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Long-term n-3 polyunsaturated fatty acids administration ameliorates arteriosclerosis by modulating T-cell activity in a rat model of small intestine transplantation

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Abstract

Background: Fish oil, rich in n-3 polyunsaturated fatty acids (n-3 PUFAs), has been found to reduce graft rejection and increase allografts survival. But these studies mainly focused on acute rejection. We imitated long-term fish oil administration to investigate the effects of n-3 PUFAs on graft arteriosclerosis, and T cells in a rat model of small intestine transplantation.
Methods: From 2 weeks pre-transplantation to the 60th day post-transplantation, the Lewis rats were supplemented by gavage with phosphate buffer saline, corn oil and fish oil respectively. Total small intestine was heterotopically transplanted from F344 to Lewis rat. Graft arteriosclerosis was assessed by histological grading of intimal thickening. The expression of CD25 and CD154, IL-2 level, and NF-κB activation in T cells were analyzed by western blotting, ELISA, and electrophoretic mobility shift assay respectively.
Results: Compared with corn oil, graft arteriosclerosis was ameliorated by fish oil significantly. The expression of CD25 and CD154, IL-2 level, and NF-κB activation were markedly reduced by fish oil.
Conclusions: Long-term n-3 PUFAs administration pre- and post-transplantation could inhibit T-cell activity by reducing CD154 expression and NF-κB activation, which might contribute to amelioration of graft arteriosclerosis.

Keywords: Fish oil; N-3 polyunsaturated fatty acids; Small intestine transplantation; CD25; CD154; IL-2; NF-κB; Arteriosclerosis; T cell

1. Introduction

Fish oil, rich in n-3 polyunsaturated fatty acids (n-3 PUFAs), e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been shown to influence the immune responses in vivo [1–4]. The prevailing dogma is that a major mechanism by which dietary n-3 fatty acids exert their effects on immune system is by down-regulation of T lymphocyte activity. Activated T lymphocytes are known to play a central role in the rejection of solid organ transplants. T-cell activation is a key event leading to chronic vascular rejection [5–7]. But the activation of T cell needs two signals, antigen recognition and co-stimulatory molecules interaction. CD154 (CD40 ligand, CD40L) is an important co-stimulatory molecule, which has been shown to play an important role in transplantation [8]. When T cells are activated, CD154 expression increases on the cell surface [9]. The CD40 and CD154 interaction increases the avidity of antigen presenting cells (APCs) and T-cell binding to enhance signals generated through the T-cell receptor (TCR). It also provides synergistic signals that modulate T-cell expansion and effector function. The co-stimulatory receptors are also important for controlling nuclear factor-kappa B (NF-κB) activation in T cells [10]. NF-κB is a critical nuclear factor for T-cell activation and IL-2 regulation [11,12].

Some studies have shown that n-3 PUFAs inhibit T-cell activation by modulating signal transduction molecules and inhibiting nuclear factor activation such as NF-κB, nuclear factor of activated T cell (NF-AT), and activating protein-1 (AP-1) [13–16]. N-3 PUFAs were also proved to regulate membrane protein expression, including adhesion and accessory molecules, on inflammatory and immunological cells [17]. Li et al. have found that n-3 PUFAs alter the expression of IL-2Rα-chain (CD25), an important surface marker for

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which expression is upregulated in response to stimulation, on human lymphocyte in vitro [18]. We have also established a rat model of chronic rejection and found fish oil was beneficial to the allograft in small intestine [19,20]. We imitated clinical long-term fish oil administration to investigate the effects of n-3 FUPAs on graft arteriosclerosis, and to identify the molecular targets by which n-3 PUFAs modulated T-cell activation in a chronic rejection rat model of small intestine transplantation.

2. Materials and methods

2.1. Materials

Corn oil and fish oil were from Sigma Chem. Co. (No. C8627 and F8020 respectively) containing EPA 139 mg/ml and DHA 136 mg/ml, respectively. Cyclosporin A (CsA) was from Novartis Pharma AG, Switzerland. Antibodies to CD25 and CD154 and secondary antibody Rabbit anti-Goat IgG were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). T-cell enrichment columns were obtained from R&D Systems (Minneapolis, MN). Enzyme-linked immunosorbent assay (ELISA) kit for IL-2 was from Biosource International Inc. (Carlsbad, CA). The enhanced chemiluminescence (ECL) western blotting detection reagents were obtained from Amersham Pharmacia Biotech (Piscataway NJ). The NF-κB oligonucleotide probe (5′-AGTTGAGGGGACTTTCCCAGGC-3′) was synthesized by FreeBiotech (Beijing, China). The electrophoretic mobility shift assay (EMSA) commercial kit was from Promega, (Madison WI).

2.2. Animals

Inbred adult male Fisher 344 (F344, $RT1^{av1}$) and Lewis (Lew, $RT1^{l}$) rats were from Vitalriver Company (Beijing, China). F344 and Lewis rats served as donors and recipients, respectively. All rats were housed individually in standard animal facilities, maintained on 12-h light/dark cycles, and fed with rat chow and tap water ad libitum for 1 week before the experiment to acclimatize the laboratory. Food was withheld from both donor and recipient animals for 24 h prior to surgery. The protocol was approved by the Animal Research Committee of the Nanjing University. All procedures were carried out in accordance with "Principles of laboratory animal care."

2.3. Surgical procedures

Heterotopic small intestine transplantation (HSIT) was performed using standard microvascular technique with some modification [21]. Briefly, the total small bowel, from the distal 1 cm of Treitz's Ligament to the terminal ileum, was harvested with a vascular pedicle consisting of the superior mesenteric artery and portal vein. In the recipient, end-to-side anastomoses were made between the recipient infra-renal aorta and donor superior mesenteric artery, and recipient caval and donor portal vein. The proximal graft end was ligated and the distal end was exteriorized as ostomy on the right abdomen wall. Animals surviving less than 5 days after transplantation were considered as a technical failure and were excluded.

2.4. Postoperative care

The rats were kept on a warming blanket and put under a heating lamp until recovery from anesthesia. They usually revived within 1–2 h after operation. All animals had access to regular food and water ad libitum.

2.5. Experimental groups

From 2 weeks pre-transplantation to the 60th day post-transplantation, the Lewis rats were supplemented by gavage with: 1) phosphate buffer saline (PBS), 0.6% V/W (ml per 100 g body weight), as control group, $n=8$; 2) corn oil (CO), (0.6% V/W), $n=8$; 3) fish oil (FO), (0.6% V/W), $n=8$. The intragastric administration of PBS, corn oil and fish oil was performed daily between 5:00 and 6:00 p.m. Rats were treated with CsA (5 mg/kg/d, IM) from day 0 to day 13 after transplantation to prevent acute rejection.

2.6. Processing the graft

Samples were retrieved under anesthesia at the 61st day post-transplantation, and rats were killed immediately thereafter. Spleens were aseptically taken from rats and portion of the ileum measuring approximately 2 cm were fixed in 10% neutral buffered formalin or snap frozen in liquid nitrogen.

2.7. Quantification of graft arteriosclerosis

Semi-quantitative histological grading of intimal thickening as an indicator of graft arteriosclerosis was performed in a blinded fashion. Intimal thickening was graded as follows: grade 0, normal artery; grade 1, intimal thickening up to approximately 50% of the perimeter of the lumen, with <20% luminal compromise; grade 2, intimal thickening involving between 50% and 100%, with <20% luminal compromise; grade 3, 20%–50% luminal compromise; grade 4, 50%–80% luminal compromise; grade 5, >80% luminal compromise [22].

2.8. T-cell purification

T cells were isolated from spleens as described previously with some modification [23]. Spleen was crushed gently and separated into single cells by squeezing in D-Hank's solution. The cells obtained were passed though a 149-μm wire mesh filter to create single-cell suspensions and centrifuged at 2000 ×*g* for 5 min at 4 °C. Pellet was added into 10 ml sterile 0.17 M Tris (hydroxymethyl aminomethane)–0.75% NH_4Cl (pH 7.5) followed by centrifugation to remove erythrocytes. The prepared rat spleen cell suspensions were loaded onto a negative-selection rat T-cell enrichment columns to purify the T-cell population (>90% CD3 positive).

2.9. Western immunoblotting

The amount of CD25 and CD154 was determined by western blotting. Equal amounts of protein (80 μg) in the sample were first resolved by SDS-polyacrylamide gel electrophoresis, then transferred electrophoretically to nitrocellulose membrane and subsequently incubated with the primary antibody. For detection, the nitrocellulose filter was incubated with a horseradish peroxidase coupled secondary antibody, followed by an enhanced chemiluminescence substrate reaction using ECL western blotting detection reagents.

2.10. Cytokine analysis

IL-2 level in T cells was measured in supernatants of T-cell lysis after centrifugation. The supernatant was harvested and stored at −80 °C until analysis by anti-rat IL-2 ELISA kit as the manufacturer's instructions described.

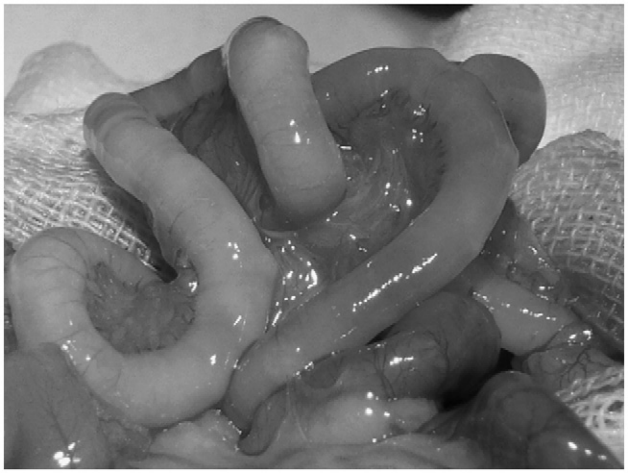

Fig. 1. Macroscopic appearance of grafts. Grafts showed a characteristic milky whitish surface, Peyer's patches appeared scarred. Length of mesenteries was moderate and mildly shortened.