Gastrin attenuates ischemia-reperfusion-induced intestinal injury in rats

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Abstract

Intestinal ischemia-reperfusion (I/R) injury is a devastating complication when the blood supply is reflowed in ischemic organs. Gastrin has critical function in regulating acid secretion, proliferation, and differentiation in the gastric mucosa. We aimed to determine whether gastrin has an effect on intestinal I/R damage. Intestinal I/R injury was induced by 60-min occlusion of the superior mesenteric artery followed by 60-min reperfusion, and the rats were induced to be hypergastrinemic by pretreated with omeprazole or directly injected with gastrin. Some hypergastrinemic rats were injected with cholecystokinin-2 (CCK-2) receptor antagonist prior to I/R operation. After the animal surgery, the intestine was collected for histological analysis. Isolated intestinal epithelial cells or crypts were harvested for RNA and protein analysis. CCK-2 receptor expression, intestinal mucosal damage, cell apoptosis, and apoptotic protein caspase-3 activity were measured. We found that high gastrin in serum significantly reduced intestinal hemorrhage, alleviated extensive epithelial disruption, decreased disintegration of lamina propria, downregulated myeloperoxidase activity, tumor necrosis factor- α , and caspase-3 activity, and lead to low mortality in response to I/R injury. On the contrary, CCK-2 receptor antagonist L365260 could markedly impair intestinal protection by gastrin on intestinal I/R. Severe edema of mucosal villi with severe intestinal crypt injury and numerous intestinal villi disintegrated were observed again in the hypergastrinemic rats with L365260. The survival in the hypergastrinemic rats after intestinal I/R injury was shortened by L365260. Finally, gastrin could remarkably upregulated intestinal CCK-2 receptor expression. Our data suggest that gastrin by omeprazole remarkably attenuated I/R induced intestinal injury by enhancing CCK-2 receptor expression and gastrin could be a potential mitigator for intestinal I/R damage in the clinical setting.

Keywords: Gastrin, intestinal mucosa, ischemia-reperfusion apoptosis, cholecystokinin-2 receptor

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Introduction

Intestinal ischemia-reperfusion (I/R) injury will high-frequently occur when subjected to acute mesenteric ischemia, hemorrhagic, traumatic, or septic shock, severe burns or some surgical procedures including hypovolemic/septic shock, neonatal necrotizing enterocolitis, organ transplantation, cardiopulmonary bypass and abdominal aortic surgery.¹ Intestinal I/R is mainly attributable to progressive exacerbation of intestinal mucosa damage, activation of inflammatory-related cells and release of inflammatory cytokines, which in turn, cause microcirculatory dysfunction in organs and finally result in cardiac, respiratory, hepatic, and renal failure.^{2,3} In the critical care circumstance, the

initiation and development of intestinal ischemia is an critical contribution in high mortality (67–80%).⁴

Gastrin is a neuroendocrine hormone presented and secreted from G-cells in the antrum of the stomach. A few cases, G-cells in the fundus and corpus of the stomach is also synthesized and secreted. It has multifunction in regulating acid secretion, cell growth, cell apoptosis, and cell division in the gastric mucosa.⁵ Gastrin has also been proved to stimulate enterochromaffin-like cells to produce histamine which contributes to the healing of experimentally induced ulcers in rat stomach.⁶ Although precursor forms of gastrin, such as progastrin and glycine-extended gastrin have been found to encourage cell growth, division and hyperproliferation in normal murine colon,^{7–9} few

report that whether gastrin has effects upon small intestinal epithelial cells as the Cholecystokinin-2 (CCK-2) receptor is lowly expressed in the tissue.^{10,11} Some researches have demonstrated that gastrin is often used as potential protein for facilitating proliferation in intestinal mucosa following ionizing radiation or chemical carcinogen.^{8,12} We will explore the effect of gastrin on the healing of intestinal epithelial cells following I/R. Therefore, this study was designed to investigate the effect of hypergastrinemia induced by omeprazole on intestinal injury after I/R and pinpoint the potential mechanisms involved in the effects of gastrin.

Materials and methods

Animals and surgery

The current study was approved by the Animal Care Committee of Sun Yat-sen University, Guangzhou, China (Chinese Council) and was performed in accordance with National Institutes of Health guidelines for the use of experimental animals. Male Sprague-Dawley rats (200–250 g) were obtained from Laboratory Animal Center of Sun Yat-sen University and monitored for pathogens involving virus (Pneumonia virus of mice, Reovirus type III, Minute virus of mice, Theiler's mouse encephalomyelitis, Mouse adenovirus, Polyoma virus) and bacteria (*Pasteurella pneumotropica*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, β -Hemolytic streptococcus, *Pseudomonas aeruginosa*) before used. They were housed in wire-bottomed cages placed in a room illuminated from 8:00 to 20:00 (12:12-h light-dark cycle) and maintained at $21 \pm 1^\circ\text{C}$. The rats were allowed access to water and chow *ad libitum*.

The rats were anesthetized for 3 h by intraperitoneal injection of 4% chloral hydrate (200 mg/kg). A laparotomy was carried out under chloral hydrate anesthesia, and the superior mesenteric artery (SMA) was occluded with a microbulldog clamp. At the end of the ischemic period, the clamp was released, and three drops of lidocaine were applied directly onto the SMA to facilitate reperfusion. After the experiment, animals were euthanized, and then the ischemia jejunum (8 cm between stomach and ileum) was carefully removed and placed on ice and was rinsed thoroughly with physiological saline and then cut into 0.5 cm in length. Some were fixed in 10% neutral buffered formalin for measurement of mucosal injury and terminal deoxynucleotidyl transferase-mediated dUDP-biotin nick-end labeling (TUNEL) assay. Some was opened longitudinally on antimesenteric border to expose the intestinal mucosa. The mucosal layer or isolated intestinal epithelial cells was harvested as previously described.^{13,14}

Groups and treatments

Animals were randomly allocated into five groups ($n=15$ per group), as follows. Sham group (Sham): the rats received continuous intravenous infusion of normal saline and sham surgical preparation, including isolation of the SMA without occlusion. Injury group (Injury) the rats received continuous intravenous infusion of normal

saline, and intestinal I/R was induced by clamping the SMA for 60 min followed by declamping (reperfusion) for 60 min. Hypergastrinemia group (Hypergastrinemia): the rats were rendered hypergastrinemic by gavage with 120 mg/kg omeprazole (AstraZeneca, Luton, UK) suspended in 0.25% methylcellulose (Sigma) one time each day for three times. Hypergastrinemia group (Hypergastrinemia + injury): the hypergastrinemic rats after three times treatment of omeprazole received surgical preparation. Besides, L365260 groups: the rats with treatment of omeprazole that additionally received 0.3 mg/kg L365260 (Santa Cruz, Santa Cruz, USA) through intrahepatic injection. Omeprazole or L365260 was given 24 h prior to intestinal I/R treatment and daily up until sacrifice.

The Gastrin group: rats were intravenously injected with three times treatment of $3 \mu\text{g/kg}$ gastrin (Sigma Chemical Co, St Louis, MO) once each day received 60 min occlusion of SMA followed by 60 min of reperfusion.

Isolation of intestinal epithelial cells

The small intestinal epithelial cells were isolated as previously described.¹⁵ Briefly, isolated small intestines were opened longitudinally, and washed with cold PBS. The tissue was chopped into around 5 mm pieces, and further washed with cold PBS. The tissue fragments were incubated in 2 mmol/L EDTA with PBS for 30 min on ice. After removal of EDTA medium, the tissue fragments were vigorously suspended by using a 10-mL pipette with cold PBS. This fraction was passed through a 70-mm cell strainer (BD Bioscience, Franklin Lake, NJ) to remove residual villous material. Cells were collected for PCR and Western blotting.

Isolation of intestinal crypts

The small intestinal epithelial crypts were isolated as previously described.¹⁶ Briefly, isolated small intestines were opened longitudinally, and washed with cold PBS. The tissue was chopped into around 5 mm pieces, and further washed with cold PBS. The tissue fragments were incubated in 2 mmol/L EDTA with PBS for 30 min on ice. After removal of EDTA medium, the tissue fragments were vigorously suspended by using a 10-mL pipette with cold PBS. The supernatant was the villous fraction and was discarded; the sediment was resuspended with PBS. After further vigorous suspension and centrifugation, the supernatant was enriched for crypts. This fraction was passed through a 70-mm cell strainer (BD Bioscience, Franklin Lake, NJ) to remove residual villous material. Isolated crypts were centrifuged at 150–200 g for 3 min to separate crypts from single cells. The final fraction consisted of essentially pure crypts for extraction of total proteins and nuclear proteins.

Enzyme-linked immunosorbent assay (ELISA) and myeloperoxidase (MPO) activity assay

The gastrin concentration of serum and TNF- α concentration in intestinal epithelial cells was measured using a commercial kit (eBioscience, San Diego, CA), according to the

manufacturer's instructions. Briefly, ELISA plates were coated with 100 μ L/well of capture antibody diluted in coating buffer and incubated overnight at room temperature (RT). Plates were washed with wash buffer and blocked for 1 h at RT with 200 μ L/well assay diluent. Then the gastrin or TNF- α standard and samples (100 μ L) were pipetted into appropriate wells. After that, the plates were sealed and incubated at RT for 2 h. After washing, 100 μ L of detection antibody was added to each well, sealed, and incubated for 1 h at RT. After washing, 100 μ L of substrate solution was added to each well and incubated for 30 min at RT in the dark. Stop solution (2N H₂SO₄, 50 μ L/well) was added and the plates were read at 450 nm (570 nm correction) on a MicroPlate Reader (BioTek, Seattle, WA). The values for results were expressed as Gastrin pg/mL protein.

The MPO activity was used as an index to reflect neutrophil migration into the small intestine.¹⁷ The intestine assay sample was homogenized and the homogenate was frozen-thawed twice, and then centrifuged at 13,000 rotations per minute (rpm) for 5 min. The resulting supernatant was assayed spectrophotometrically for MPO activity. One unit of MPO was defined as that degrading 1 μ mol peroxide per minute at 25°C. Results were expressed as unit per gram protein of intestinal epithelial cells.

RNA extraction and real-time PCR

RNA was extracted from 100 mg mucosal scraping using TRIzol Reagent (Invitrogen, Carlsbad, CA) as per manufacturer's instructions. First strand cDNA was synthesized from 1.5 μ g total RNA using ReverTra Ace kit (TOYOBO, Japan) as per manufacturer's instructions. An ABI Prism 7000 sequence detection system (Applied Biosystems, Bedford, MA) was then used for real-time PCR experiments to quantitate the gene expression of CCK-2 receptor and β -actin for each sample. Reactions were performed in a 20 μ L volume with TaKaRa TaqTM (TaKaRa, Japan). The PCR conditions included a denaturation step at 94°C for 5 min. Amplification was carried out for 35 cycles (denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s). Quantification was performed by using the 7000 SDS instrument software (Applied Biosystems) for relative quantification of gene expression. Primer sequences used were as follows: CCK-2 receptor forward primer 5'-AGCTGGGGAAGACAGTGAT-3'; CCK-2 receptor reverse primer 5'-GGGGTTGACACAAGCAGA-3'; β -actin forward primer 5'-GAAATCGTGCGT GACATCAAA G-3'; β -actin reverse primer 5'-TGTAGTTTCATGGATGCCACA G-3'. Primers were supplied by Invitrogen. Results were expressed in fold change in mRNA expression from sham-operated rats.

Morphological analysis and mucosal injury score

After animal experiment, the tissue samples were immediately fixed in 10% neutral buffered formalin, and then embedded in paraffin and sectioned. Sample sections were processed with hematoxylin-eosin staining, and examined by light microscopy, according to the criteria described by Chiu *et al.*¹⁸ as follows: grade 0, normal mucosa; grade 1, development of subepithelial spaces

near the tips of the villi with capillary congestion; grade 2, extension of the subepithelial space with moderate epithelial lifting from the lamina propria; grade 3, significant epithelial lifting along the length of the villi with a few denuded villous tips; grade 4, denuded villi with exposed lamina propria and dilated capillaries; and grade 5, disintegration of the lamina propria, hemorrhage, and ulceration. Twenty visual fields at $\times 200$ magnification were evaluated for each sample slides, and the final injury scoring was a gross assessment of the degree of mucosal damage. All slides were evaluated by two examiners in a blinded fashion.

Analysis of Bax and cytochrome c translocation

To detect Bax and cytochrome c translocation, intestinal mucosal sample was used to isolated mitochondrial and cytosolic fractions by the differential centrifugation method as described previously.¹³ Briefly, after washed by ice cold PBS, the samples were resuspended in homogenization buffer (0.25 mol/L sucrose, 10 mmol/L HEPES, pH 7.4, and 1 mmol/L EGTA). The homogenate was subjected to centrifugation at 1000 g for 15 min at 4°C to separate the nuclei and unbroken cells. The supernatant was subsequently centrifuged at 10,000g to harvest the cytosolic fraction (supernatant) and the mitochondrial fraction (pellet). The mitochondrial fraction was resuspended in homogenization buffer. Both fractions were analyzed by Western blotting for Bax (Abcam, England).

TUNEL assay and apoptotic index analysis

Sample sections were used to detect cell apoptosis. Fragmented DNA of apoptotic cell was stained with Fluorescent TUNEL method by using an in situ cell death detection kit (Roche, Switzerland). The apoptotic index was calculated randomly in a minimum of 100 crypts and analyzed in six separate samples. The apoptotic index was determined by dividing the number of apoptotic cells by the total number of cells in the crypt column.

Western blot analysis

CCK-2 receptor and apoptotic proteins caspase-3 were analyzed by Western blotting. Equal quantities (20 μ g) of lysates were electrophoresed in a SDS-PAGE and then transferred onto a nitrocellulose membrane (Bio-Rad). After blocking with PBS containing 0.1% polyoxyethylene sorbitan monolaurate (Tween 20, Sigma) and 5% skim milk for 1 h, the membrane was incubated with a rabbit polyclonal anti-CCK-2 receptor antibody (1:500; Santa Cruz), a rabbit polyclonal anti-caspase-3 antibody (1:1000; Cell Signaling Technology), at 4°C overnight. Antigen-antibody complexes were detected with horseradish peroxidase-conjugated anti-rabbit IgG (1:6000; Santa Cruz Biotechnology). Detection of chemiluminescence was performed using ECL Western blotting detection reagents (Amersham Pharmacia Biotech, Piscataway, NJ).

Statistical and survival analysis

The data are expressed as means $\pm$ SD. Data were evaluated by one-way ANOVA in which multiple comparisons were performed by using the method of least significant difference *t* test. Differences were considered significant if $P < 0.05$. The rats ($n = 20$ per group) were used to detect survival time. Two hours after reperfusion, rats with surgical operation were transferred to their individual cages monitored by 10 min interval, allowed free access to food and water, and sacrificed by lethal sodium pentobarbital injection when presented by being in move-stopping condition with faintly breathing. The survival data were analyzed using a log-rank test with the SPSS 17.0 software.

Results

Gastrin improved I/R-induced intestinal injury in rats

Based on the results (Figure 1a, b), omeprazole with 120 mg/kg efficiently inducing hypergastrinemia was chosen to investigate the effect of gastrin on intestinal injury induced by I/R. In the Sham group, the villi and crypts were normal. By contrast, severe edema of mucosal villi accompanied with severe intestinal crypt injury and a large number of intestinal villi disintegrated were observed in the injury group with 60 or 30 min of ischemia following reperfusion, which were significantly reduced in the hypergastrinemic group and gastrin group (Figure 1c, d, g, Supplementary Figure 1a, b). Moreover, diamine oxidase

in serum, an indicator for intestinal disintegrability and a response to inflammation, was greatly diminished in the hypergastrinemic group, compared with the injury group (Figure 1e and Supplementary Figure 1e, f). The MPO activity, an index to the reflection of neutrophil migration into the small intestine, and inflammatory factor TNF- α were sharply reduced in the hypergastrinemic group and gastrin group (Supplementary Figure 1f, h). These explain that the survival rate in the hypergastrinemic group showed significant longer than that in the injury group (Figure 1f). This indicates that gastrin ameliorates I/R-induced intestinal injury.

Gastrin increased intestinal CCK-2 receptor expression

CCK-2 receptor expression in epithelial cells from small intestine of rats was examined by real-time RT-PCR and Western blotting. CCK-2 mRNA was significantly induced by 120 mg/kg omeprazole suspended in 0.25% methylcellulose at three days and gastrin infusion in epithelial cells from small intestine or colon (Figure 2a, b and Supplementary Figure 1i). CCK-2 protein expression was significantly elevated at the third day in the hypergastrinemic group in epithelial cells from small intestine and colon compared with the untreated group (Figure 2c, d). These imply that gastrin could induce intestinal CCK-2 receptor expression.

Figure 1 Gastrin improved ischemia-reperfusion (I/R)-induced intestinal injury in rats. (a) ELISA analysis of serum gastrin protein with treatment of 50 mg/kg omeprazole in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 15$ in each group. (b) ELISA analysis of serum gastrin protein with treatment of 120 mg/kg omeprazole in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 15$ in each group. (c) Morphologic changes of intestinal mucosa in mice with or without 60 min of ischemia following 60 min reperfusion. Representative sections of small intestine for hematoxylin and eosin (H&E) staining ($\times 200$) were showed. (d) The evaluation of gut injury with Chiu's scores under light microscopy ($\times 200$) in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 15$ in each group. (e) The changes of diamine oxidase concentration in serum in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 15$ in each group. (f) Effects of gastrin on survival in mice with or without 60 min of ischemia following reperfusion. Results were compared by Kaplan-Meier log-rank test, $n = 30$ in each group. (g) The evaluation of gut injury with Chiu's scores under light microscopy ($\times 200$) in mice with 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 10$ in each group. (h) The changes of MPO activity in small intestinal epithelial cells in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD; $n = 6$ in each group. (A color version of this figure is available in the online journal.)

Gastrin protected intestinal injury induced by I/R via upregulating CCK-2 receptor in rats

To investigate whether gastrin signals via CCK-2 receptor to protect intestinal injury induced by I/R, L365260, CCK-2 receptor antagonist, was injected in hypergastrinemic mice. We found that L365260 markedly downregulated CCK-2 receptor expression enhanced by gastrin and distinctly promoted apoptotic protein caspase-3 activity (Figure 3a, b, c). Morphologically, L365260 remarkably impaired gastrin intestinal protection and caused intestinal crypt injury with edema of mucosal villi, a large amount of disintegrated intestinal villi and shortened villi much

severer than that in the hypergastrinemic mice (Figure 3d). Consequently, hypergastrinemic mice treated with L365260 resulted in a significant decrease in survival rate, compared with the mice without L365260 (data not shown). All strongly suggest that gastrin exerts intestinal protection on intestinal I/R injury via potentiating CCK-2 receptor.

Gastrin decreased crypt cell apoptosis in response to I/R injury in rats

We have demonstrated that gastrin has intestinal benefit in intestinal I/R such as sustaining integrity of villi and

Figure 2 Gastrin increased intestinal CCK-2 receptor expression. (a) CCK-2 receptor mRNA expression in epithelial cells from small intestine in groups with or without 60 min of ischemia following 60 min reperfusion was determined by quantitative PCR. Values are means $\pm$ SD, $n = 15$ in each group. (b) CCK-2 receptor mRNA expression in epithelial cells from colon in groups with or without 60 min of ischemia following 60 min reperfusion was determined by quantitative PCR. Values are means $\pm$ SD, $n = 15$ in each group. (c) CCK-2 receptor protein expression in epithelial cells from small intestine and colon in groups with or without 60 min of ischemia following 60 min reperfusion were evaluated by Western blotting. β -Actin was used as the control for loading. Four independent experiments were performed. (d) Quantitative analysis of Western blotting of CCK-2 receptor in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 15$ in each group.

Figure 3 Gastrin protected intestinal injury induced by I/R via upregulating CCK-2 receptor in rats. (a) CCK-2 receptor protein expression in epithelial cells from small intestine were evaluated by Western blotting. β -Actin was used as the control for loading. (b) Quantification analysis of Western blotting of CCK-2 receptor. Values are means $\pm$ SD, $n = 15$ in each group. (c) CCK-2 receptor mRNA expression in epithelial cells from small intestine in groups was determined by quantitative PCR. Values are means $\pm$ SD, $n = 15$ in each group. (d) Morphologic changes of intestinal mucosa. Representative sections of small intestine for hematoxylin and eosin (H&E) staining ($\times 200$) were showed. (A color version of this figure is available in the online journal.)

restraining intestinal apoptosis. Intestinal crypt is closely associated with restoration of intestinal epithelium following insult or disturbance. It is reasonable to believe that gastrin has a protective effect on intestinal crypt after the insult to I/R. Our results showed that gastrin significantly lessened apoptotic cells in crypts after intestinal I/R, compared with untreated group (Figure 4a, b and Supplementary Figure 1d, g). Next, we detected CCK-2 receptor and apoptotic caspase-3 activity and found that in isolated intestinal crypts, CCK-2 receptor expression was greatly enhanced, while caspase-3 activity was decreased, in the hypergastrinemic mice compared with untreated group (Figure 4c, d and Supplementary Figure 1c). These reveal that gastrin defends intestinal crypts against I/R-induced apoptosis.

Gastrin allayed I/R-induced mitochondrial-mediated apoptosis via CCK-2R

To examine the mechanisms of CCK-2R-mediated antiapoptosis following I/R, we analyzed several mitochondria-related events in intestinal epithelial cells. I/R injury induced cytosolic release of cytochrome c and mitochondrial translocation in intestinal epithelial cells, which had

been virtually blocked by omeprazole treatment (Figure 5a, b). However, omeprazole-induced mitochondrial protection was reversed by the CCK-2R inhibitor L365260 (Figure 5a, b). These data reveal that gastrin reduces mitochondrial-related events following intestinal I/R injury.

Discussion

I/R-induced intestinal injury contributes to many critically intensive medical events, such as shock, severe infection, cardiopulmonary bypass, small intestine transplantation, and abdominal aortic artery surgery, which are troublesome and less efficient therapies in clinical medical procedure. However, the mechanism of I/R induced intestinal injury remains to be elusive. In the current study, we found for the first time that gastrin induced by omeprazole in serum remarkably alleviated I/R induced intestinal injury and morphological analysis showed that intestinal hemorrhage, extensive epithelial disruption and disintegration of lamina propria were significantly improved in the hypergastrinemic mice. Moreover, previous studies indicate that inflammatory response, oxidative injury, and neutrophil infiltration were involved in the pathogenesis of intestinal I/R injury. The current data showed that gastrin reduced

Figure 4 Gastrin decreased crypt cell apoptosis in response to I/R injury in rats. (a) The degree of apoptosis in small intestinal crypts in groups with or without 60 min of ischemia following 60 min reperfusion was measured 24 h after I/R injury using TUNEL fluorescent staining (green); magnifications, $\times 400$. (b) Quantification of the apoptotic index in the crypts in mice with or without 60 min of ischemia following 60 min reperfusion measured by TUNEL staining. Values are means $\pm$ SD; $n = 15$ in each group. (c) CCK-2 receptor and active caspase-3 protein expression in small intestinal crypts in groups with or without 60 min of ischemia following 60 min reperfusion were evaluated by Western blotting. β -Actin was used as the control for loading. (d) Quantification analysis of Western blotting of CCK-2 receptor in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 15$ in each group. (A color version of this figure is available in the online journal.)

Figure 5 Gastrin allayed I/R induced mitochondrial-mediated apoptosis via CCK-2R. (a) Bax were analyzed in the cytosolic and mitochondrial fractions of epithelial cells from small intestine in groups with or without 60 min of ischemia following 60 min reperfusion. β -Actin and COX IV were used as the controls for loading and fractionation. (b) Quantification analysis of Western blotting of Bax translocation in mice with or without 60 min of ischemia following 60 min reperfusion. Values are means $\pm$ SD, $n = 15$ in each group.

diamine oxidase in serum, which normally mostly exist in the intestinal epithelial cells, suggesting that gastrin could confer its intestinal protection by partially inhibiting inflammatory response. Therefore, morphological intestinal improvement and decreased response to inflammation by gastrin significantly prolonged the survival in the hypergastrinemic rats.

Accumulated evidences suggest that intestinal CCK-2 receptor is overexpressed when gastrointestinal epithelial cells are subjected to injury or inflammation.¹⁹ For example, markedly increased expression of CCK-2 receptor by three times in the epithelium of Barrett esophagus individuals and also escalated proliferation of mucosal biopsies from patients with Barrett esophagus.²⁰ But in the current study, intestinal I/R injury did not increase basic CCK-2 receptor expression in rats. The possible reasons are the timing of tissue collection, different strains of rats or variation between stomach and gut. More importantly, L365260, the CCK-2 receptor antagonist, compromised intestinal protection by gastrin in rats and diminished the survival in the hypergastrinemic rats. So, the CCK-2 receptor is the critical mediator for intestinal protection in intestinal I/R injury in the hypergastrinemic rats.

Intestinal mucosal epithelial cells are the main physical component of the intestinal mucosal barrier. Programmed cell death is a main pattern of cell-destruction in I/R-induced intestinal epithelial cell injury.^{21,22} Several reports have demonstrated that prophylactic antiapoptotic treatment is an effective therapeutic strategy for the prevention of intestinal I/R injury.^{23,24} In this study, we showed that gastrin could attenuate intestinal crypt cell apoptosis, as evidenced by decline in the apoptotic index, caspase-3 activity and intestinal morphological injury. The antiapoptotic effect of gastrin might be associated with CCK-2 signaling activity. It has been reported that CCK-2 pathway could enhance cell proliferation in intestinal epithelium,²⁵ which could counteract apoptotic pathway such as Bax expression.

There are two issues to be explained. In our research, we found that 120 mg/kg omeprazole-induced hypergastrinemia has a protective effect on intestinal injury induced by I/R. It tells us that elevated gastrin in serum could prevent ischemia-stimulated intestinal injury, although the dose of omeprazole used in the research is much higher than the one administered in human disease. Gavage of 120 mg/kg omeprazole three times each day did not cause liver dysfunction of rats such as high level of alanine transaminase or aspartate transaminase (data not shown). In future, potential chemicals may be created to effectively and efficiently increase gastrin in serum with appropriate dose for human to exert intestinal protection. In addition, L365260 is believed to be specificity for the CCK-2 receptor.^{25,26} In our research, L365260, a CCK-2 receptor antagonist, indeed down-regulated the expression of CCK-2R. There may be an uncharted mechanism that could explain this effect. Our next work will focus on the detail-unknown effect. However, the unexplained effect does not challenge the blockade of L365260 on the binding of gastrin with CCK-2R.

In summary, this study reports that high gastrin in serum induced by omeprazole improves intestinal injury following I/R, partly by prohibiting the systemic inflammation

and intestinal crypt cells apoptosis via promoting CCK-2 receptor expression. Our results identify gastrin as a potential protein for providing effective intestinal protection in intestinal I/R damage.

Author contributions: ZL and YL contributed equally to this work; ZL, JX, and HZ designed the research; ZL, YL, YC, DZ, and AZ performed the research; YL, ZL, and CY analyzed the data; JX and HZ contributed reagents/materials/analysis tools; ZL and YL wrote the paper.

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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.

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