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ORIGINAL ARTICLE

Basic Study

Hydrogen gas and preservation of intestinal stem cells in mesenteric ischemia and reperfusion

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Abstract

BACKGROUND

Patients with mesenteric ischemia frequently suffer from bowel necrosis even after revascularization. Hydrogen gas has showed promising effects for ischemia-reperfusion injury by reducing reactive oxygen species in various animal and clinical studies. We examined intestinal tissue injury by ischemia and reperfusion under continuous initiation of 3% hydrogen gas.

AIM

To clarify the treatment effects and target cells of hydrogen gas for mesenteric ischemia.

METHODS

Three rat groups underwent 60-min mesenteric artery occlusion (ischemia), 60-min reperfusion following 60-min occlusion (reperfusion), or ischemia-reperfusion with the same duration under continuous 3% hydrogen gas inhalation (hydrogen). The distal ileum was harvested. Immunofluorescence staining with caspase-3 and leucine-rich repeat-containing G-protein-coupled 5 (LGR5), a specific marker of intestinal stem cell, was conducted to evaluate the injury location and cell types protected by hydrogen. mRNA expressions of LGR5, olfactomedin 4 (OLFM4), hairy and enhancer of split 1, Jagged 2, and Neurogenic locus notch homolog protein 1 were measured by quantitative polymerase chain reaction. Tissue oxidative stress was analyzed with immunostaining for 8-hydroxy-2'-deoxyguanosine (8-OHdG). Systemic oxidative stress was evaluated by plasma 8-OHdG.

RESULTS

Ischemia damaged the epithelial layer at the tip of the villi, whereas reperfusion induced extensive apoptosis of the cells at the crypt base, which were identified as intestinal stem cells with double immunofluorescence stain. Hydrogen mitigated such apoptosis at the crypt base, and the LGR5 expression of the tissues was higher in the hydrogen group than in the reperfusion group. OLFM4 was also relatively higher in the hydrogen group, whereas other measured RNAs were comparable between the groups. 8-OHdG concentration was high in the reperfusion group, which was reduced by hydrogen, particularly at the crypt base. Serum 8-OHdG concentrations were relatively higher in both reperfusion and hydrogen groups without significance.

CONCLUSION

This study demonstrated that hydrogen gas inhalation preserves intestinal stem cells and mitigates oxidative stress caused by mesenteric ischemia and reperfusion.

Key Words: Hydrogen molecule; Intestinal ischemia; Ischemia-reperfusion injury; Tissue protection; Non-operative management; Leucine-rich repeat-containing G-protein-coupled 5

Core Tip: Distal ileum of rats was observed after 60-min mesenteric artery occlusion (ischemia), 60-min reperfusion following 60-min occlusion (reperfusion), or ischemia-reperfusion with the same duration under continuous 3% hydrogen gas inhalation (hydrogen). Immunofluorescence staining with caspase-3 and leucine-rich repeat-containing G-protein-coupled 5 (LGR5) (a specific marker of intestinal stem cell) identified ischemia damaged the epithelial layer at the tip of the villi, whereas reperfusion induced extensive apoptosis of intestinal stem cells that was mitigated by hydrogen. In addition, quantitative polymerase chain reaction revealed the LGR5 expression of the tissues was higher in rats with hydrogen inhalation than in those with reperfusion injury.

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INTRODUCTION

Mesenteric ischemia is caused by insufficient blood flow in the mesenteric artery to meet the metabolic demand of the visceral organs[1]. Despite the relatively low incidence of mesenteric ischemia, delayed diagnosis caused by vague symptoms sometimes results in devastating complications, such as intestinal necrosis, abdominal sepsis, and mortality[1-3]. While rapid recirculation of mesenteric vessels by endovascular or surgical intervention has been critical, increasing the arterial flow would be the only method of preserving the intestines when hemodynamic instability causes mesenteric ischemia[1,4,5]. Notably, some patients develop bowel necrosis even after revascularization of the mesenteric artery[6].

In the last decades, hydrogen gas (molecular hydrogen) has emerged as an attractive medicinal agent for various diseases, specifically for ischemic diseases that require revascularization[7-9]. Some animal studies have shown that hydrogen had anti-inflammatory effects and reduced the reactive oxygen species (ROS) that are produced during ischemia-reperfusion injury[7,8,10,11]. Hydrogen inhalation was associated with the reduction of necrotic tissues in the animal model of cerebral and myocardial ischemia[7,8]. Moreover, several clinical investigations have reported that hydrogen gas inhalation was beneficial on acute myocardial infarction and out-of-hospital cardiac arrest, which are typical ischemia-reperfusion injuries[12,13].

Furthermore, studies on the safety of hydrogen gas have identified that hydrogen can be supplied to patients using a simple device without any adverse events, suggesting its high feasibility for clinical use [12-14]. Although the physiological mechanisms of the possible therapeutic benefits remain unclear, recent studies have suggested that the antioxidant effects of hydrogen would be introduced by cell signal transduction, preventing cellular apoptosis[15,16]. Another study reported that oxidation-reduction reactions that involve molecular hydrogen occur only with strong fatal ROS rather than with weak or beneficial ROS[14].

Accordingly, as mesenteric ischemia with revascularization is an ischemia-reperfusion injury, we aimed to clarify the favorable effects of hydrogen gas inhalation for mesenteric ischemia as a novel

noninvasive treatment. In this study, we examined the degree of tissue damage in the intestines following ischemia and reperfusion and the tissue-protective effects of continuous initiation of 3% hydrogen gas. Moreover, several cell markers were substantially measured for the differentiation of hydrogen-target cells to elucidate the pathophysiological mechanisms of hydrogen.

MATERIALS AND METHODS

Animals

The protocol used in this study was approved by the Research Council and Animal Care and Use Committee of the Research Institute of Keio University in Tokyo, Japan (approval number 21013-0) and was performed in accordance with the guidelines for the care and use of laboratory animals established by the Japanese Pharmacological Society and the National Institutes of Health.

Eight-week-old male Sprague-Dawley rats (250-270 g) were purchased from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan) and kept in a temperature- and light-controlled room (20 °C, 12-h light/dark cycle). The rats had free access to food and water. Before the procedure, they were intraperitoneally anesthetized with a combination of 0.3 mg/kg medetomidine, 2.0 mg/kg midazolam, and 2.5 mg/kg butorphanol. They were appropriately anesthetized throughout the procedure.

Hydrogen gas preparation

Hydrogen gas (3%) was prepared using a hydrogen gas supply device (Nihon Kohden Co., Tokyo, Japan)[14] and administered to rats at a rate of 0.2 L/min. The hydrogen gas stored in the device was mixed with air, and the targeted concentration was measured inside the supply device. The gas flow rate was adjusted at the output port of the device and validated with a flow meter attached to the respiratory circuit.

Experimental protocol

The rats were allocated to three groups: ischemia (control 1), reperfusion (control 2), and hydrogen groups. The ischemia group (n = 10) underwent a median laparotomy and dissection of the superior mesenteric artery. The artery was then occluded at the root by a double microclamp for 60 min. The marginal arteries of the intestines were also ligated at the ileocolic junction and at 15 cm proximal from the junction to achieve complete ischemia of the terminal ileum. The reperfusion group (n = 11) similarly underwent 60-min occlusion of the superior mesenteric artery and ligation of the marginal arteries. The occlusion was then released by declamping of the mesenteric artery, and reperfusion of the intestine was observed for 60 min without closing the abdomen[17,18]. The hydrogen group (n = 9) was connected to the respiratory circuit using a gas supply hood that covered the face and head of the rats, in which spontaneous respiration was maintained without using mechanical ventilation[14]. After hydrogen gas inhalation, the rats underwent the same surgical procedures as those in the reperfusion group.

In each rat, intestinal ischemia was confirmed by the paleness of the distal ileum and pulselessness of the mesenteric artery. The procedures of the tree groups were conducted simultaneously to reduce potential confounders by procedures. All animals were sacrificed immediately after the aforementioned procedures. A 2-cm-long ileum was then excised at 6 cm proximal from the ileocolic junction, which was processed for histological evaluation and ribonucleic acid (RNA) extraction. Blood samples were also obtained directly from the left ventricle[19]. Details of study protocol were not pre-registered nor published.

Histological evaluation

Tissues were fixed in 4% neutral-buffered paraformaldehyde, and 4 μm paraffin-embedded sections were prepared. Hematoxylin and eosin (H&E) staining was performed to evaluate histopathological changes that were visually compared between the three groups (ischemia, ischemia-reperfusion, and ischemia-reperfusion under hydrogen gas inhalation).

For immunostaining, samples were deparaffinized and rehydrated, and endogenous peroxidase activity was then suppressed using 0.3% hydrogen peroxide (H_2O_2) in methanol. Nonspecific binding was blocked with bovine serum albumin (BSA) for 30 min. Primary rabbit antirat antibody against active caspase-3 (CST 9661S; Cell Signaling Technology, Beverly, MA, United States) or against 8-hydroxy-2'-deoxyguanosine (8-OHdG) that is an oxidized nucleoside of DNA induced by ROS (N45.1; Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan) was applied with 1:200 dilution and then incubated overnight at 4 °C. After washing, sections were incubated with biotinylated antirabbit antibodies (Vector Laboratories, Burlingame, CA, United States) diluted to 1:200 in a blocking serum for 30 min. Sections were subjected to peroxidase along with 3,3'-diaminobenzidine-tetrahydrochloride and H_2O_2 (Elite ABC Kit and DAB Substrate Kit; Vector Laboratories). Slides were washed and counter-stained with Gill's hematoxylin (Accustain; Sigma-Aldrich, St. Louis, MO, United States), and samples were microphotographed at Zeiss Axioscope2 (Carl Zeiss, Oberkochen, Germany).

Frozen sections of the harvested tissues were incubated with one of the following primary antibodies diluted in 0.1% BSA/phosphate-buffered saline (PBS): LGR5 as an intestinal stem cell marker[20] (1:200, bs-1117R Bioss Antibodies Inc., MA, United States) and active caspase-3 (1:200). After washing with PBS/0.1% Tween 20 (Wako Pure Chemical Industries, Japan), sections were incubated with secondary antibodies for 1 h at room temperature using Alexa Fluor 488-conjugated (1:500, Invitrogen, CA, United States) or TAS fluorescence systems (NEL 702, PerkinElmer Life Sciences Inc., MA, United States). After counterstaining with Hoechst 33258 (94403, Sigma-Aldrich, MO, United States) to visualize nuclei, images were obtained with a BZ9000 (Keyence, Osaka, Japan).

RNA extraction, cDNA synthesis, polymerase chain reaction, and real-time polymerase chain reaction

Total RNA was isolated using a miRNeasy Mini Kit (Qiagen, Valencia, CA, United States) and converted to cDNA using the High-Capacity Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, United States), according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) was performed using a QuantiFast SYBR Green PCR Kit (Qiagen), according to the manufacturer's instructions. The PCR primers used in this study were as follows: LGR5 forward, 5'-TGTCATGTGAGCTGGATGG-3' and reverse, 5'-ATGCAGGAGACTGGCAGGTA-3'; OLFM4 forward, 5'-GTGGACAGAAGGTGGTACTCTG-3' and reverse, 5'- GCTGGACATACTCCTTCACCTTA-3'; Hes1 forward, 5'-ATAAACCCTCAACTGCTCCGT-3' and reverse, 5'-CCATGATAGGCTTTGATGACTTTCT-3'; Jag2 forward, 5'-CCACACCAGATGAGGAGCTG-3' and reverse, 5'-CAGAACTTGTTGCAGGTGGC-3'; Notch1 forward, 5'-TGGTCTCAACTGCCAGAACC-3' and reverse, 5'-CACTCGCAGTG-GTACTGTGT-3'; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward, 5'-TTGTGCAGT-GCCAGCCTC-3' and reverse, 5'-GGTAACCAGGCGTCCGATAC-3'. Expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to levels of the internal control GAPDH[19,21,22]. Real-time PCR was performed by a researcher who was blinded to the group allocation.

Evaluation of systemic oxidative injury

Serum samples were prepared from blood using Nanosep® Centrifugal Devices with Omega™ Membrane 10K (Pall Corporation, NY, United States), according to the manufacturer's instructions. The supernatant was used to determine 8-OHdG by a competitive enzyme-linked immunosorbent assay (Highly Sensitive 8-OHdG Check; Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan) [20].

Statistical analysis

Descriptive statistics are presented as median (interquartile range) or number (percentage). Intergroup comparisons of mRNA expressions and 8-OHdG concentrations were performed using analysis of variance with Tukey-Kramer as post-hoc test and/or Kruskal-Wallis tests, as appropriate. All statistical tests used a α error rate of 0.05 and were two-sided. All statistical analyses were conducted using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, United States), and Microsoft Excel (Microsoft, Redmond, WA, United States).

RESULTS

Intestinal mucosal injury

Histological sections with H&E staining showed morphologic changes in the intestinal mucosa of the ischemia group (Figure 1A), in which pyknosis was observed in the epithelial layer. Mucosa was more severely injured in the reperfusion group (Figure 1B), and extensive pyknosis in the epithelial layer, denudation of the tip of the villi, and capillary congestion were observed. Conversely, the hydrogen groups showed similar degree of injury to the ischemia group (Figure 1C), with mild epithelial pyknosis and denudation of the villi.

Intestinal stem cell injury by ischemia-reperfusion injury

Immunofluorescence analyses with caspase-3 antibodies revealed that the intestinal mucosa of the ischemia group was mainly injured at the epithelial layer closed to the tip of the villi (Figure 2A). In the reperfusion group, apoptosis was extensively identified at the crypt base, where intestinal stem cells exist[23,24], in addition to injuries at the whole epithelial layer (Figure 2B). Apoptosis at the crypt base was limited in the hydrogen group (Figure 2C), whereas epithelial injury was observed at the villi with a half side of the digestive tract.

In the ischemia group, immunofluorescence analyses using simultaneous staining of caspase-3 and LGR5, a specific protein for intestinal stem cell, revealed that LGR5-positive cells at the crypt base did not undergo apoptosis [caspase-3 (red) and LGR5 (green) were separately stained; Figure 3A]. Conversely, multiple LGR5-positive cells underwent apoptosis after reperfusion (there were double-stained cells with caspase-3 and LGR5; Figure 3B).

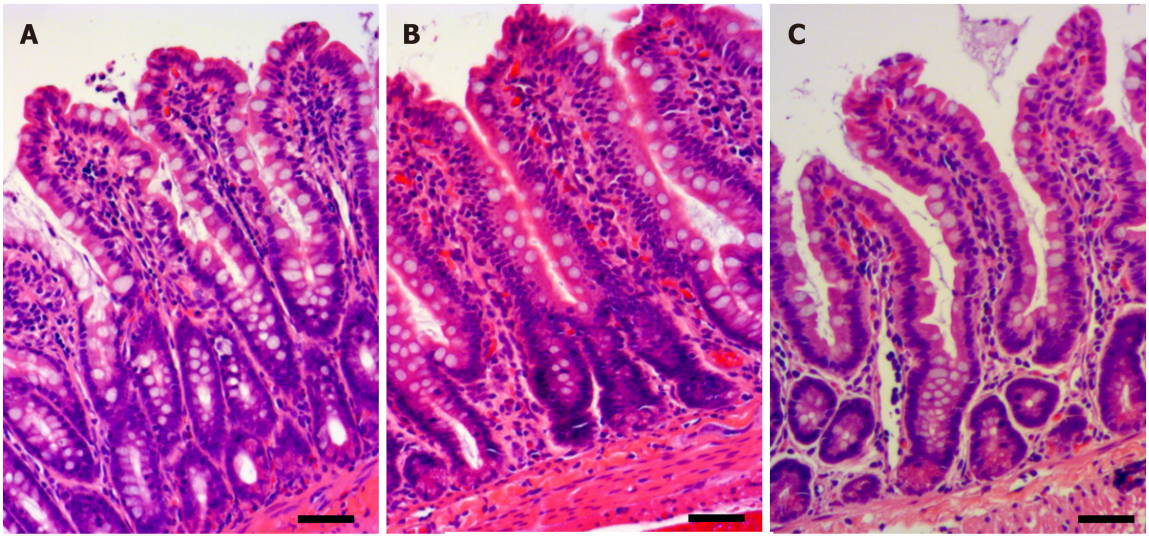

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Figure 1 Intestinal mucosal injury with ischemia-reperfusion injury. A: Histological sections with H&E stain showed pyknosis in the epithelial layer in the ischemia group; B: Extensive pyknosis in the epithelial layer, denudation of the tip of the villi, and capillary congestion in the reperfusion group; C: Mild epithelial pyknosis and denudation of the villi in the hydrogen group. Scale bar: 50 μm.

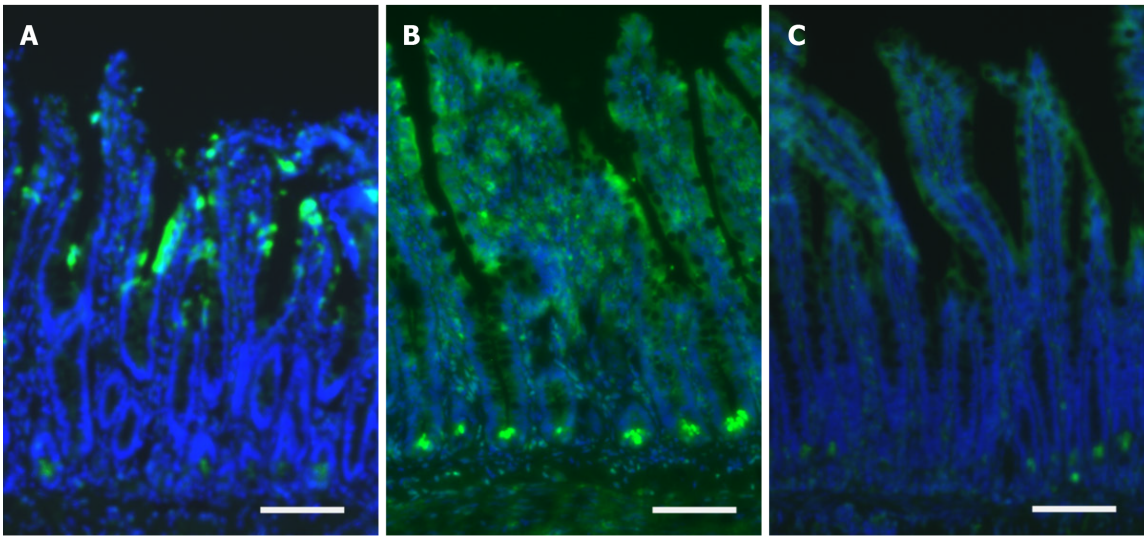

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Figure 2 Intestinal stem cell injury by ischemia-reperfusion injury. A: Immunofluorescence staining with caspase-3 (green) revealed mucosal injury at the epithelial layer closed to the tip of the villi in the ischemia group; B: Extensive apoptosis was identified at the crypt base and the whole epithelial layer in the reperfusion group; C: Apoptosis at the crypt base was limited in the hydrogen group. Scale bar: 100 μm.

Quantitative analysis of RNA with RT-PCR

Quantitative analyses of RNA in the homogenized intestinal tissues were conducted on LGR5, OLFM4, Hes1, Jag2, and Notch1, and Figure 4 summarizes these RNA expressions in the ischemia, reperfusion, and hydrogen groups. The expression of LGR5 was significantly lower in the reperfusion group than in the ischemia group, whereas the expression of LGR5 was higher in the hydrogen group than in the reperfusion group (Figure 4A). In addition, the expression of OLFM4 was relatively higher in the hydrogen group than in the reperfusion group, although the differences were not significant.

Conversely, the expression of Hes1, a transcriptional repressor of genes, was relatively high in the reperfusion group than in the ischemia and hydrogen groups (Figure 4B). Expressions of Jag2 (a ligand in Notch signaling for cell fate decision) and Notch1 (a transmembrane receptor in Notch signaling) were comparable among the ischemia, reperfusion, and hydrogen groups (Figure 4B).

Mucosal and systemic oxidative injuries

Immunostaining for 8-OHdG of intestinal tissue showed that the ischemia group had limited oxidative

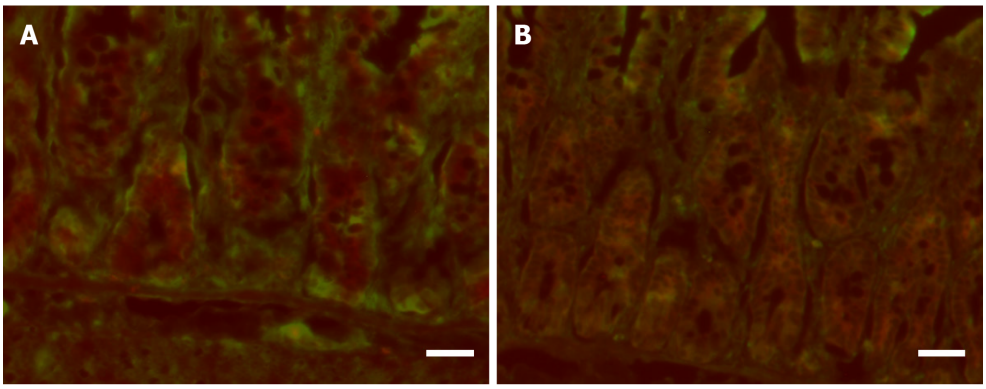

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Figure 3 Intestinal stem cell with double immunofluorescence staining. A: Double immunofluorescence staining with caspase-3 (red) and leucine-rich repeat-containing G-protein-coupled 5 (LGR5) (green) showed that LGR5-positive cells at the crypt base did not undergo apoptosis in the ischemia group (caspase-3 and LGR5 were separately stained); B: Multiple LGR5-positive cells underwent apoptosis after reperfusion (there were double-stained cells with caspase-3 and LGR5). Scale bar: 50 μm.

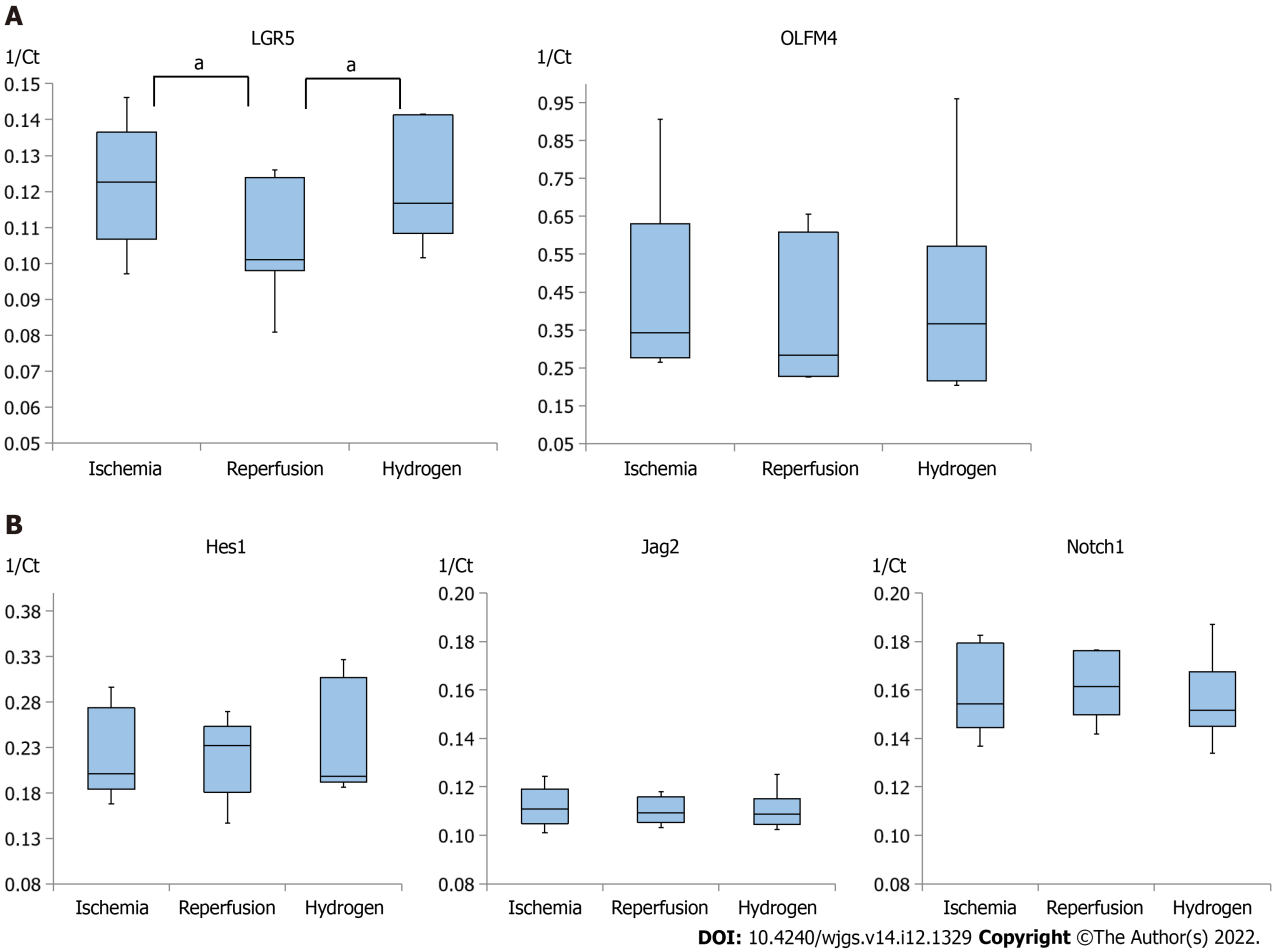

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Figure 4 Quantitative analysis of RNA. A: Leucine-rich repeat-containing G-protein-coupled 5 expression was significantly lower in the reperfusion group than in the ischemia group, but it was significantly higher in the hydrogen group than in the reperfusion group. Olfactomedin 4 expression was also relatively higher in the hydrogen group than in the reperfusion group, although the differences were not significant; B: Hes1 expression was relatively high in the reperfusion group than in the ischemia and hydrogen groups, whereas Jag2 and Notch1 expressions were comparable between the ischemia, reperfusion, and hydrogen groups. $^{a}P < 0.05$. LGR5: Leucine-rich repeat-containing G-protein-coupled 5; OLFM4: Olfactomedin 4; Hes1: Hairy and enhancer of split 1; Jag2: Jagged 2; Notch1: Neurogenic locus notch homolog protein 1.

stress at the intestinal mucosa, although considerable pyknosis occurred in the epithelial layer, particularly at the tip of the villi (Figure 5A). Conversely, the reperfusion group had extensive oxidative injury throughout the epithelium, including at the crypt base (Figure 5B). The hydrogen group had

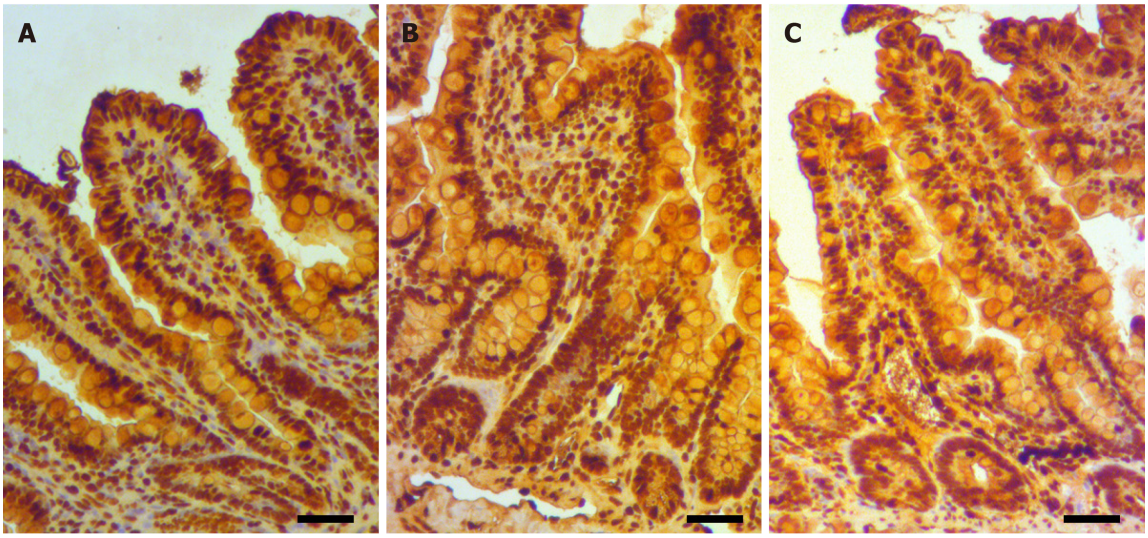

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Figure 5 Mucosal oxidative injury in the ischemia-reperfusion model. A: Immunostaining for 8-hydroxy-2'-deoxyguanosine showed limited oxidative stress at the mucosa in the ischemia group, although there was considerable pyknosis at the tip of the villi; B: Extensive oxidative injury throughout the epithelium, including the crypt base, in the reperfusion group; C: Mild-to-moderate oxidative injury at the mucosa of the crypt base, along with pyknosis with the denudation at the tip of the villi, in the hydrogen group. Scale bar: 50 μm.

mild-to-moderate oxidative injury at the mucosa of the crypt base, along with pyknosis with the denudation at the tip of the villi (Figure 5C).

Regarding systematic oxidative injury, the serum 8-OHdG concentrations immediately after the intervention in each group (ischemia, ischemia and reperfusion, and ischemia and reperfusion under hydrogen gas inhalation) are summarized in Figure 6. Despite the lack of significance, 8-OHdG concentration was higher in the reperfusion and hydrogen groups than in the ischemia group.

DISCUSSION

In this study, the tissue-protective effects of continuous hydrogen gas inhalation were histologically identified in the model of ischemic-reperfusion injury at mesentery. In addition, hydrogen protected intestinal stem cells from oxidative stress following ischemia-reperfusion injury, which has not been reported as therapeutic effect of hydrogen in previous studies. Notably, the intestinal stem cells were not injured by ischemia alone (ischemia without reperfusion), and therefore, hydrogen would provide tissue-protective effect only when reperfusion happens, rather than only ischemic injury exists.

Previous clinical and animal studies have suggested that hydrogen gas has anti-oxidative and anti-inflammatory effects on several critical diseases, such as cerebral infarction, myocardial infarction, and post-cardiac arrest syndrome[7,10,12]. Although pathophysiological mechanisms underlying these therapeutic effects remain unclear, hydrogen would attenuate excessive neutrophil activation and reduce hydroxyl radicals produced following an ischemia-reperfusion injury[25,26]. In this study, fewer oxidized nucleosides of DNA (8-OHdG) were observed at the crypt base of the intestines during continuous inhalation of hydrogen gas, which suggests that hydrogen mitigated ROS toxicity.

Immunofluorescence assay using LGR5 suggested that the intestinal stem cells would be a target of the therapeutic effects of hydrogen, at least under mesenteric ischemia and reperfusion. Stem cells have unique features, one of which is self-proliferation under adequate ischemic stimuli, whereas differentiated enterocytes undergo apoptosis because of ischemia-induced energy depletion[23,27]. In a study on the association between ROS and intestinal stem cells, modest ROS following ischemia would signal proliferation and differentiation of stem cells[27]. However, the same study suggested that high levels of ROS can induce intestinal stem cell apoptosis, which is similar to the observations in this study. We showed relative preservation of intestinal stem cells with ischemic stress alone and extensive apoptosis of stem cells with reperfusion that would have introduced massive ROS. Therefore, our results might indicate that hydrogen reduces excessive ROS caused by ischemia-reperfusion stimuli and prevents apoptosis of intestinal stem cells.

The protective effects of hydrogen on intestinal stem cells are also indicated by the higher LGR5 expression with hydrogen gas inhalation in the quantitative measurement of RNA. OLFM4 is a robust marker of LGR5-positive stem cells[28], and in this study, its expression was relatively higher in the hydrogen group than in the reperfusion group. Jag2/Notch1/Hes1 expressions have been reported to increase with epithelial cell proliferation following ischemia-reperfusion injury in the intestines[19].

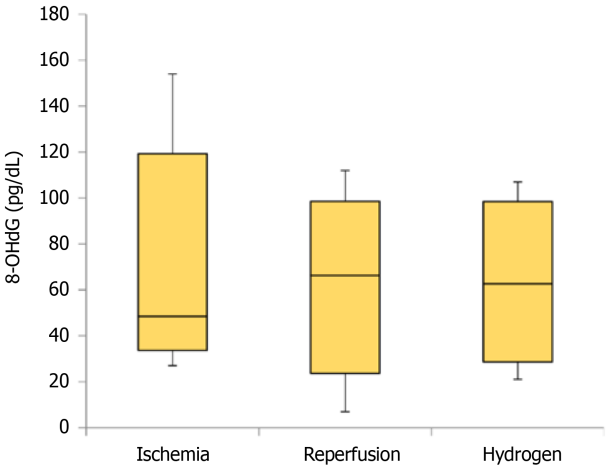

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Figure 6 Systematic oxidative injury in the ischemia-reperfusion model. Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentration immediately after each intervention (ischemia, ischemia-reperfusion, and ischemia-reperfusion under hydrogen gas inhalation) was measured. Although not significant, the 8-OHdG concentration was higher in the reperfusion and hydrogen groups than in the ischemia group. 8-OHdG: 8-hydroxy-2'-deoxyguanosine.

Expressions of Jag2 and Notch1 were comparable between the three groups, and the expression of Hes1 was slightly high in the reperfusion group, suggesting that proliferation signals at the epithelium under ischemia would be similar regardless of reperfusion or hydrogen inhalation.

Systemic oxidative stress was not different between the reperfusion and hydrogen groups. Therefore, hydrogen would not systematically affect the total amount of ROS in the body. Although hydrogen may reduce ROS in other tissues or organs in addition to the intestinal mucosa, such possible effects were not examined in this study. Moreover, the mechanisms of the reduction of ROS toxicity by hydrogen were not assessed. Future studies should focus on these topics to develop a noninvasive novel therapy using hydrogen gas.

CONCLUSION

This study reported on the tissue-protective effects of continuous hydrogen gas inhalation in ischemia-reperfusion injury in the intestines. The target cells of hydrogen might be intestinal stem cells, which are injured by excessive ROS caused by reperfusion following ischemia rather than by ischemic stress alone. The pathophysiological mechanisms for ROS reduction by hydrogen in stem cells should be further clarified in future studies.

ARTICLE HIGHLIGHTS

Research background

Mesenteric ischemia introduces unfavorable clinical outcomes particularly when bowel necrosis is diagnosed, and it can happen even after revascularization. However, promising treatment has not been developed to prevent bowel necrosis after revascularization.

Research motivation

Hydrogen gas inhalation has showed tissue preserving effects for several ischemia-reperfusion injuries by reducing reactive oxygen species (ROS) in various animal and clinical studies. In addition, the safety of hydrogen gas was shown by clinical studies that examined the efficacy of hydrogen on myocardial infarction and post-cardiac arrest syndrome. Therefore, hydrogen gas for mesenteric ischemia can be a novel noninvasive treatment.

Research objectives

This study aimed to clarify the favorable effects of hydrogen gas inhalation for mesenteric ischemia and reperfusion. We hypothesized that the degree of tissue damage in the intestines following ischemia and reperfusion would be mitigated by continuous initiation of 3% hydrogen gas.

Research methods

Rats were allocated to three groups: ischemia (control 1) that underwent 60-min occlusion of mesenteric artery by clamping under laparotomy, reperfusion (control 2) that underwent the ischemia procedure and 60-min release of occlusion, and hydrogen that underwent the ischemia and reperfusion under 0.3% hydrogen gas inhalation at a rate of 0.2 L/min. Then, the tissue damages at the ileum were histologically evaluated, using immunostaining against caspase-3, 8-hydroxy-2'-deoxyguanosine, and leucine-rich repeat-containing G-protein-coupled 5 (LGR5). Several mRNA, including LGR5, were quantitatively measured with RT-PCR.

Research results

The reperfusion procedure introduced intestinal tissue destruction, which was mitigated by hydrogen gas inhalation. In addition, the intestinal tissue injury by the reperfusion involved intestinal stem cell that was marked by LGR5, whereas the ischemia without reperfusion did not affect the stem cell. The expression of LGR5 was significantly lower in the reperfusion group than in the ischemia group, whereas the expression of LGR5 was higher in the hydrogen group than in the reperfusion group.

Research conclusions

This study reported on the tissue-protective effects of continuous hydrogen gas inhalation in the ischemia and reperfusion injury at the intestine. The target cells of hydrogen might be intestinal stem cells that are injured by excessive ROS caused by reperfusion following ischemia.

Research perspectives

Hydrogen may reduce ROS in other tissues in addition to the intestinal mucosa, which should be examined in the future study. Moreover, the mechanisms of the reduction of ROS toxicity by hydrogen should be revealed to validate the hydrogen as a noninvasive novel treatment.

FOOTNOTES

Author contributions: Yamamoto R, Suzuki S, Homma K, Yamaguchi S, and Sujino T designed the experiment; Yamamoto R, Suzuki S, and Homma K performed the experiment and analyzed the data; Sasaki J supervised the experiment; Yamamoto R and Suzuki S wrote the original manuscript; Yamamoto R, Suzuki S, and Homma K revised the manuscript; all authors reviewed the manuscript.

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