Bradykinin B
\textsubscript{2} Receptor Antagonist FR173657 Ameliorates Small Bowel Ischemia–Reperfusion Injury in Dogs

KAZUHISA ARAKAWA, MD,* IZUMI TAKEYOSHI, MD,* YOSHIHIKO AKAO, MD,* OSAMU TOTSUKA, MD,* KOSHI MATSUMOTO, MD,† and YASUO MORISHITA, MD*

Bradykinin mediates acute inflammation by increasing microvascular permeability, vasodilation, leukocyte migration and accumulation, and the production of arachidonic acid via phospholipase A\textsubscript{2} activation. Arachidonic acid metabolites, or eicosanoids, are potent modulators of biological functions, particularly inflammation. Bradykinin exerts its inflammatory effects via the bradykinin B\textsubscript{2} receptor. The aim of this study was to evaluate the effect of a bradykinin B\textsubscript{2} receptor antagonist, FR173657 (FR), on intestinal ischemia-reperfusion (I/R) injury. Twenty-eight mongrel dogs were divided into four groups (n = 7 per group). Group I underwent I/R alone, Group II underwent I/R and received FR treatment, Group III was sham operated, and Group IV was sham operated and received FR treatment. The FR treatment consisted of FR continuously from 30 min prior to ischemia to 2 hr after reperfusion. In the I/R procedure, the superior mesenteric artery (SMA) and vein were clamped for 2 hr and then released to permit reperfusion for 12 hr. The intramucosal pH (pH\textsubscript{i}), SMA blood flow, and mucosal tissue blood flow were measured during the reperfusion period. The serum thromboxane B\textsubscript{2} and 6-keto-prostaglandin F\textsubscript{1α} levels were determined, and tissue samples were examined histologically. Results showed that tissue blood flow, pH\textsubscript{i}, and SMA blood flow after reperfusion were maintained in Group II in comparison with Group I. Histopathological examination showed less severe mucosal damage after reperfusion in Group II than in Group I. The serum thromboxane B\textsubscript{2} and 6-keto-prostaglandin in F\textsubscript{1α} levels were significantly lower in Group II than in Group I (P < 0.05). We conclude that FR treatment appears to have clear protective effects on small bowel I/R injury by inhibiting the release of eicosanoids.

**KEY WORDS:** small intestine; ischemia–reperfusion injury; bradykinin B\textsubscript{2} receptor antagonist; FR173657.

Small bowel transplantation (SBT) is an important therapy for patients with intestinal failure. In SBT, ischemia and reperfusion (I/R) of the graft are inevitable consequences of the surgical process. As the small bowel is susceptible to I/R injury, the minimization of I/R injury to the graft is a crucial factor in determining the success of SBT. I/R causes the activation of neutrophils and the production of oxygen free radicals, cytokines, nitric oxide, and arachidonic acid metabolites, such as thromboxane (TX) B\textsubscript{2} and leukotriene (LT) B\textsubscript{4} (1–5). Intestinal I/R injury is characterized by the infiltration of neutrophils, platelet aggregation, vasoconstriction, and changes in capillary permeability. These changes result, in part, from altered arachidonic acid metabolism (5). Arachidonic acid products are synthesized via a complex enzyme cascade that is regulated by the principal enzyme phospholipase A\textsubscript{2} (PLA\textsubscript{2}).

*From the *Second Department of Surgery, Gunma University School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, and †Department of Pathology, Nippon Medical School, 1-396 Kosugi-chou, Nakaharaku, Kawasaki, Kanagawa 211-8533, Japan.

Address for reprint requests: Izumi Takeyoshi, MD, Second Department of Surgery, Gunma University School of Medicine 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan; takeyosi@showa.gunma-u.ac.jp.

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PLA₂ causes progressive degradation of phospholipids during ischemia (6, 7), which leads to the accumulation of cytotoxic products, such as free fatty acids and lysophospholipids, in ischemic tissues. These products are further metabolized to precursors of specific pro-inflammatory lipid mediators, which include platelet-activating factor (PAF) and eicosanoids such as prostaglandins (PG) and LTs. PLA₂ is activated by increased levels of intracellular or extracellular calcium, which are induced by the inflammatory mediator bradykinin (8–10).

Bradykinin and related peptides are believed to be key mediators of inflammatory diseases. During acute inflammation, bradykinin acts via the bradykinin B₂ receptor (11). Bradykinin B₂ receptor antagonists have demonstrated benefits in the treatment of pancreatitis (12) and pancreatic (13) and pulmonary (14) I/R injury. Therefore, we hypothesized that the bradykinin B₂ receptor antagonist FR173657 (FR) might decrease the production of eicosanoids and attenuate small intestinal I/R injury. The aim of this study was to evaluate the effect of FR on small intestinal I/R injury in an in situ warm ischemia model in dogs.

MATERIALS AND METHODS

Animals. Twenty-eight adult mongrel dogs of both sexes, weighing 12 to 18 kg, were used in this study. The dogs were fasted but had free access to water for 24 hr prior to the experiment. All of the animals were cared for in accordance with the guidelines set forth in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85-23; revised 1985). This study was also approved by the Animal Care and Experimentation Committee, Gunma University, Showa Campus.

Surgical Procedures. After intramuscular administration of ketamine hydrochloride (2 mg/kg), the animals were anesthetized with intravenous pentobarbital sodium (15 mg/kg). Endotracheal intubation was performed, and the animals were ventilated mechanically at a tidal volume of 20 ml/kg and a rate of 10 breaths/min. Anesthesia was maintained with inhalation of 1 to 2% halothane, and muscular relaxation was obtained with additional pancuronium bromide (0.1 mg/kg).

The surgery was performed under sterile conditions. A polyethylene catheter was positioned in the abdominal aorta through the right femoral artery and connected to a pressure transducer, to record arterial pressure. Another polyethylene catheter was inserted into the inferior vena cava via the right femoral vein, for infusion of lactated Ringers solution at a rate of 30 ml/kg/hr for 6 hr after reperfusion, and thereafter at a rate of 15 ml/kg/hr, to compensate for fluid loss. A third polyethylene catheter was positioned in the superior mesenteric vein (SMV) through the left gastric vein, to allow for blood sampling.

Laparotomy was performed via a midline incision after the blood pressure and respiration parameters had stabilized. The small bowel was isolated with the vascular pedicle, and both the superior mesenteric artery (SMA) and the SMV were cleared from the surrounding lymph nodes, plexuses, and tissues. The proximal jejunum and distal ileum were resected in order to interrupt the intramural blood flow. Warm ischemia was induced by clamping the SMA and SMV for 2 hr. One hour after reperfusion, proximal intestinal continuity was restored with an end-to-end anastomosis. The resected distal intestine was used as a stoma for taking whole-thickness tissue samples. During the 6-hr experimental period, the abdominal cavity was closed and opened temporarily for each measurement. After 6 hr of reperfusion, the abdominal wall was closed again, and all the animals recovered from anesthesia. The experiment was discontinued at 12 hr after reperfusion. Catecholamines were not used at any point during the experiment.

Experimental Groups. The animals were divided into four groups (n = 7 per group). The Group I animals underwent I/R and received vehicle alone. The Group II animals received FR173657 (0.06 mg/kg/hr) continuously beginning 30 min prior to the onset of ischemia and continuing for 2 hr after reperfusion. The Group III animals underwent a sham operation for a comparable time, under the same anesthesia. The Group IV animals underwent a sham operation and received FR173657.

Superior Mesenteric Artery Blood Flow. SMA blood flow was measured prior to ischemia and at 1, 3, and 6 hr after reperfusion, using an electromagnetic blood flowmeter (Model MFV-3100; Nihonkohden Co., Ltd., Tokyo). SMA blood flow is expressed as the percentage of the level that was determined prior to ischemia.

Tissue Blood Flow Measurements. Tissue blood flow was measured in the small bowel mucosa using a laser Doppler flow meter (ALF 21; ADVANCE Co., Ltd., Tokyo) prior to ischemia and at 1, 3, and 6 hr after reperfusion. Each measurement was made at three points by inserting a probe through an incision in the bowel wall and placing it against the antimesenteric side of the bowel lumen. The laser probe reading reflects tissue blood flow within about 1.0 mm of the surface of the bowel wall. Tissue blood flow was calculated as the mean of the three measurements and is expressed as the percentage of the level that was determined prior to ischemia.

Intramucosal pH Measurements. Intramucosal pH (pHi) was measured prior to ischemia, during ischemia, and at 1, 3, 6, and 12 hr after reperfusion. This method has been described previously (15). In brief, a tonometer (Trip; Tonometrics, Helsinki, Finland) was inserted into the small bowel lumen through a small incision in the bowel wall and secured with a purse-string suture. Within 40 min, the PCO₂ of the saline in the balloon had equilibrated with the intraluminal PCO₂, which reflects the mucosal PCO₂ of the bowel. The HCO₃⁻ concentration in the bowel wall was assumed to be the same as the HCO₃⁻ concentration in the arterial blood. The saline PCO₂ and the HCO₃⁻ concentration in the artery were determined with a blood gas analyzer (Stat Profile M; Nova Biomedical Co., Waltham, MA) and were used to calculate the pHi using the Henderson–Hasselbach equation:

\[ \text{pHi} = 6.1 + \log[\text{arterial HCO}_3^-/(0.03 \times \text{saline PCO}_2)] \]

Histology. Whole-thickness specimens of the ileum were harvested prior to ischemia, just before reperfusion, and at 1, 3, 6, and 12 hr after reperfusion. The intestinal sections for histology were fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned at 3- to 5-μm thickness, and stained with hematoxylin and eosin or with naphthol AS-D chloroacetate esterase to identify leukocytes. The degree of intestinal mucosal injury was evaluated on a graded scale from 0 to 5, as originally described by Chiu et al. (16). Grade 0 is defined as normal mucosa. With increasing damage, the subepithelial space of the villi