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Activation of NF-κB pathway in oral buccal mucosa during small intestinal ischemia-reperfusion injury

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ABSTRACT

Background: Intestinal ischemia-reperfusion injury induces intestinal mucosal barrier disruption, systemic inflammatory response syndrome, multiorgan failure, and death. The major pathway for the systemic inflammatory responses depends on nuclear factor kappa B (NF-κB). However, direct measuring of NF-κB in injured tissues is not routinely available. Our aim was to determine whether NF-κB pathway in buccal mucosa is activated during intestinal ischemia-reperfusion injury.

Materials and methods: Male Sprague-Dawley rats were prepared for the animal experiment. Superior mesenteric artery (SMA) was exposed and clamped for 30 min in the intestinal ischemia-reperfusion (IR) group. SMA was exposed only in control group. Serum, buccal mucosa, and small intestinal mucosa were harvested in 90 min after reperfusion in IR or 120 min after SMA exposure in control group. Serum cytokine levels and tissue NF-κB pathway activities were measured.

Results: Serum TNF-α (5.49 ± 2.72 *versus* 1.77 ± 1.20 pg/mL, $P = 0.002$) and interleukin-6 (232.32 ± 29.98 *versus* 115.92 ± 17.81 pg/mL, $P = 0.002$) levels were significantly higher in IR than control group. Intestinal mucosal cytoplasmic phosphorylated inhibitor kappa B (IκB)/IκB ratio, nuclear NF-κB expression, and NF-κB DNA-binding activity were significantly higher in IR than control group. Buccal mucosal cytoplasmic phosphorylated IκB/IκB ratio, nuclear NF-κB expression, and NF-κB DNA-binding activity were also higher in IR than control group.

Conclusion: Buccal mucosal NF-κB pathway was activated by intestinal ischemia-reperfusion injury. The present study suggests that buccal mucosal may be considered as an indicator for the assessment of intestinal ischemia-reperfusion injury.

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1. Introduction

Diminished blood supply results in an ischemic injury to the tissues with active metabolism. When blood flow is restored, additional cell damage called reperfusion injury is induced. Reperfusion injury often brings about more severe tissue damage than the initial ischemic injury [1]. The restored blood flow introduces a cascade of reperfusion damage as follows: release of reactive oxygen species and stored irons; micro-vasculatures damage; inflammatory cytokines release; complements activation; and neutrophilinfiltration [2–7].

The intestine is more vulnerable to the ischemia-reperfusion injury (IRI) than other internal organs [8,9]. Villous enterocyte is especially fragile to the IRI because of its hemodynamic characteristics and degree of differentiation [10–12]. Cascade reaction caused by the IRI deteriorates preventive functions of intestinal mucous. Intestinal contents, bacteria, and bacterial byproducts infiltrate mesenteric lymph nodes and portal vein by intestinal mucosa. Progressive and systemic inflammatory reactions are induced by increased resistance of microvasculature and destruction of the intestinal mucosa. This systemic inflammatory response syndrome damages distant organs other than the intestine and gives rise to the multiple organ failure (MOF) and death in the end [13]. Therefore, the intestine is also called a motor of the MOF [14].

The following clinical conditions can cause the intestinal IRI: necrotizing enterocolitis; midgut volvulus; intussusceptions; mesenteric ischemic disease; small bowel transplantation; aortic aneurysm surgery; cardiopulmonary bypass; hemodynamic shock; and sepsis [15–18]. To evaluate the severity of the intestinal IRI, direct observation of the intestine is the most accurate. It is, however, difficult and impractical in the clinical setting. Currently, it is indirectly assessed by the damage of distant organs. Available indirect methods include: (1) measurement of general inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6); (2) evaluation of lung function by PaO_2/FiO_2 ratio (PaO_2: partial pressure of oxygen in arterial blood, FiO_2: fraction of inspired oxygen); (3) assessment of liver function by alanine transaminase or aspartate transaminase. The nuclear factor kappa B (NF-κB) pathway plays a pivotal role in initiation and progression of systemic inflammatory reaction of the intestinal IRI. Activation of the NF-κB is the most appropriate marker to investigate the severity of the IRI. It can directly assess the injured intestine. It also indirectly evaluates the damage by systemic immune reaction in the distant organs induced by the IRI.

Oral buccal mucosa does not exhibit distinctive hemodynamic characteristics like intestinal villous cells. It is, however, prone to ischemic injury during systemic shock because of its unique differentiation state of cell turnover [19]. Microvascular blood flow of sublingual mucosa is decreased in the distributive shock such as sepsis. Recent researches revealed that measuring microvascular blood flow of sublingual mucosa is helpful in determining the severity of the sepsis or reaction to the treatment [20,21]. Ischemic injury of intestinal mucosa plays a key role in aggravation of the sepsis to the MOF or death. Blood flow of intestinal or oral mucosa can be used to monitor change in the intestinal blood flow. Oral mucosa such as sublingual mucosa is useful in evaluating the severity of the IRI. Oral mucosa can be obtained easily and noninvasively.

In this study, it was postulated that the intestinal IRI can be detected with oral buccal mucosa because systemic immune response induced by the intestinal IRI activates the NF-κB pathway of the oral buccal mucosa.

The aim of this study was to investigate the usefulness of oral buccal mucosa in the evaluation of the intestinal IRI by verifying the activation of the NF-κB pathway of the oral buccal mucosa. It was intended to provide a foundation to develop methods to assess severity of the IRI in various clinical situations.

2. Materials and methods

2.1. Experiment animals

Specific pathogen-free male Sprague–Dawley rats (280–330 g; Orient Bio Incorporated, Seongnam, Korea) were used. Experiment animals were fed with forage with water available for more than 2 wk. Ambient lighting was repeatedly turned on and off at every 12 h to get rats to be accustomed to the surrounding environment. Rats were fasted 8 h before the experiment and only water was available. Rats were divided into test and control groups of six animals each. Optimal aseptic technique was employed during the entire test procedures. Temperature of rats was monitored with rectal thermometer and was maintained at $37 \pm 0.5^{\circ}C$ with infrared lamp.

In test group, rats were anesthetized with 100% oxygen and 3% isoflurane (Forane solution; Choongwae Pharmacy, Seoul, Korea) in the rodent circuit controller. Appropriate level of anesthesia was obtained by intramuscular injection of 15 mg/kg of Zoletil (zolazepam/tiletamine; Virbac Laboratories, Carros cedex, France) in the femur of rats. Abdominal hairs were trimmed. Laparotomy was performed after surgical site was disinfected with povidone-iodine solution. The superior mesenteric artery (SMA) was isolated without any damage to the peritoneal organs and blood vessels. The SMA was clamped with a bulldog clamp. Adequate blockage of SMA was confirmed as the color of the intestine turned pale (Fig. 1). To minimize water and heat loss, abdominal cavity was closed with 3-0 nylon sutures. Laparotomy was carried out again in 30 min. The bulldog clamp was removed and reperfusion of the SMA was verified by the scarlet color of the small intestine (Fig. 1). Abdominal cavity was sutured again and 50 mg/kg of saline was subcutaneously injected to the abdominal area of rats. Rats were put in the cage, and water and food were provided ad libitum for 90 min.

In the control group, laparotomy was performed after anesthesia as described above. The SMA was separated. In contrast with the test group, the artery was not clamped. Abdominal cavity was sutured with the clamp left in the peritoneum. The same amount of saline was injected. Then, the rats were placed in the cage. Water and food were also provided ad libitum for 90 min.

2.2. Specimen collection

After 30 min of ischemic injury and 90 min of reperfusion injury, a median incision was made to expose the abdominal