vein to the liver. Although the mechanisms controlling liver circulation have been profoundly studied, the effects of gut bacteria-derived molecules on portal blood pressure have not been established. We hypothesized that the colon-derived H_2S may affect arterial blood pressure (ABP) and portal blood pressure. Since portal hypertension is one of the most common complications contributing to the high mortality rate in liver cirrhotic patients, we investigated the effects of the colon-derived H_2S in healthy and in cirrhotic animals.

Materials and methods

Ethical approval, animals, and model of liver cirrhosis

The experiments were performed according to Directive 2010/63 EU on the protection of animals used for scientific purposes and approved by the I Local Bioethical Committee in Warsaw (permission: 103/2016). Rats were housed in groups (3-4 animals) in polypropylene cages with environmental enrichment, 12-h light/12-h dark cycle, temperature 22–23°C, humidity 45–55%, food and water *ad libitum*.

Measurements were performed on 28-week-old, male, Sprague Dawley rats ($n = 69$, healthy rats) and Sprague Dawley rats with liver cirrhosis ($n = 54$, cirrhotic rats) fed a standard laboratory diet *ad libitum*. Liver cirrhosis was induced by administering thioacetamide in a drinking water 0.3 g/L for 18 weeks, as previously described.[8,9] Cirrhosis was confirmed by histopathological examination with hematoxylin and eosin staining, using a standard light microscope Olympus BX41 and CellSens software (Olympus Corporation, Tokyo, Japan).

Surgical preparation

Rats were anaesthetized with urethane 1.5 g/kg of bw i.p. First, an arterial catheter was implanted into the aorta via the femoral artery for ABP measurements (all the experimental series). Next, a venous catheter was implanted into the femoral vein for peripheral infusions (series evaluating hemodynamic effects of H_2S administration into peripheral vein). Next, the portal vein was catheterized for portal blood pressure (PBP) measurements (all the experimental series). Specifically, skin and muscles were cut from the xiphoid to the navel. The portal vein and the superior mesenteric vein were dissected from adjacent tissue and stabilized with ligatures. A polyurethane T-shape catheter (ID 0.6 mm, OD 1 mm) with the tip made of a 27 G, 0.5 cm long, blunt needle was inserted into the portal vein via the superior mesenteric vein. The extravascular part of the catheter was secured to the mesentery with a single surgical knot and an adhesive glue. Subsequently, the abdominal cavity was closed. The hemodynamic measurements were performed with the Biopac MP 150 recording system (Biopac Systems, Goleta, USA).

Hemodynamic measurements

The measurements started 60 min after the induction of anesthesia.

Hemodynamic effects of H_2S administration into the colon. Intracolonic infusions were performed by means of a flexible catheter inserted into the colon, 8 cm from the anus.

A pilot dose-response study. In healthy rats, ABP and PBP were recorded for 15 min at baseline and 20 min after intracolonic infusions of Na_2S at the following doses: 0.08 mmol Na_2S/kg/b.w. ($n = 7$), 0.8 mmol Na_2S/kg/b.w. ($n = 7$), 2.4 mmol Na_2S/kg/b.w. ($n = 7$).

The effect of intracolonic infusions on portal blood flow and ABP. The experiments were performed in healthy rats ($n = 7$). Measurements of portal vein blood flow (PBF) were conducted using the transit time ultrasound set-up (T106, Transonic System Inc., Ithaca, NY, USA). PBF and ABP were measured at baseline, for 30 min after intracolonic administration of 0.5 mL of saline, and 30 min after intracolonic administration of Na_2S at a dose of 2.4 mmol/kg/b.w.

Hemodynamic effects of H_2S administration into the colon in healthy and cirrhotic rats. In healthy rats, ABP and PBP were recorded for 15 min at baseline and 70 min after the intracolonic infusions of either a vehicle (0.5 mL of 0.9% saline) or 0.8 mmol Na_2S/kg/b.w. ($n = 6$) or 2.4 mmol Na_2S/kg/b.w. ($n = 7$). The same experimental series were performed in cirrhotic rats, i.e. vehicle infusion ($n = 6$), 0.8 mmol Na_2S/kg/b.w. ($n = 6$), and 2.4 mmol of Na_2S/kg/b.w. ($n = 6$).

Hemodynamic effects of H_2S administration into the portal vein. ABP and PBP were recorded for 15 min at baseline and 45 min after the treatment. Infusions were performed by the T-shape catheter inserted into the portal vein. Healthy rats were treated with either a vehicle (0.2 mL of 0.9% saline) or 0.04 mmol Na_2S/kg/b.w. ($n = 6$) or 0.4 mmol Na_2S/kg/b.w. ($n = 6$). The same experimental series were performed in cirrhotic rats, i.e. vehicle infusion ($n = 6$), or 0.04 mmol Na_2S/kg/b.w. ($n = 6$) or 0.4 mmol Na_2S/kg/b.w. ($n = 6$).

Hemodynamic effects of H_2S administration into the peripheral vein. Infusions were performed by means of the catheter inserted into the femoral vein. ABP and PBP were recorded for 15 min at baseline and 30 min after the treatment. Healthy rats were treated with either a vehicle (0.2 mL of 0.9% saline) or 0.04 mmol Na_2S/kg/b.w. ($n = 6$) or 0.4 mmol Na_2S/kg/b.w. ($n = 6$). The same experimental series were performed in cirrhotic rats, i.e. vehicle infusion ($n = 6$), or 0.04 mmol of Na_2S/kg/b.w. ($n = 6$) or 0.4 mmol of Na_2S/kg/b.w. ($n = 6$).

Determination of the H_2S level in liver homogenates

The frozen liver samples were homogenized at a ratio of 1/10 with ice-cold 50 mM phosphate buffer pH 8.0 for 1 min at 8000–9500 r/min using a blender homogenizer. H_2S level was determined in homogenates after 1 h of incubation with L-cysteine, using the method described in Kartha *et al.*[10] with modifications described by Bronowicka-Adamska *et al.*[11] Methylene blue was spectrophotometrically detected at 670 nm. The standard curve was linear at the concentration range of 0–250 μM with a correlation coefficient of 0.994.

RT-PCR

Isolation of total RNA. Total RNA was extracted from the liver using TRIzol reagent (Invitrogen, CA, USA) following the manufacturer's instructions. RNA concentration was estimated by measuring the absorbance at 260 nm. Isolated RNA was characterized for quantity and integrity and was stored at −80°C for RT-PCR analysis.

Reverse transcription of RNA. Reaction volume was 20 μL and components were used including 3 μg of total RNA, Oligo d(T) primer 0.5 μg/μl (1 μl; Thermo Scientific), GoScript™ 5× Reaction buffer (4 μl, Promega Corporation), $MgCl_2$ (3 μl), GoScript™ Reverse Transcriptase 160 U/μl (1 μl, Promega Corporation), RNase Inhibitor 20 U/μl (1 μl, Thermo Scientific) and dNTP mix 10 mM (1 μL, Thermo Scientific). RNA was mixed with Oligo d(T) primer and was heated for 5 min at 70°C. Then, samples were incubated in the reaction mixture for 5 min at 25°C, 60 min at 42°C, and 15 min at 70°C.

Polymerase chain reaction. PCR was performed using 2 μl of cDNA, 0.2 μM of each primer, 0.04 U/μl of DNA polymerase (Thermo Scientific) in 10 mM buffer Tris–HCl pH 8.8 (supplemented with 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100), 0.2 mM of dNTP mix (Thermo Scientific), and H_2O-DEPC in total reaction volume of 25 μl. Primer sequences were: 5′CTCTATCGAGCCGCTG GTCTC3′ (sense) and 5′TCGTAAGGCGAAGTCGTGTC3′ (antisense) for TST, 200bp; 5′TTTGTATACAGCCGC TCTGGA3′ (sense) and 5′ACAAGCTTGGTCTGTGGTG T3′ (antisense) for CTH, 290bp; 5′CTGTGAAGGGCTA TCGCTGC3′(sense) and 5′CTGGCATTGCGGTACTGG TC3′ (antisense) for CBS, 205bp; 5′TCCTGGGTGGAGTG GTACAT3′ (sense) and 5′GTGAAACAAGCTAGGT GGGC3′ (antisense) for MPST, 339bp; 5′ACCCGCGAGT ACAACCTTCTT3′ (sense) and 5′GCCGTGTTCAATGG GGTACT3′ (antisense) for β-actin, 285bp.

PCR products were analyzed by electrophoresis on 2.0% agarose gel stained with ethidium bromide, visualized under ultraviolet light, and analyzed by densitometric analysis using the system of documentation and computer analysis UVI-KS 4000i/ImagePC (Syngen Biotech).

Compounds

Urethane, Na_2S, L-cysteine, pyridoxal phosphate (PLP), sodium dihydrogen phosphate dihydrate pure, sodium sulfite, agarose, and N,N-dimethyl-p-phenylenediamine sulfate salt were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ferric chloride, zinc acetate dehydrate pure, trichloroacetic acid (TCA), and sodium hydroxide were from Polskie Odczynniki Chemiczne S.A. (Gliwice, Poland).

Statistics

Mean ABP and PBP were calculated on the blood pressure tracing by AcqKnowledge 4.3.1 (Biopac Systems, Goleta, USA). For the evaluation of ABP, PBP, and PBF response to the treatment, the average over 1 min baseline was compared with the averages over 1 min after the treatment by means of one-way analysis of variance (ANOVA) for repeated measures. Differences between the groups were evaluated by multivariate ANOVA, followed by Tukey's *post hoc* test or *t*-test, when appropriate. The Kolmogorov–Smirnov test was used to test normality of the distribution. A value of two-sided $P < 0.05$ was considered significant. Means ± SE are presented. Analyses were conducted using STATISTICA 12.0 (Stat Soft, Krakow, Poland).

Results

Hemodynamic and histological characterization of healthy and cirrhotic rats

At baseline, PBP was significantly higher in cirrhotic rats (11.1 ± 0.2, $n = 30$) than in healthy rats (6.0 ± 0.3, $n = 30$), $P < 0.05$. In contrast, mean ABP was significantly lower in cirrhotic rats (84.9 ± 1.9, $n = 30$) than in healthy rats (101.3 ± 2.0, $n = 30$), $P < 0.05$.

Histopathological examination showed no pathological changes in livers of rats that drank tap water (healthy rats). In rats treated with thioacetamide the following pathological findings were present: large hepatic nodules separated by bands of connective tissue, hyperplasia of connective tissue (around portal fields and on the periphery of nodules), and extension of central veins and portal venules. In the parenchyma, hepatocytes with degenerative changes dominated. The changes can be classified as an incomplete septal cirrhosis or early cirrhosis, with the features of macronodular cirrhosis (Figure 1).

There was no significant difference between healthy and cirrhotic rats in liver test, underlining the chronic cirrhotic process without decompensated liver failure. Healthy rats ($n = 12$): AST(U/L) 97.5 ± 11.3, ALT(U/L) 60.7 ± 3.6, total protein (g/dL) 5.27 ± 0.07, albumins (g/dL) 3.65 ± 0.04; and Cirrhotic rats ($n = 12$): AST(U/L) 110.8 ± 12.7, ALT (U/L) 64.8 ± 3.4, total protein (g/dL) 5.31 ± 0.08, albumins (g/dL) 3.76 ± 0.07.

Hemodynamic effects of H_2S administration into the colon

A pilot dose-response study. In healthy rats, intracolonic administration of Na_2S produced a significant increase in PBP in all the series, i.e. 0.08 mmol/kg/b.w. ($F_{2,12} = 18.84$, $P < 0.05$), 0.8 mmol/kg/b.w. ($F_{2,12} = 4.69$, $P < 0.05$), and 2.4 mmol/kg/b.w. ($F_{2,12} = 6.61$, $P < 0.05$), which was followed by a decrease in PBP (Figure 2(a)). The changes in PBP were

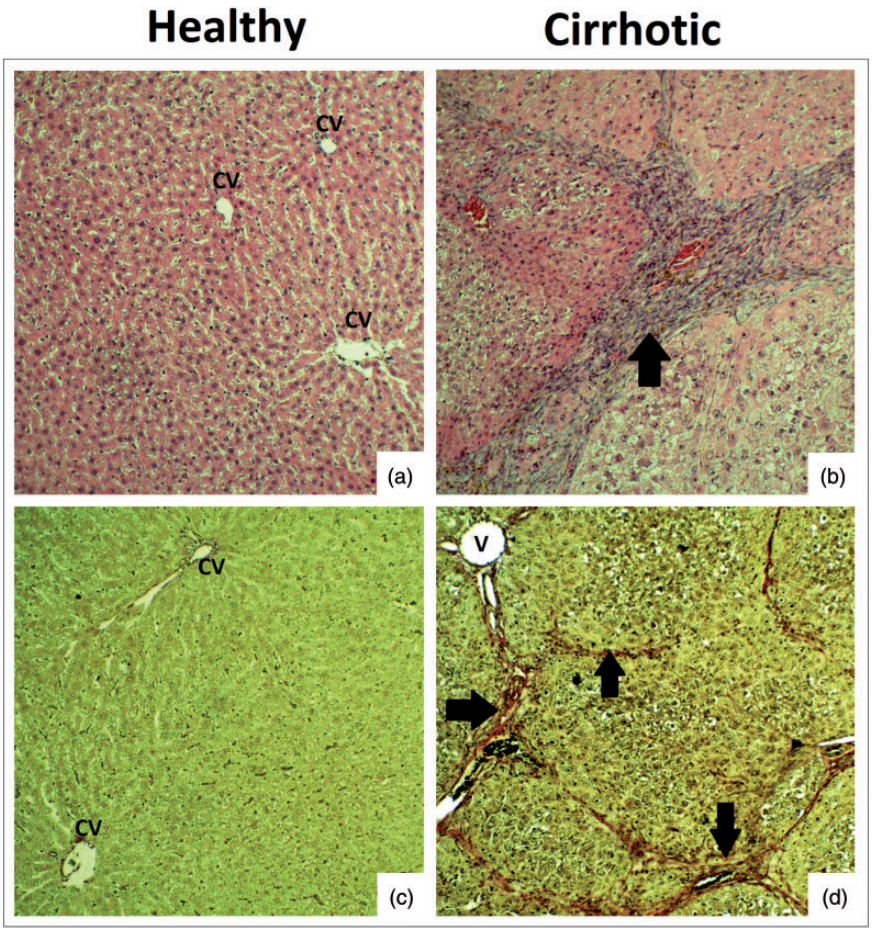

Figure 1. Histopathology of the liver in healthy and cirrhotic rats. (a, b) Liver parenchyma with hematoxylin and eosin (×10 objective lens). (c, d) Fibrosis of liver parenchyma with van Gieson stain (×10 objective lens). CV: central veins of liver; V: extended lumen of portal venules. Arrows – Hyperplasia of connective tissue in the liver parenchyma. (A color version of this figure is available in the online journal.)

accompanied by a significant decrease in ABP, i.e. 0.08 mmol/kg/b.w. ($F_{6,36} = 4.17$, $P < 0.05$), 0.8 mmol/kg/b.w. ($F_{6,36} = 10.66$, $P < 0.05$), and 2.4 mmol/kg/b.w. ($F_{6,36} = 48.40$, $P < 0.05$) (Figure 2(b)).

Changes in PBF and ABP. Intracolonic administration of the vehicle did not affect PBF and ABP. In contrast, Na_2S at a dose of 2.4 mmol/kg/b.w. produced a significant decrease in PBF ($F_{5,30} = 5.23$, $P < 0.05$) and ABP ($F_{5,30} = 12.92$, $P < 0.05$) (Figure 3).

Hemodynamic effects of H_2S administration into the colon in healthy and cirrhotic rats

Changes in PBP. Administration of the vehicle did not produce a significant change in PBP.

In healthy rats, Na_2S at a dose of 0.8 mmol/kg/b.w. ($F_{3,15} = 10.37$, $P < 0.05$) and 2.4 mmol/kg/b.w. ($F_{3,18} = 3.91$, $P < 0.05$) produced a significant increase in PBP (Figure 4 (a)). There were significant differences between the vehicle and 0.8 mmol/kg/b.w. and 2.4 mmol/kg/b.w. series ($F_{2,16} = 11.43$, $P < 0.05$).

Cirrhotic rats showed a significant increase in PBP after administration of Na_2S at a dose of 0.8 mmol/kg/b.w. ($F_{4,20} = 8.61$, $P < 0.05$), and 2.4 mmol/kg/b.w. ($F_{4,20} = 26.72$, $P < 0.05$) (Figure 4(b)). In the latter series, all rats died due to significant hypotension. There were significant differences between the vehicle and 0.8 mmol/kg/b.w. and 2.4 mmol/kg/b.w. series ($F_{2,15} = 6.61$, $P < 0.05$).

Cirrhotic group treated with Na_2S at a dose of 2.4 mmol/kg/b.w. showed a significantly higher increase in PBP than the healthy group treated with Na_2S at the same dose ($F_{1,11} = 7.27$, $P < 0.05$) (Figure 4(a) and (b)).

Changes in ABP. Administration of the vehicle did not affect ABP.

In healthy rats, Na_2S at a dose of 0.8 mmol/kg/b.w. ($F_{9,45} = 10.48$, $P < 0.05$) and 2.4 mmol/kg/b.w. ($F_{9,54} = 24.90$, $P < 0.05$) produced a significant decrease in ABP (Figure 4(c)). There were significant differences between the vehicle and 0.8 mmol/kg/b.w. and 2.4 mmol/kg/b.w. series ($F_{2,16} = 15.68$, $P < 0.05$).

Cirrhotic rats exhibited a significant decrease in ABP after administration of Na_2S at a dose of 0.8 mmol/kg/b.w. ($F_{9,45} = 10.48$, $P < 0.05$) and 2.4 mmol/kg/b.w. ($F_{9,45} = 27.51$, $P < 0.05$) (Figure 4(d)). In the latter series, all rats died due to significant hypotension. There were significant differences between the vehicle and 0.8 mmol/kg/b.w. and 2.4 mmol/kg/b.w. series ($F_{2,16} = 19.34$, $P < 0.05$).

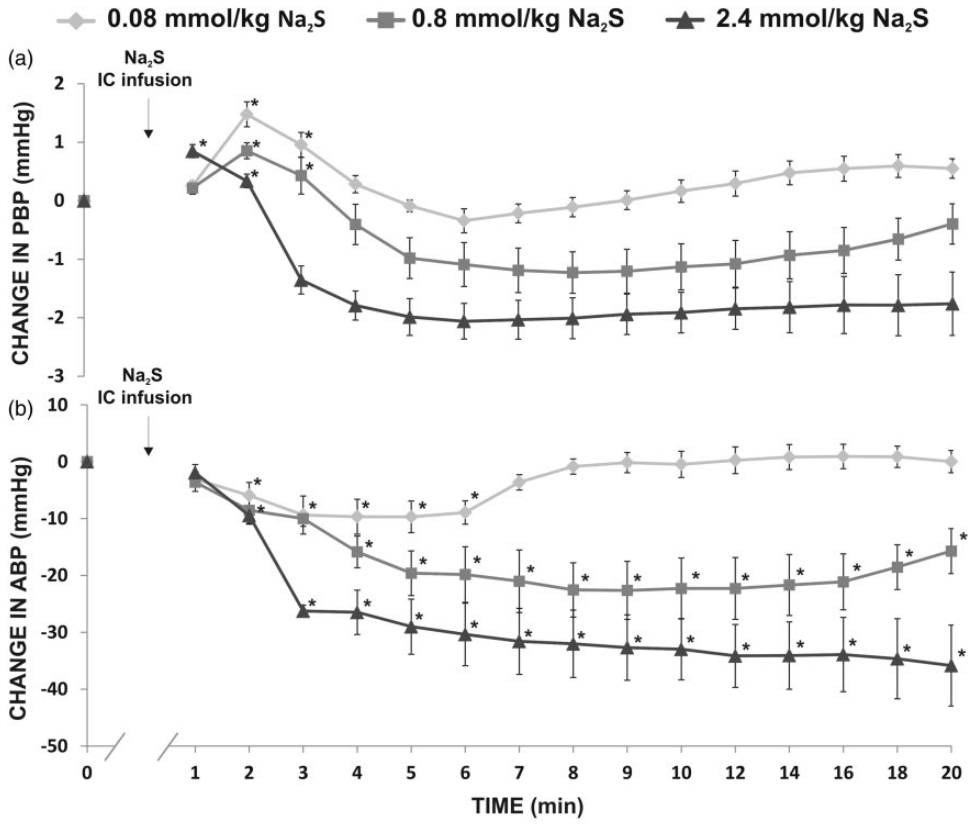

Figure 2. Changes in (a) portal blood pressure (PBP, mmHg), and (b) mean arterial blood pressure (ABP, mmHg) after intracolonic (IC) infusion of Na_2S, a fast-releasing H_2S donor in healthy rats ($n = 7$).
*$P<0.05$ – vs. baseline.

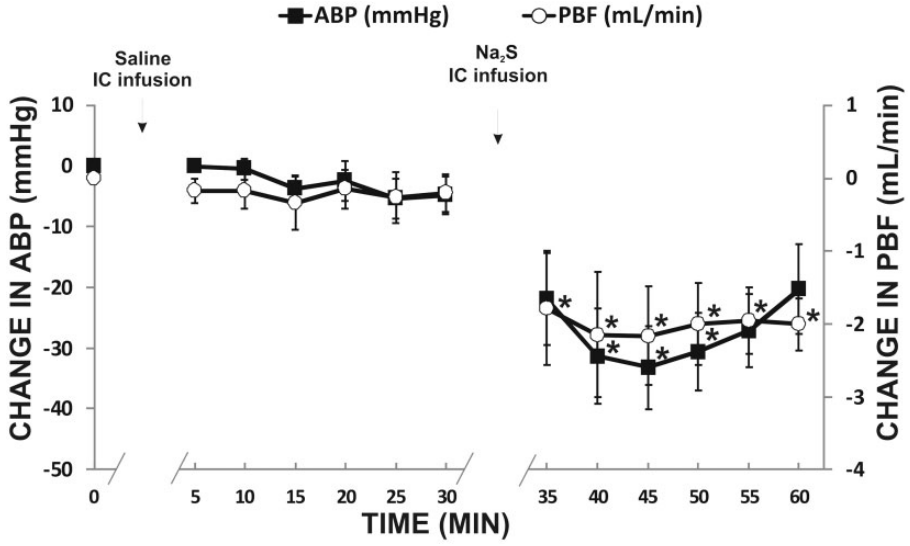

Figure 3. Changes in portal vein blood flow (PBF, mL/min) and mean arterial blood pressure (ABP, mmHg) after the intracolonic (IC) infusion of the vehicle and Na_2S, a fast-releasing H_2S donor, in rats ($n = 7$).
*$P<0.05$ – vs. baseline.

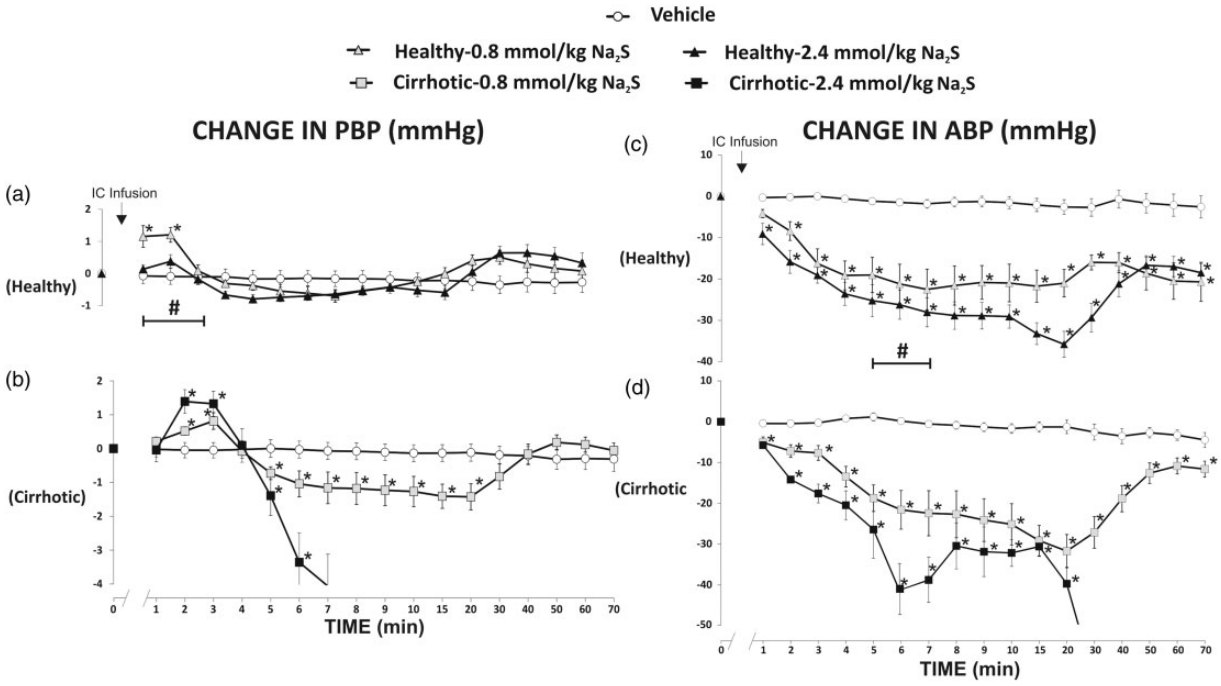

Figure 4. Changes in portal blood pressure (PBP, mmHg) and mean arterial blood pressure (ABP, mmHg) after the intracolonic (IC) infusion of either saline (vehicle) or Na_2S, a fast-releasing H_2S donor.
Healthy rats: Na_2S 0.8 mmol/kg/b.w. (n=6); Na_2S 2.4 mmol/kg/b.w. (n=7), cirrhotic rats: Na_2S 0.8 mmol/kg/b.w. (n=6); Na_2S 2.4 mmol/kg/b.w. (n=6).
*$P<0.05$ – vs. baseline. $^{\#}P<0.05$ – Healthy – 2.4 mmol/kg/b.w. of Na_2S series vs. Cirrhotic – 2.4 mmol/kg/b.w. of Na_2S series.

Cirrhotic rats treated with Na_2S at a dose of 2.4 mmol/kg/b.w. showed a significantly greater decrease in ABP than healthy rats ($F_{1,11} = 6.87$, $P < 0.05$) (Figure 4(c) and (d)).

Hemodynamic effects of H_2S administration into the portal vein

Changes in PBP. Administration of the vehicle did not produce a significant change in PBP.

In healthy rats, Na_2S at a dose of 0.04 mmol/kg/b.w. produced a not significant increase in PBP, whereas Na_2S at a dose of 0.4 mmol/kg/b.w. produced a significant increase in PBP ($F_{7,35} = 29.99$, $P < 0.05$) (Figure 5(a)). There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10}$ =16.77, $P < 0.05$).

In cirrhotic rats, Na_2S at a dose of 0.04 mmol/kg/b.w. caused a significant increase in PBP ($F_{7,35} = 13.27$, $P < 0.05$). Administration of Na_2S at a dose of 0.4 mmol/kg/b.w. resulted in a biphasic response, i.e. a transient decrease in PBP followed by a significant increase in PBP ($F_{7,35} = 63.94$, $P < 0.05$) (Figure 5(b)). There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10} = 59.70$, $P < 0.05$).

Cirrhotic rats showed significantly greater increase in PBP than healthy rats after administration of Na_2S at a dose of 0.4 mmol/kg/b.w. ($F_{1,10} = 6.33$, $P < 0.05$) (Figure 5 (a) and (b)).

Changes in mean ABP. Administration of the vehicle did not affect ABP.

In healthy rats, Na_2S at a dose of 0.04 mmol/kg/b.w. ($F_{5,25} = 4.18$, $P < 0.05$) and 0.4 mmol/kg/b.w. ($F_{3,15} = 26.04$, $P < 0.05$) produced a significant decrease in ABP (Figure 5 (c)). There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10} = 22.46$, $P < 0.05$).

In cirrhotic rats, there was a significant decrease in ABP after administration of Na_2S at a dose of 0.04 mmol/kg/b.w. ($F_{5,25} = 7.15$, $P < 0.05$) and 0.4 mmol/kg/b.w. ($F_{5,25} = 29.16$, $P < 0.05$) (Figure 5(d)). There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10} = 66.92$, $P < 0.05$).

Cirrhotic rats showed significantly greater decrease in ABP than healthy rats in 0.4 mmol/kg/b.w. series ($F_{1,10} = 5.15$ $P < 0.05$) (Figure 5(c) and (d)).

Hemodynamic effects of H_2S administration into the peripheral vein

Changes in PBP. Administration of the vehicle did not affect PBP.

In healthy rats, Na_2S at a dose of 0.04 mmol/kg/b.w. ($F_{2,10} = 3.83$, $P < 0.05$) and 0.4 mmol/kg/b.w. ($F_{5,25} = 27.73$, $P < 0.05$) produced a significant decrease in PBP (Figure 6 (a)). In the latter series, all rats died due to a significant hypotension after 15 min. There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10} = 38.65$, $P < 0.05$).

In cirrhotic rats, there was a significant decrease in PBP after administration of Na_2S at a dose of 0.04 mmol/kg/b.w. ($F_{2,10} = 4.77$, $P < 0.05$) and 0.4 mmol/kg/b.w. ($F_{5,25} = 28.46$, $P < 0.05$) and in the latter series all rats died due to a significant hypotension after 10 min (Figure 6(b)).

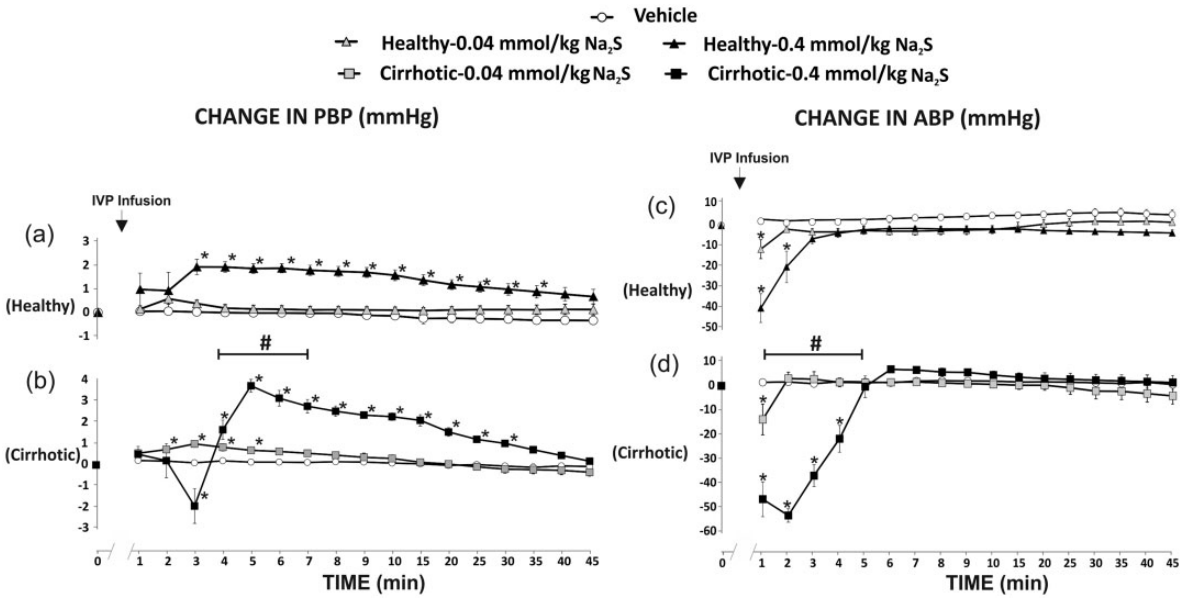

Figure 5. Changes in portal blood pressure (PBP, mmHg) and mean arterial blood pressure (ABP, mmHg) after the administration of either saline (vehicle) or Na_2S, a fast-releasing H_2S donor, into the portal vein (IVP).
Healthy rats: Na_2S 0.8 mmol/kg/b.w. (n=6); Na_2S 2.4 mmol/kg/b.w. (n=6), cirrhotic rats: Na_2S 0.8 mmol/kg/b.w. (n=6); Na_2S 2.4 mmol/kg/b.w. (n=6).
*$P<0.05$ – vs. baseline. #$P<0.05$ – Healthy – 0.4 mmol/kg/b.w. of Na_2S series vs. Cirrhotic – 0.4 mmol/kg/b.w. of Na_2S series.

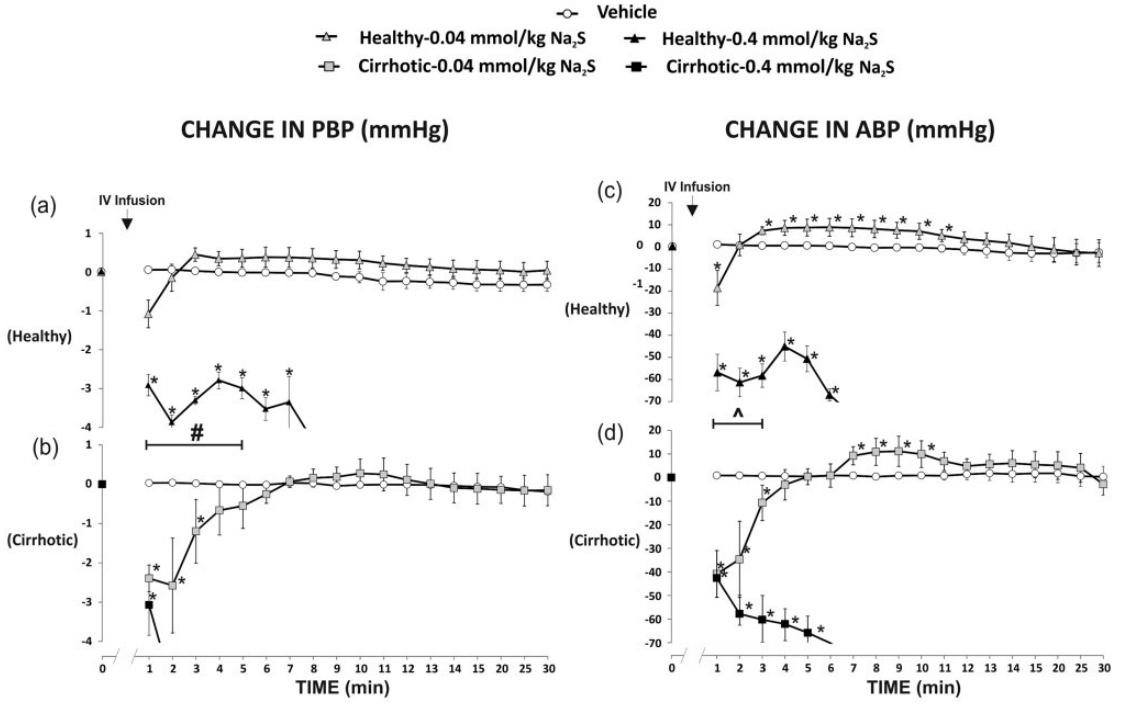

Figure 6. Changes in portal blood pressure (PBP, mmHg) and mean arterial blood pressure (ABP, mmHg) after the administration of either saline (vehicle) or Na_2S, a fast-releasing H_2S donor, into the femoral vein (IV).
Healthy rats: Na_2S 0.8 mmol/kg/b.w. (n=6); Na_2S 2.4 mmol/kg/b.w. (n=6), cirrhotic rats Na_2S 0.8 mmol/kg/b.w. (n=6); Na_2S 2.4 mmol/kg/b.w. (n=6).
*$P<0.05$ – vs. baseline. #$P< 0.05$ – Healthy – 0.4 mmol/kg/b.w. of Na_2S series vs. Cirrhotic – 0.4 mmol/kg/b.w. of Na_2S series. ^$P<0.05$ – Healthy – 0.04 mmol/kg/b.w. of Na_2S series vs. Cirrhotic – 0.04 mmol/kg/b.w. of Na_2S series.

There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10} = 42.03$, $P < 0.05$).

Namely, cirrhotic rats showed a significantly greater decrease in PBP than healthy rats ($F_{1,10} = 7.18$, $P < 0.05$) in 0.4 mmol/kg/b.w. series (Figure 6(a) and (b)).

Changes in ABP. Administration of the vehicle did not affect ABP.

In healthy rats, Na_2S at a dose of 0.04 mmol/kg/b.w. ($F_{3,15} = 3.92$, $P < 0.05$) and 0.4 mmol/kg/b.w. ($F_{5,25} = 19.61$, $P < 0.05$) produced a significant decrease in ABP

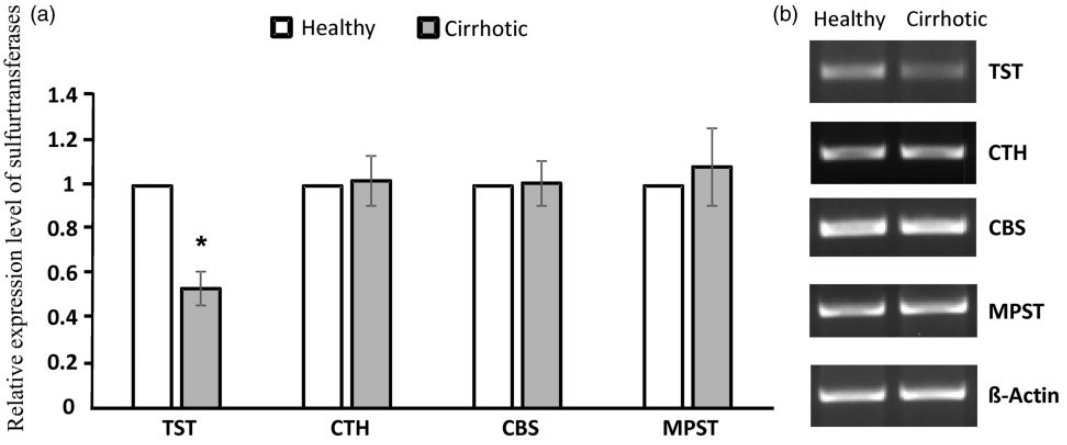

Figure 7. Relative expression level of sulfurtransferases in rat liver in healthy group and cirrhotic group. TST: rhodanese; CTH: γ-cystathionase; CBS: cystathionine-β-synthase; MPST: 3-mercaptopyruvate sulfurtransferase.
(a) Densities of bands were normalized using β-actin. Each point represents the mean $\pm$ SE of three independent experiments; healthy rats (n=7); cirrhotic rats (n=7), *$P < 0.05$ – healthy vs. cirrhotic rats. (b) Representative results of RT-PCR analysis for sulfurtransferases.

(Figure 6(c)). There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10} = 27.66$, $P < 0.05$).

Cirrhotic rats showed a significant decrease in ABP after administration of Na_2S at a dose of 0.04 mmol/kg/b.w. ($F_{3,15} = 4.79$, $P < 0.05$) and 0.4 mmol/kg/b.w. ($F_{5,25} = 19.55$, $P < 0.05$) (Figure 6(d)). There were significant differences between the vehicle and 0.4 mmol/kg/b.w. series ($F_{1,10} = 30.06$, $P < 0.05$).

The between-group analysis revealed a significant difference between healthy and cirrhotic groups after administration of Na_2S at a dose of 0.4 mmol/kg/b.w. Namely, cirrhotic rats that received Na_2S at a dose of 0.04 mmol/kg/b.w. showed a significantly greater decrease in ABP than healthy rats ($F_{1,10} = 5.19$, $P < 0.05$) (Figure 6(c) and (d)).

H_2S generation

Livers of cirrhotic rats showed a significantly higher concentration of H_2S after 1 h of incubation with L-cysteine (17.8 ± 1.6 nmol H_2S/g of tissue, $n = 14$) than livers of healthy rats (10.4 ± 0.9 nmol H_2S/g of tissue, $n = 15$).

Expression of sulfurtransferases

In cirrhotic rats, the expression of TST in liver was decreased twofold as compared to the healthy group. We did not observe any statistically significant differences in the expression of mRNA of CTH, CBS, and MPST genes between healthy and cirrhotic rats (Figure 7).

Discussion

A new finding of our study is that colon-administered H_2S increases PBP while decreasing systemic ABP in rats. Furthermore, we found that cirrhotic rats show an augmented hemodynamic response to H_2S, which is associated with a significantly lower expression of rhodanese, an enzyme converting H_2S to sulfate.

H_2S has been found to play an important role in the control of ABP[12]; however, the mechanisms are not clear. This is in part because H_2S may exert its biological effects via its derivatives such as S-nitrosothiols, thiosulfates as well as interacting with nitric oxide and carbon monoxide.[13,14] Interestingly, similarly to epinephrine, H_2S may act as a vasoconstrictor or as a vasorelaxant, depending on a type of vascular bed and the dose.[15] In rodents, H_2S donors have been found to produce a significant hypotensive effect, associated with peripheral vasodilatation.[12] Likewise, in the present study Na_2S, the most commonly used H_2S donor administered intravenously lowered ABP.

Hemodynamic studies on H_2S have thus far focused on the effects of H_2S produced enzymatically by blood vessels, the heart, kidneys, or the brain. However, recent studies suggest that the gut-derived H_2S may also contribute to the regulation of systemic ABP.[5] As a matter of fact, large intestine may represent the largest pool of H_2S in the body, since H_2S is produced by both intestinal tissue and gut bacteria.[6,7] Accordingly, the estimated concentration of H_2S in the blood and other tissues has been reported to be within the micromolar[16] or nanomolar range,[17] in contrast to millimolar concentrations in the intestines.[18,19]

Previously, Ebrahimkhani *et al.*,[20] hypothesized that H_2S may be responsible for changes in the portal circulation. However, to the best of our knowledge, the effect of colon-derived H_2S on PBP has not been studied.

Recently, we have found that colonic administration of H_2S decreases systemic ABP, which is associated with an increased portal blood levels of thiosulfate and sulfane sulfur, products of H_2S oxidation.[5] Before entering the systemic circulation, blood collected from the colon passes the liver. Therefore, in the present study, we evaluated if colon-derived H_2S may affect the liver circulation. In particular, we assessed the effect of H_2S on concomitant changes in PBP and systemic ABP, a major factor influencing PBP by changing intestinal blood flow.

Portal blood pressure depends on blood volume flowing from portal vein and hepatic artery, and on the resistance offered by the terminal portal venules, the sinusoids, and the roots of hepatic venules (liver resistance). The liver does

not control PBF, which is simply the outflow from intestines, spleen, and pancreas. However, changes in PBF produce compensatory response from hepatic artery, a mechanism known as hepatic arterial buffer response. Interestingly, it has been suggested that this mechanism may also involve H_2S signaling.[21]

In the present study, we have found that intracolonic administration of H_2S increases PBP, which is accompanied by a drop in systemic ABP. Furthermore, we have found that rats infused with H_2S into the colon showed a similar pattern of hemodynamic response to rats infused with H_2S directly into the portal vein. Besides, the intracolonic administration of H_2S decreased PBF. These suggest that an increase in PBP after intracolonic administration of H_2S was caused by an increase in the liver resistance. This concept may also be supported by studies by Norris and collaborators, who found the constriction of liver sinusoids after administration of H_2S into the splenic vein.[22]

Interestingly, in our study, the highest PBP was present in rats treated with smaller doses of H_2S which produced a smaller systemic hypotension. Furthermore, PBP was increasing as systemic ABP was returning to baseline values after the H_2S treatment. These data strongly suggest that the H_2S-driven increase in PBP was reduced by the concomitant H_2S-induced systemic hypotension (Figure 8).

In contrast to rats infused with H_2S into the colon or into the portal vein, rats infused with H_2S into the femoral vein showed a significant decrease in both, ABP, and PBP. These findings show that the H_2S-dependent increase in PBP requires a direct delivery of H_2S from the colon. Otherwise, the systemic hypotension produced by H_2S outweighs the pressor effect of H_2S on portal circulation. This is likely due to the hypotension-driven decrease in the liver blood flow and/or too small amount of H_2S reaching portal circulation after systemic administration of the H_2S donor.

Interestingly, there are some studies suggesting that H_2S may decrease PBP. Distrutti *et al.*[23] and Fiorucci *et al.*[24] found that NaHS, another fast-releasing H_2S donor, reversed hepatic vasoconstriction induced by norepinephrine and homocysteine. However, those experiments were performed in a perfused liver model which may not fully reflect physiological conditions, and the effect of NaHS could depend on the increased concentration of noradrenaline and cysteine. Tan *et al.*[25] found that intraperitoneal treatment with NaHS, twice a day reduced portal pressure in CCL_4-induced liver cirrhosis. However, the authors did not evaluate the effect of H_2S on PBP in healthy animals, and did not record ABP. Therefore, a decrease in PBP could be secondary to systemic hypotension. Furthermore, it is of note, that although the authors used NaHS, a fast-releasing H_2S donor, they observed long-term effects (days). This may suggest that their findings were dependent on other than H_2S-dependent effects. In this respect, NaHS similar to Na_2S dissociates rapidly in plasma leading to instant formation of H_2S and a short-lasting increase in plasma level of H_2S and its derivatives.[26] This is reflected in short-term changes in hemodynamic parameters after a single parenteral administration of H_2S donors.[27]

Portal hypertension is a major complication of liver cirrhosis. Cirrhotic patients often present systemic hypotension caused by peripheral vasodilatation, which is mediated by nitric oxide and other vasorelaxant factors[28,29]; however, the mechanisms have not been fully elucidated. Interestingly, in our study, the intracolonic treatment of healthy animals with H_2S produced similar hemodynamic characteristics, i.e. an increased PBP and a decreased systemic ABP. Moreover, the hemodynamic response to H_2S was greater in cirrhotic than in healthy rats.

The greater hemodynamic response to H_2S in cirrhotic rats seems to be caused by a reduced metabolism of H_2S in cirrhotic livers. H_2S is synthetized in the liver by 3-mercapropyruvate sulfurtransferase (MPST), cystathionine β-synthase (CBS), and gamma-cystathionase (CTH).[30] Here, we found that cirrhotic livers showed higher concentration of H_2S after liver homogenate incubation with L-cysteine, a substrate for H_2S synthesis. However, there are no significant difference between healthy and cirrhotic rats in the liver expression of MPST, CBS, and CTH. These data, together with Km values for the enzymes, which were substantially above intracellular cysteine concentrations,[31] suggest that the higher concentration of H_2S in cirrhotic livers was due to the smaller rate of H_2S degradation rather than the higher enzymatic production of H_2S. In this respect, in our study, cirrhotic livers showed downregulated expression of rhodanese which converts H_2S into sulfate, thereby diminishing H_2S level.[32] Rhodanese is an enzyme located in the mitochondria[33] and is susceptible to oxidative stress[34] which accompanies liver injury.[35]

The importance of the liver, and likely rhodanese, in detoxifying or buffering the amount of the gut-derived H_2S entering the systemic circulation was notably evident in rats treated with higher doses of H_2S. Namely, cirrhotic rats developed a significant systemic hypotension leading to death, whereas healthy rats showed only a transient hypotension.

Taking together, our findings imply that the gut-derived H_2S contributes to the regulation of PBP and ABP. Moreover, it may produce harmful hemodynamic effects in liver cirrhosis by augmenting portal hypertension and systemic hypotension.

A limitation of our study is that thioacetamide-induced liver cirrhosis may not reflect the whole spectrum of liver

Figure 8. A proposed interplay between the effects of H_2S on systemic arterial blood pressure (arterial pressure) and portal blood pressure (portal pressure). On the one hand, H_2S increases portal pressure increasing liver resistance. On the other hand, H_2S decreases portal blood pressure indirectly, by lowering systemic arterial pressure and intestinal blood flow.

cirrhosis caused by numerous factors, such viral infections that may differently affect biochemical activity of the liver.[36] However, in our study, histological and hemodynamic characteristics of cirrhotic rats were like those found in liver cirrhosis of various origin in humans.

In conclusion, colon-administered H_2S increases PBP while decreasing systemic ABP in rats. Importantly, the hemodynamic effects are more pronounced in cirrhotic animals which exhibit decreased expression of rhodanese, an enzyme transforming H_2S to other sulfur compound. Therefore, the gut-derived H_2S may contribute to the control of portal and ABPs, and may be involved in the etiology of portal hypertension and systemic hypotension in liver cirrhosis. Further studies on hemodynamic effects of gut-derived molecules, such as H_2S, are needed.

Authors' contributions: The experiments were performed at the Laboratory of Experimental Physiology and Pathophysiology, Laboratory of the Centre for Preclinical Research, Medical University of Warsaw, Poland and the Laboratory of Medical Biochemistry, Jagiellonian University Medical College, Krakow, Poland.

Conception or design of the work – TH, MU. Acquisition, analysis, or interpretation of data for the work; TH, HJ, MW, KJ, MO, MU. Drafting the work – TH, HJ, MW, KJ, MO, MU.

All authors have approved the final version of the manuscript and agreed to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This work was supported by the National Science Centre, Poland grant no. UMO-2016/22/E/NZ5/00647.

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(Received September 15, 2017, Accepted October 23, 2017)