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Original article

N-3 PUFAs attenuate ischemia/reperfusion induced intestinal barrier injury by activating I-FABP-PPARγ pathway☆

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SUMMARY

Background & aims: This study was designed to investigate whether n-3 PUFAs attenuate ischemia/reperfusion (I/R) induced intestinal barrier injury by activating I-FABP-PPARγ pathway.
Methods: 24 Male Sprague-Dawley rats were assigned to 4 groups: control group, I/R group, pretreated with n-3 PUFAs for 7 days before I/R (group 3), pretreated with peroxisome proliferator-activated receptor (PPARγ) agonist 30 min before I/R (group 4). The serum and intestinal mucosa samples were collected.
Results: I/R disrupted the structure of intestinal tight junctions (TJs) and reduced occludin expression. The intestinal fatty acid binding protein (I-FABP) was elevated in plasma while decreased in cells. PPARγ expression in nucleus of intestinal mucosa was attenuated. N-3 PUFAs attenuated the damaged TJ structure and elevated occludin, intracellular I-FABP and PPARγ expression. A PPARγ agonist had the same effect as n-3 PUFAs.
Conclusions: The intestinal barrier is severely damaged after I/R, which is related to the redistribution of I-FABP. Our findings firstly indicate that n-3 PUFAs protect the intestinal barrier by modifying intracellular I-FABP, activating the PPARγ pathway, and then upregulating TJ protein expression.

1. Introduction

In critical illness, it has been found that early hypovolemia leads to splanchnic hypoperfusion. The latter causes ischemic and reperfusion (I/R) injury, which is the possible mechanism of intestinal barrier injury.[1–4] The intestinal mucosa, especially in the region of villi, is preferentially susceptible to hypoxia, ischemia and reperfusion because of its high-energy demand. The gut serves as a barrier against living organisms and antigens within its lumen, and the barrier function depends on tight junctions (TJs) between intact epithelial cells.[5] Previous studies reported that defects in intestinal TJs permitted bacteria or microbial products, such as endotoxin, to cross from the lumen into the systemic compartment after severe trauma and hemorrhagic shock, which was regarded as an initial factor of "secondary insult" and might contribute to the development of multiple organ dysfunction syndrome.[3,4] Therefore, in the early phase of critical illness, if we can attenuate intestinal barrier injury, the secondary insult may be inhibited and morbidity and mortality may be reduced.

Intestinal fatty acid binding protein (I-FABP) is a 15-kd protein that is uniquely located at the tips of intestinal mucosal villi. It constitutes approximately 2%–3% of enterocyte proteins and is generally undetectable in the peripheral circulation. When intestinal ischemia exceeded 2 h, I-FABP is released from damaged intestinal mucosa cytoplasm, which may lead to the increase of plasma concentration.[6–8] In previous work, we proved that I-FABP could be used for assessing intestinal I/R injury in the early hypovolemia of severe acute pancreatitis patients.[9] I-FABP is not only a sensitive and organ-specific biochemical marker for intestinal mucosal injury, but also a carrier protein for long chain fatty acids to transport from cytoplasm to nuclei. It can

Abbreviations: n-3 PUFAs, n-3 polyunsaturated fatty acids; I-FABP, Intestinal fatty acid binding protein; I/R, Ischemia and reperfusion; PPARγ, Peroxisome proliferator-activated receptor γ; TJ, Tight junction.

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mediate protection of ischemic myocardium with long chain fatty acids.[10]

Nowadays, n-3 polyunsaturated fatty acids (PUFAs), the active ingredients of fish oil, are considered to have benefits on critical ill patients, such as small bowel transplantation, and cerebral I/R injury.[9,11] Studies have observed that supplementation with n-3 PUFAs effectively prevented the distortion of TJ morphology and reduction of TJ proteins.[12] However, there is no information on whether the intestinal barrier injury after I/R is affected by n-3 PUFAs, and the molecular mechanism underlying this effect if it occurs.

As it is important as an intracellular carrier of long chain fatty acids, we hypothesize that I-FABP is involved in the protection on intestinal barrier by n-3 PUFAs. Thus we designed this study to demonstrate whether n-3 PUFAs could protect the intestinal barrier in the intestinal I/R injury model and further to investigate the possible mechanism of the protection.

2. Materials and methods

The procedures were approved by the Animal Research Committee of Nanjing University, and complied with the Principles of laboratory animal care (NIH publication No. 86-23, revised 1985).

2.1. Animal model of intestinal ischemia and reperfusion (I/R)

Male Sprague-Dawley rats (200–250 g) were housed in a temperature-controlled room on a 12-hr light/dark cycle and fed a standard Purina rat chow diet. They were fasted with free access to water overnight before laparotomy.

Operative procedures were performed using standard sterile technique under general anesthesia with ketamine (100 mg/kg, i.p.). All intestinal I/R rats were subjected to laparotomy using a midline incision about 3 cm and identified the principal branches of superior mesenteric artery (SMA). The SMA was occluded for 30 min, and subsequently reperfused for 2 h. Control animals underwent the same procedure without occluding SMA.

The twenty-four rats were divided into 4 groups ($n = 6$ per group): group 1 comprised control animals, group 2 comprised I/R animals. The animals in Group 3 were supplemented with n-3 PUFAs (FO, Sigma Chemical, St. Louis, product number F8020) by gavage for 7 days. On the morning of the 8th day, the intestinal I/R injury was induced. The dosage of FO was 0.6% V/W (mL per 100 g body weight). The FO contained eicosapentaenoic acid (EPA,

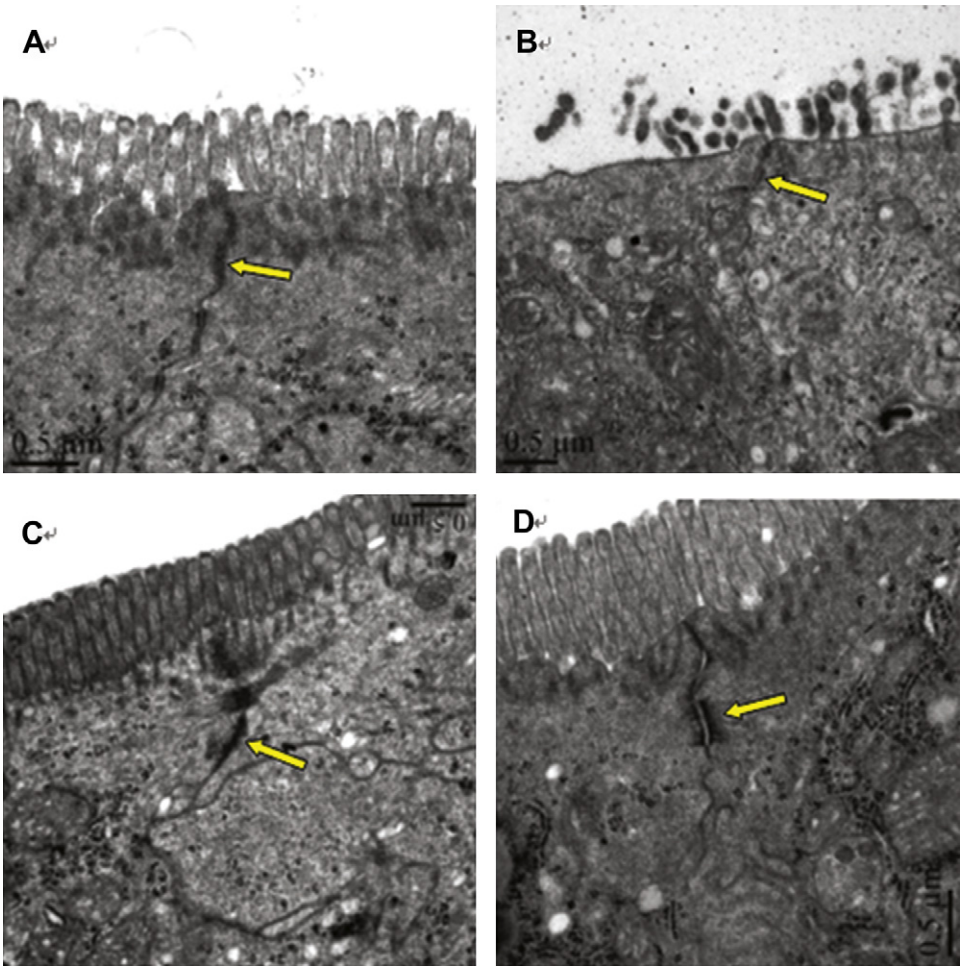

Fig. 1. Transmission electron microscopy of intestine. Transmission electron microscopy of rat intestine from the control group (group 1, A), the I/R group (group 2, B), the fish oil pretreatment group (group 3, C), and the PPARγ agonist pretreatment group (group 4, D). (A) Control group. Microvilli and TJ (→) were intact. (B) I/R group. After 30 min ischemia and sequent 2 h reperfusion, microvilli were loose and falling over, TJ (→) was disrupted. (C) Fish oil pretreatment group. Rats were pretreated with fish oil for 1 week and then suffered from I/R injury. Microvilli and TJ (→) were intact. (D) The PPARγ agonist pretreatment group. Rats were pretreated with PPARγ agonist (15d-PGJ_2) and then suffered from I/R injury. Microvilli and TJ (→) were intact. Scale bars = 500 nM.