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Interstitial Cells of Cajal in Dysmotility in Intestinal Ischemia and Reperfusion Injury in Rats

Naoki Shimojima, M.D.,*[,1] Toshio Nakaki, M.D., Ph.D.,† Yasuhide Morikawa, M.D., Ph.D.,* Ken Hoshino, M.D., Ph.D.,* Hiroshi Ozaki, D.V.M., Ph.D.,‡ Masatoshi Hori, D.V.M., Ph.D.,‡ and Masaki Kitajima, M.D., Ph.D.*

*Department of Surgery, Keio University School of Medicine; †Department of Pharmacology, Teikyo University School of Medicine; and ‡Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo, Japan

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Background. **Intestinal ischemia and reperfusion (I/R) injury is an obligatory occurrence in small bowel transplantation. I/R may impair the normal gastrointestinal motility. Interstitial cells of Cajal (ICC) are known as pacemaker cells in the gastrointestinal tract. The aim of this study was to assess the role of ICC in the gastrointestinal motility in a rat model of I/R injury.**

Materials and methods. **Wistar rats were subjected to 30- or 80-min intestinal ischemia by occluding the mesenteric vessels followed by reperfusion. Small intestinal segments were resected at 12 h or 4 days. The spontaneous mechanical activity was evaluated by organ bath technique. Immunopositivity of c-Kit and PGP9.5 at the level of the myenteric plexus was evaluated as markers of ICC and enteric nerves, respectively.**

Results. **In the bowel segment with 80-min ischemia followed by 12-h reperfusion, muscles showed a 25% reduction ($P < 0.05$) in the frequency of contractions compared to that with 30-min ischemia followed by 12-h reperfusion, whereas amplitude of contractions was not significantly different. This change was associated with a 70% decrease ($P < 0.01$) of c-Kit immunopositivity. These changes of intestinal motility pattern and distribution of c-Kit-positive cells were both recovered from 80-min ischemia followed by 4 days reperfusion. In contrast, the immunopositivity of PGP9.5 was not affected in any I/R injury group.**

Conclusions. **Transient functional changes in ICC were induced by prolonged I/R injury but they recovered after 4 days, suggesting a central role of ICC in both disrupting and restoring the normal gastrointestinal motility in I/R injury.** **© 2006 Elsevier Inc. All rights reserved.**

Key Words: **intestine; gastrointestinal motility; contraction; pacemaker; c-Kit; enteric nervous system; small bowel transplantation.**

INTRODUCTION

Ischemia reperfusion (I/R) injury is an obligatory factor in small bowel transplantation because the small intestine is one of the most susceptible organs to ischemia. In an I/R injury model of the small intestine, mucosal changes and the role of muscular layers in the local and systemic inflammatory response have been documented [1]. Some investigators have reported the alterations of gastrointestinal motility [2–7]. However, the role of the interstitial cells of Cajal (ICC), which are the pacemakers in gastrointestinal tract, in I/R injury has not been investigated.

ICC were first described by Ramón y Cajal, a Spanish anatomist, over 100 years ago [8]. Further investigations in the last two decades have clarified the roles of ICC [9–13]. In the small intestine, ICC form an extensive network between the circular and longitudinal muscle layers at the level of the myenteric plexus (ICC-MY), and a second population of ICC is found at the level of the deep muscular plexus in the circular muscle layer (ICC-DMP). ICC-MY are thought to be the main ICC required for smooth muscle pacing in the small intestine [14, 15]. Today, the role of ICC should be considered in addition to the enteric ganglia and smooth muscles when assessing the gastrointestinal motility.

c-Kit tyrosine kinase, which is expressed on the membrane of ICC, is essential for the development of

[1] To whom correspondence and reprint requests should be addressed at Division of Pediatric Surgery, Department of Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: mojimoji@sc.itc.keio.ac.jp.

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ICC [14, 16–19] and maintenance of their phenotype [20]. Recently, we reported that imatinib, a potent inhibitor of c-Kit tyrosine kinase, dose-dependently abolished spontaneous contractile activity in ring preparation of adult murine small intestine [21]. This result means that c-Kit signaling of ICC plays an essential role in the spontaneous mechanical activity of intestine. In morphological studies, ICC can be detected as the c-Kit-positive cells in the circular and longitudinal muscle layers and at the level of the myenteric plexus in the stomach, at the levels of the myenteric plexus and deep muscular plexus in the circular muscle layer in the small intestine, and at the level of the myenteric plexus and submucosal surface of the circular muscle layer in the colon [18, 22].

The aim of this study was to assess the role of ICC in the gastrointestinal motility in a rat model of I/R injury. We evaluated the intestinal spontaneous mechanical contractions, which were presumably driven by ICC, and the morphological alterations of the network of ICC-MY by immunohistochemical staining for c-Kit in three different conditions of I/R injury. This is the first report documenting the function and morphology of ICC in I/R injury.

MATERIALS AND METHODS

Animals

Male Wistar rats (200–240 g) were purchased from Crea Japan, Inc. (Tokyo, Japan). All animals were housed under specific pathogen-free conditions in our animal facilities. They were maintained using a 12-h light/dark cycle and had free access to chow and water. All experimental procedures complied with the guidelines set forth by Keio University and were approved by the institutional ethics committee.

Operative Procedures

Operations for experimental animals were performed under general anesthesia by isoflurane inhalation. After the laparotomy by midline incision, the cecum was placed at right lower position and the small bowel was retracted to the left side so that the mesentery was well expanded and the superior mesenteric artery and vein were well identified. The pancreas was separated from the ascending mesocolon. The vascular arcades between the descending branch of the right colic artery and the ascending branch of the ileocolic artery, between the descending branch of the ileocolic artery and the last ileal artery, and between the jejunal arteries just proximal and distal to the point of the superior mesenteric artery occlusion were ligated to interrupt the collateral flow. In addition, for interruption of the collateral flow within the intestinal wall, bulldog clamps were applied to the jejunum and terminal ileum at the same levels of previous ligations for vascular arcades. Then, the superior mesenteric artery was occluded just proximal to the right colic artery by vascular microclip. The right colic artery and vein and the ileocolic artery and vein were also occluded. Adequate degree of ischemia was confirmed by complete disappearance of mesenteric arterial pulsation and the intestinal color turning immediately pale. After the planned ischemic period, vascular microclips and bulldog clamps were removed and blood reperfusion of the intestine was confirmed by immediate change of intestinal color from white to pink and recovery of mesenteric arterial pulsations. The intestines were carefully returned to the abdomen and the incision was closed by two-layer continuous 3-0 silk sutures.

Experimental Groups

Rats were divided into three groups according to the ischemia and reperfusion periods: ischemia for 30 min followed by reperfusion for 12 h (I_{30}/R_{12h}); ischemia for 80 min followed by reperfusion for 12 h (I_{80}/R_{12h}); and ischemia for 80 min followed by reperfusion for 4 days (I_{80}/R_{4d}) ($N = 5$ in each group). Sham control animals ($N = 5$ in each group) were treated in an identical fashion except for vascular clamping.

After each reperfusion period, animals were sacrificed by decapitation and the ileum was harvested.

Recordings of Spontaneous Mechanical Activity

Intestinal rings of approximately 5 mm of ileum were set in organ chambers with a volume of 10 mL buffer. The rings were suspended with hooks, which were connected to isometric transducers that recorded the mechanical tension generated by the circular muscles. A smoothing function of the recorder was not used.

All experiments were performed in Krebs-Ringer bicarbonate buffer (pH 7.4) at 37.0–37.5°C that was constantly saturated with a mixture of 95% O_2 and 5% CO_2 as described previously [23]. The composition of the buffer was as follows (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 11. After an equilibration period of 30 min, spontaneous mechanical contractions were recorded.

We confirmed that tetrodotoxin (0.1 μM) did not abolish spontaneous rhythmic contractions, suggesting that the basal mechanical activities were not of neuronal origin (data not shown).

The frequency and amplitude were calculated as intestinal motility parameters. Frequency of contractions was counted for 5 min, and the average number of contractions per minute in each sample was calculated. Amplitude was analyzed as area under the curve for 5 min and calculated in milligrams per 5 min in each sample.

Histopathology

We performed hematoxylin and eosin staining to evaluate the severity of the I/R injury. Ileum segments were fixed in 4% paraformaldehyde and embedded in paraffin. Longitudinal sections of 5 μm thickness were stained with hematoxylin and eosin and examined by light microscopy.

Immunohistochemical Staining

Immunohistochemical staining was performed against c-Kit and PGP9.5 as markers of ICC and enteric nerves, respectively. Full-thickness specimens of approximately 15 mm length were resected and opened along the mesenteric border. Specimens were stretched to approximately 150% and fixed with 2% periodate-lysine paraformaldehyde for 6 h and 4% paraformaldehyde for 1 h for c-Kit and PGP9.5 staining, respectively. Whole-mount preparation was made of each specimen using fine-pointed forceps and microsurgical scissors under the microscope in a PBS-filled dish.

For the c-Kit staining, specimens were incubated with 0.3% Triton X in 10% normal rabbit serum for 60 min and incubated with goat anti c-Kit polyclonal antibody (sc-1494; 1:200 in 0.05 M Tris buffer; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at 4°C overnight. After rinsing in PBS three times for 30 min, immunoreactivity was detected using Alexa Fluor 488 rabbit anti-goat IgG (1:500 in 0.05 M Tris buffer; Molecular Probes, Eugene, OR) at room temperature for 2 h, followed by rinsing in PBS twice for 30 min.

For the PGP9.5 staining, specimens were incubated with 0.3% Triton X in 10% normal goat serum for 60 min and incubated in rabbit anti-PGP9.5 polyclonal antibody (1:5000 in 0.05 M Tris buffer;

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