Role of matrix metalloproteinase-8 as a mediator of injury in intestinal ischemia and reperfusion

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ABSTRACT: Acute mesenteric ischemia is associated with high morbidity and mortality. In recent studies, we found that the intestine is an important source of matrix metalloproteinase (MMP)8 during intestinal injury. We hypothesized that genetic ablation or pharmacological inhibition of MMP8 would reduce intestinal injury in mice subjected to intestinal ischemia–reperfusion (I/R) injury. Male mice aged 8–12 wk were subjected to intestinal I/R injury by transient occlusion of the superior mesenteric artery for 30 min. MMP8 was inhibited by genetic and pharmacological approaches. In vivo study endpoints included several functional, histological, and biochemical assays. Intestinal sections were assessed for barrier function and expression of tight junction proteins. I/R injury led to increased intestinal and systemic expression of MMP8. This increase was associated with increased intestinal neutrophil infiltration, epithelial injury, and permeability. I/R injury was associated with increased systemic inflammation and weight loss. These parameters were ameliorated by inhibiting MMP8. I/R injury caused a loss of the tight junction protein claudin-3, which was ameliorated by genetic ablation of MMP8. MMP8 plays an important role in intestinal I/R injury through mechanisms involving increased inflammation and loss of claudin-3. Inhibition of MMP8 is a potential therapeutic strategy in this setting.—Daly, M. C., Atkinson, S. J., Varisco, B. M., Klingbeil L., Hake, P., Lahni, P., Piraino, G., Wu, D., Hogan, S. P., Zingarelli, B., Wong, H. R. Role of matrix metalloproteinase-8 as a mediator of injury in intestinal ischemia and reperfusion. FASEB J. 30, 000–000 (2016). www.fasebj.org

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Intestinal mucosa has a high metabolic rate and is particularly sensitive to the effects of decreased perfusion (1). Ischemia of the intestine is associated with high morbidity and mortality, despite appropriate intensive care. Arterial ischemia initiates alterations in tissues by disrupting the oxygen supply and hindering aerobic energy metabolism (2). It is associated with intestinal barrier function loss and triggers a systemic inflammatory response. Reperfusion of the damaged tissue further exacerbates the injury and increases the risk of developing shock and multiorgan system failure (3). Although well studied, the pathophysiological mechanisms underlying intestinal ischemia–reperfusion (I/R) injury and proper therapeutic approaches are not completely understood.

Matrix metalloproteinase (MMP)8 is a neutrophil-derived collagenase involved in the degradation of type I collagen. It also plays a role in neutrophil cell migration and chemotaxis, chemokine modulation, and cleavage of noncollagen proteolytic targets (4–6). An imbalance between matrix metalloproteinases and their tissue inhibitors is thought to play an important role in the pathophysiology of intestinal inflammatory conditions (7).

We recently demonstrated that the intestine is an important source of MMP8 in animal models of sepsis involving polymicrobial peritonitis with concomitant intestinal injury (8). Based on these findings and the biological overlaps between sepsis and intestinal ischemia–reperfusion (I/R), we now test the hypothesis that MMP8 is an important mediator of intestinal I/R injury.

MATERIALS AND METHODS

Murine model

All aspects of this study complied with the Guide for the Care and Use of Laboratory Animals published by the U.S. National

ABBREVIATIONS: ABC, avidin-biotin peroxidase complex; BSA, bovine serum albumin; Dlgl, Drosophila disc large tumor suppressor; H&E, hematoxylin and eosin; I/R, ischemia–reperfusion; MMP, matrix metalloproteinase; MPO, myeloperoxidase; PSD95, postsynaptic density protein 95; SMA, superior mesenteric artery; WT, wild-type; ZO-1, zonula occludens-1

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Institutes of Health (8th Ed, 2011) and met the approval of the Institutional Animal Care and Use Committee. MMP8-null mice on a C57BL/6 background were provided by Dr. Steven Shapiro, University of Pittsburgh. Wild-type (WT) C57BL/6 mice were obtained from Charles Rivers Laboratories (Wilmington, MA, USA). All mice were fed standard rodent chow and maintained on 12 h light/dark cycles.

Murine model of I/R injury

Male mice aged 8–12 wk were anesthetized with isoflurane 1–3% at 1–1.5 L/min, and a midline laparotomy was performed. The superior mesenteric artery (SMA) was isolated and clamped for 30 min with a vascular clamp. Control mice were subjected to a laparotomy with no clamping of the SMA. Moist gauze was placed over the exposed intestine for the duration of the ischemic period. After 30 min, the vascular clamp was removed. The abdominal cavity was closed with interrupted 6-0 silk sutures. The skin was closed with Gluture Tissue Adhesive (Abbott Laboratories, Abbott Park, IL, USA). All mice received a 1 ml s.c. injection of 0.9% normal saline into the scrub of the neck for resuscitation immediately after skin closure. Isoflurane anesthetic was discontinued, and the animals were returned to a cage with access to water and standard rodent chow. The mice were monitored for 7 d with daily weight measurements or were euthanized at 1, 2, 3, and 4 h for procurement of biological specimens. Animals received an intraperitoneal injection of sodium pentobarbital 50 mg/kg body weight. Cardiac puncture was performed with withdrawal of 0.8–1.0 ml whole blood. Lung, liver, kidney, spleen, heart, and intestinal tissues were collected for analysis.

The MMP8 inhibitor [(3R)-(+)\{-2-(4-methoxybenzenesulfonyl)\}-1,2,3,4-tetrahydroisquinoline-3-hydroxamate] (EMD Millipore, Billerica, MA, USA) was dissolved in 1% DMSO in PBS to a final concentration of 0.03 mg/ml. Mice treated with MMP8 inhibitor received a 1 ml s.c. injection at the dose of 0.3 mg/kg body weight at 12-h intervals starting 24 h before clamping of the SMA. The third and final dose was administered immediately after abdominal closure. Mice treated with vehicle received 3 intraperitoneal injections of 1% DMSO in PBS at a dose of 0.3 mg/kg body weight at 12-h intervals starting 24 h before clamping of the SMA.

Measurement of myeloperoxidase activity

Myeloperoxidase (MPO) activity was measured as an indication of neutrophil infiltration in lung and intestinal tissue. Lung tissue was homogenized in a buffer containing 0.5% hexadecyltrimethylammonium bromide in 10 mM 3-(N-morpholino)propanesulfonic acid. After homogenization, tissue was centrifuged for 30 min at 4000 rpm at 4°C and supernatant was collected. The supernatant was then mixed with 3,3,5,5 tetramethylbenzidine for 30 min at 4000 rpm at 4°C and supernatant was collected. The panesulfonic acid. After homogenization, tissue was centrifuged for analysis.

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Histology

Intestinal tissue (distal ileum) was harvested and placed immediately in 10% neutral-buffered formalin. The tissue was then embedded in formalin, sectioned, and stained with hematoxylin and eosin (H&E). Light microscopy was used to evaluate cross sections for tissue damage and inflammation. Four blinded, independent observers graded 25 slides of each group based on the following villous injury severity scale (9): a score of 0 indicated normal mucosa; a score of 1 represented development of subepithelial Gruenahren’s space and vacuolization at the villus tip; a score of 2 demonstrated extension of the subepithelial space with moderate lifting of the epithelial layer from the lamina propria; a score of 3 indicated massive subepithelial lifting/sloughing and increased vacuolization from the tip to midportion of villi; a score of 4 represented epithelial lifting and vacuolization from the tip to lower portion of villi; and a score of 5 corresponded to mucosal ulceration and disintegration of the lamina propria.

MMP8 serum protein concentrations

MMP-8 serum protein concentrations were measured on serum samples collected at 4 h using a magnetic bead-based immunoassay (HSP4MAG-63K, EMS-Millipore) and a Luminex multiplex system (Austin, TX, USA) according to instructions from the manufacturer.

In vivo measurement of intestinal permeability

Mice were kept on solid food restriction with water ad libitum for 6 h before surgery. Intestinal mucosal permeability to fluorescein isothiocyanate-conjugated dextran (FD-40, molecular weight 40 kDa; Sigma-Aldrich, St. Louis, MO, USA) was measured in vivo. Animals underwent oral gavage with 20 ml/kg body weight 30 min before induction of anesthesia. At the time of death (4 h after reperfusion and 5 h after gavage), blood was collected and centrifuged at 3000 rpm at 4°C for 20 min. Serum samples were subsequently assayed with a fluorescence spectrophotometer with an excitation wavelength of 485 nm and an emission wavelength of 525 nm. To determine the concentration of FITC-dextran, a standard curve was obtained through serial dilution in normal mouse plasma.

Bacterial clearance

At 4 h after ischemia, blood samples were obtained via direct cardiac puncture using sterile technique. With a sterile 22-gauge needle, 3 ml of sterile was injected through the fascia into the peritoneal cavity and gently aspirated. Serial dilutions of blood and peritoneal fluid lavage were plated on nutrient agar and incubated overnight at 37°C. Colony counts were performed to assess bacterial burden.

Immunofluorescence of tight junction proteins

During organ harvest, the small intestine was flushed with 30 ml 0.9% normal saline. Distal ileum segments (~1 cm in length) were excised and placed in foam cassettes in 4% paraformaldehyde for 24 h. The cassettes were placed in a 30% sucrose solution for an additional 24 h. Frozen intestinal sections were fixed in 10% aceton for 10 min, rinsed in PBS, and blocked with 10% bovine serum albumin (BSA)/PBS for 1 h at room temperature. The slides were then incubated with primary antibodies as follows: rabbit anti-claudin-3 (Thermo Fisher Scientific, Rockford, IL, USA); rabbit anti-ZO-1 (Thermo Fisher Scientific) at a dilution of 1:50 in 10% BSA/PBS. Sections were incubated with isotype control alone in place of primary antibody as a negative control. After 16 h incubation at 4°C, sections were washed with PBS and incubated with goat anti-rabbit Alexa Fluor 594 (Thermo Fisher Scientific) at a dilution of 1:50 in 1× PBS for 60 min at room temperature. Slides were then washed in PBS and counterstained with DAPI dihydrochloride/Supplement G.
solution (Fluoromount-G; Sigma-Aldrich). Representative images were captured with an A1 LUNA inverted confocal microscope (×10 magnification; Nikon, Melville, NY, USA). For quantitative image analysis, the sections were tile-scanned at ×4 magnification with a Ti-E SpectraX widefield inverted microscope (Nikon) with a Zyla 4.2 SCMOS monochrome camera with LED illumination source (Andor, Belfast, United Kingdom). All slides were stained and imaged together using the same settings. Quantitative image analysis was performed using Elements (Nikon) by a blinded observer who used tissue autofluorescence (green) to identify villi that were cut longitudinally and contained a lacteal so as to be sample villi that were sectioned similarly and not biased toward villi with greater or lesser signal and to avoid signal that came from luminal contents, glands, and lymphoid tissue (Supplemental Fig. S1). The mean fluorescent intensity of the red channel of ROIs was subtracted from background and this value was used for quantification of claudin-3 and ZO-1.

Immunohistochemistry
Small intestine sections (distal ileum) harvested at 1, 2, 3, and 4 h after ischemia were fixed and embedded in paraffin, and 5 μm thick sections were cut and mounted onto slides. The paraffin sections were deparaffinized in xylene, rehydrated, and placed in a citrate buffer (pH 6.0) which was placed in a 100°C water bath for 45 min for antigen retrieval. The sections were incubated in 0.5% hydrogen peroxide in methyl alcohol for 15 min at room temperature. Sections were then washed in PBS and incubated in normal goat serum for 2 h to block nonspecific binding of secondary antibodies. The sections were incubated overnight at 4°C with rabbit anti-MMP8 at 1:250 dilution in blocking serum (Abcam, Cambridge, MA, USA) and then in donkey anti-rabbit secondary antibody at 1:200 dilution in blocking serum for 1 h (Sigma-Aldrich). Immunohistochemistry was performed by the avidin-biotin peroxidase complex (ABC) method. The Vectastain Elite ABC kit was used, as instructed by the manufacturer (Vector Laboratories, Burlingame, CA, USA). Immunoreactivity was detected with 3,3′-diaminobenzidine as a peroxidase substrate, and the sections were counterstained with Nuclear fast Red (Sigma-Aldrich). After rinsing in distilled water, the tissue was dehydrated and subsequently rinsed with xylene. A coverslip was placed with Permount (Thermo Fisher Scientific). Slides that had not been incubated with primary antibody were included as negative controls. Photomicrographs were taken using a Axio ImagerA.2 (Zeiss, Thornwood, NY, USA).

Data analysis
Statistical analyses were conducted using SigmaStat Software (Systat Software, Inc., San Jose, CA, USA). Data are represented as means ± SEM or medians with interquartile range. For multiple group analysis at a single time point, 1-way ANOVA with Student-Newman-Keuls correction was used. If data failed to follow a normal distribution, a Mann-Whitney Rank Sum test or an ANOVA on ranks test was performed. When 2 groups were compared at the same time point, a Student’s t test was performed.

Figure 1. Intestinal permeability as measured by FITC-dextran after I/R injury in (A) WT mice treated with MMP8 inhibitor and WT mice treated with vehicle; (B) untreated WT mice and MMP8-null mice. Box plots represent the 25th percentile, median, and 75th percentile; error bars define the 10th and 90th percentiles (n = 35–42). *P < 0.05. Values reported as fluorescence fold increase relative to respective sham. Absolute change in weight over 7 d recovery following I/R injury in (C) WT mice treated with MMP8 inhibitor or vehicle. (D) MMP8-null mice, and WT mice (WT + MMP8 inhibitor, n = 12; WT + vehicle, n = 11; MMP8-null, n = 13; WT mice, n = 12); values reported as mean ± SEM; *P < 0.05.
RESULTS

Inhibition of MMP8 prevents intestinal barrier dysfunction following I/R injury

Intestinal permeability was evaluated by the fluorescent tracer FITC dextran (FD-40; Sigma-Aldrich). WT mice treated with MMP8 inhibitor showed significantly reduced intestinal permeability to FITC dextran, as compared to WT mice treated with vehicle (Fig. 1A). Likewise, MMP8-null mice had reduced intestinal permeability to FD-40 compared with WT mice (Fig. 1B). No bacterial translocation was observed when blood and peritoneal lavage fluid were analyzed (data not shown).

Mice subjected to I/R injury were monitored and weighed daily for 7 d, and absolute weight change was calculated. I/R injury caused an initial weight loss, with recovery in 5 d. WT mice treated with MMP8 inhibitor at 0.3 mg/kg showed a faster recovery compared with WT mice that received vehicle (Fig. 1C). Likewise, MMP8-null mice had less initial weight loss and recovered more quickly as compared to WT mice (Fig. 1D).

Collectively, these data demonstrate that I/R leads to functional abnormalities as measured by intestinal permeability and weight loss. These abnormalities are ameliorated by inhibition of MMP8.

I/R injury leads to increased expression of intestinal and systemic MMP8

Cytoplasmic expression of MMP8 in the distal ileum of WT mice is indicated at 1, 2, 3, and 4 h after intestinal I/R (Fig. 2A). MMP8 expression peaked at 3 h after ischemia, with expression still evident at 4 h. Sections from WT sham mice showed faint staining. Sections from MMP8-null sham mice and MMP8-null mice that underwent intestinal I/R injury had no positive staining.

WT mice subjected to I/R injury had significantly higher MMP8 serum concentrations compared to sham mice. As expected, MMP8-null mice had undetectable serum protein concentrations (Fig. 2B). WT mice treated with the MMP8 inhibitor and subjected to I/R injury had similar significant increases of MMP8 serum concentrations as that seen in WT mice treated with vehicle and subjected to I/R (Fig. 1C), because the MMP8 inhibitor inhibits MMP8 activity, rather than its expression. There were no significant differences in MMP8 serum concentrations after I/R injury among WT mice that received MMP8 inhibitor, as compared with WT mice that received vehicle. The collective data demonstrate that intestinal I/R leads to increased local and systemic expression of MMP8.
Inhibition of MMP8 decreases neutrophil infiltration in the intestine and lung and prevents intestinal damage

Morbidity and mortality from intestinal ischemia is partly due to an excessive host inflammatory response to injury. We assessed whole lung and intestinal inflammation by measuring MPO activity as a surrogate of neutrophil infiltration, 4 h after reperfusion. MMP8-null mice had a significant reduction of intestinal MPO activity after I/R injury as compared to WT mice (Fig. 3A). WT mice treated with MMP8 inhibitor also had a significant reduction of intestinal neutrophil infiltration as compared with wild-type mice treated with vehicle (Fig. 3B). A significant decrease in lung MPO activity was recorded in MMP8-null mice after I/R vs. that in WT mice (Fig. 3C). There was no significant difference in lung neutrophil infiltration between WT mice treated with MMP8 inhibitor and WT mice treated with vehicle (Fig. 3D).

Light microscopy was used to evaluate H&E-stained distal ileum sections (Fig. 3E). Normal villi with intact epithelial cells were observed in the WT sham group. WT mice subjected to I/R injury showed severe villous injury with mucosal ulceration and disintegration of the lamina propria. In contrast, MMP8-null mice subjected to I/R injury demonstrated mild injury as did WT mice treated with the MMP8 inhibitor and subjected to I/R injury. The semiquantitative results of the histopathology in the respective experimental groups are shown in Fig. 3F, G.

These data show that intestinal I/R leads to significant histologic injury, in association with increased local and systemic inflammation, as measured by neutrophil infiltration. Inhibition of MMP8, either through genetic ablation or pharmacologic means, significantly reduces histologic injury in association with attenuated inflammation.

**Figure 3.** Severity of intestinal injury and systemic inflammatory response after I/R injury. MOP activity measured as an indication of neutrophil infiltration in lung and intestinal tissue. A) Intestinal neutrophil infiltration after I/R injury in MMP8-null mice and untreated WT mice; (B) WT mice treated with MMP8 inhibitor (WT + MMP8 Inh) and and WT mice treated with vehicle (WT + vehicle). C) Lung MPO activity after intestinal I/R injury in MMP8-null mice, WT mice, D) WT + MMP8 inhibitor and WT + vehicle. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol hydrogen peroxide/min at 37°C, expressed in units per 100 mg of tissue. Box plots represent the 25th percentile, median, and 75th percentile; error bars define the 10th and 90th percentiles (n=38–46). *P ≤ 0.05 vs. WT mice. E) Photomicrographs depicting H&E-stained intestinal sections. Representative sections from WT sham mice, WT mice which underwent I/R injury, MMP8-null I/R mice, and WT mice + MMP8 inhibitor I/R. Original magnification, ×40. F, G) Median histologic grade of WT sham mice, and the following groups after I/R injury: WT + vehicle I/R, WT + MMP8 inhibitor (Inh) I/R, WT I/R, and MMP8-null I/R; box plots represent the 25th percentile, median, and 75th percentile; error bars define the 10th and 90th percentiles (n=26-30); *P < 0.05.
Genetic ablation of MMP8 preserves claudin-3 expression

The intestinal epithelium serves as a barrier and is maintained by tight junction proteins, such as cadherins, claudins, occludin, and junction adhesion molecules (10). Impairment of the epithelial barrier is a critical component to the severity of intestinal injury. Claudins are anchored to the cytoskeleton via interactions with PDZ-binding domain proteins, including zonula occludens (ZO)-1, -2 and -3. We used immunofluorescence to visualize changes in tight junction protein expression during I/R injury. Figure 4 shows representative images of claudin-3 expression among the experimental groups. Expression is shown as predominantly apical staining. Qualitatively, claudin-3 expression was reduced after I/R injury, relative to the respective sham-treated animals, and this effect was ameliorated by either genetic ablation or pharmacologic inhibition of MMP8. To further evaluate this observation, we quantified claudin-3 expression using image analysis conducted in a blinded manner. Figure 5A shows that ischemia reperfusion injury significantly decreased claudin-3 expression in WT animals, but not in MMP8-null animals. When we used a pharmacological approach, we saw a similar pattern among vehicle-treated animals and animals treated with the MMP8 inhibitor, respectively, but the differences were not statistically significant. No difference was seen among the groups for ZO-1 expression (data not shown).

These data demonstrate that I/R leads to loss of claudin-3 and that genetic ablation of MMP8 reverses this process. Therefore, MMP8 appears to play a role in intestinal ischemia reperfusion injury by a mechanism involving disruption of claudin-3.

DISCUSSION

The results of our study suggest a novel role for MMP8 in intestinal I/R injury. MMP8 was expressed systemically and locally in the intestinal epithelium. Locally, peak expression of MMP8 was seen in intestinal sections at 3 h after ischemia. Genetic ablation or pharmacologic inhibition of MMP8 led to a reduction of mucosal injury as evidenced by histology. Inhibition of MMP8 decreased both acute and longer term functional consequences of intestinal I/R injury. Pharmacological inhibition of MMP8 caused an acute decrease in intestinal permeability induced by I/R as measured by permeability to FITC-dextran. Increased permeability induced by I/R may provide a route for bacterial translocation, although we did not observe this in our study. In the longer term, inhibition of MMP8 led to less weight loss and faster recovery in body weight.

The exact mechanism by which MMP8 contributes to intestinal injury requires further exploration. One potential mechanism involves increased inflammation, as demonstrated in our study examining the role of MMP8 in sepsis (8). However, the role of MMP8 in modulating inflammation is complex and dependent on the experimental model. In some experimental models MMP8 plays a proinflammatory role. For example, in a murine model of TNFα-mediated hepatitis, genetic ablation of
MMP8 protects against liver failure (11). In other studies, MMP8-null mice injected subcutaneously with either chemical carcinogens (12) or LPS (13) showed less local neutrophil infiltration than did WT mice. In contrast, there are studies indicating that MMP8 has an anti-inflammatory role. For example, intratracheal installation of LPS leads to greater neutrophil infiltration of the lung in MMP8-null mice, compared to WT mice (14). Similarly, lung injury caused by bleomycin or hyperoxia is associated with greater neutrophil infiltration in MMP8-null mice (15). Inflammation is a known cause of I/R injury and our data are consistent with this concept. Our model shows evidence of increased inflammation after intestinal I/R injury, as measured by neutrophil infiltration. Inhibition of MMP8 reversed the inflammatory response in this setting. Of note, we did not see a difference in lung neutrophil infiltration between WT mice treated with vehicle and WT mice treated with MMP8 inhibitor. It is possible that the lack of significance is related to the dosage of MMP8 inhibitor. The MMP8 inhibitor dose of 0.3 mg/kg body weight was chosen because there was evidence of an effect seen in both histologic and permeability analysis at this dosage. Although the inhibitor may have incompletely inhibited the activity of multiple matrix metalloproteinases, our MMP8 knockout data demonstrates an important and specific role for MMP8 in increasing intestinal barrier permeability in ischemia reperfusion injury.

Other proteases have been identified as mediators of inflammation in this setting. Inhibition of PAR-activating serine proteases have been shown to reduce intestinal inflammation resulting from I/R injury (16). Trypsin has been associated with activation of MMP9 resulting in an increased inflammatory response after injury (17).

One contributing factor to this increased permeability appears to be MMP8-mediated degradation of claudin-3, a critical component of the epithelial barrier. It has been demonstrated that MMP2, -3, -9, and -13 contribute to the disruption of tight junction proteins and degradation of the extracellular matrix in several I/R models. For example, in brain ischemia, MMP9 and -13 were associated with cleavage of claudin-5, occludin, and ZO-1. In renal I/R injury, MMP14 results in proteolysis of E-cadherin and MMP9 was associated with cleavage of ZO-1, and occludin (18). However, the effects of MMP8 on epithelial barrier function and tissue integrity have not been described.

To our knowledge, our study is the first to identify claudin-3 as a potential substrate of MMP8. We have demonstrated decreases in claudin-3 after I/R injury and that claudin-3 expression is preserved in MMP8-null animals. However, we were unable to fully replicate this finding when using an MMP8 inhibitor—perhaps because of incomplete inhibition of activity. Accordingly, further study is needed to definitively identify claudin-3 as a substrate of MMP8.

We note the limitations of our study. We focused our investigation on early gut dysfunction, 4 h after intestinal I/R injury. However, our functional studies of weight loss suggest that the role of MMP8 extends beyond this acute period. Another limitation surrounds the specificity of the MMP8 inhibitor. We previously showed that treatment of MMP8-null mice with this MMP8 inhibitor did not decrease the inflammatory response as compared to MMP8-null mice treated with vehicle (19). Despite this, there is evidence of overlap among individual MMPs in modulation of various signaling pathways (20). It is challenging to demonstrate complete specificity with no off-target effects. Furthermore, because the MMP8 inhibitor is solubilized in DMSO and PBS, it is possible that DMSO has a generalized anti-inflammatory effect causing an artificial augmentation of the results in mice treated with MMP8 inhibitor. Finally, our pretreatment strategy with the MMP8 inhibitor is not clinically relevant. We conducted our experiments in this manner as a complementary approach to our MMP8-null mice. Nonetheless, future work should focus on strictly postinjury treatment with MMP8 inhibitor to evaluate potential clinical relevance.

MMP8 inhibition has been proposed as a potential therapeutic target for several diseases, including sepsis (8, 19, 21), inflammatory bowel disease (22), chronic obstructive pulmonary disease (23), cerebral ischemia (18), acute myocardial infarction (24), and acute lung injury (25). Success depends on target specificity of the compound. Our study identified MMP8 as a potential therapeutic strategy in the setting of intestinal I/R injury.

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AUTHOR CONTRIBUTIONS

M. Daly, B. Varisco, B. Zingarelli, and H. Wong designed the research; M. Daly and P. Hake maintained the animal colony; M. Daly, S. Atkinson, B. Varisco, L. Klingbeil, P. Hake, P. Lahni, G. Piraino, D. Wu, and H. Wong performed the research; M. Daly, B. Varisco, S. Hogan, B. Zingarelli, and H. Wong analyzed the data; M. Daly, B. Varisco, B. Zingarelli, and H. Wong wrote the manuscript; M. Daly, S. Atkinson, B. Varisco, L. Klingbeil, P. Hake, P. Lahni, G. Piraino, D. Wu, S. Hogan, B. Zingarelli, and H. Wong performed critical revisions of the manuscript.

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