Intravenous Infusion of n-3 Polyunsaturated Fatty Acids (43411)

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Abstract. **Dietary supplementation with n-3 polyunsaturated fatty acids (PUFA) is regarded as beneficial for the prevention and treatment of atherosclerosis and thrombosis and chronic inflammatory diseases like rheumatoid arthritis and psoriasis. It may be possible to treat some acute diseases like acute myocardial infarction or acute rejection of grafted organs if it is possible to make n-3 PUFA take effect quickly (in hours instead of days). Three sets of experiments were done.**

In Experiment 1, emulsion of trieicosapentaenoyl-glycerol (EPA-TG) and tridocosahexaenoyl-glycerol was infused through rabbit ear veins, and the leukotriene B_4/B_5 production from polymorphonuclear leukocytes was measured at different time points by high-performance liquid chromatography. In Experiment 2, delayed type hypersensitivity (DTH) of mice was measured with sheep red blood cells as an antigen. Pure n-3 PUFA emulsions or a control solution were infused through tail veins just before the second challenge of the antigen. DTH was measured 24 hr after the second challenge. In Experiment 3, human natural killer cell activity was measured using K562 target cells before and after the infusion of pure EPA-TG emulsion to an antecubital vein.

Leukotriene B_4 production by rabbit polymorphonuclear leukocytes was depressed by 40% by EPA-TG infusion. DTH was suppressed almost completely by n-3 PUFA infusion. Natural killer cell activity was suppressed almost completely by EPA-TG infusion in 8 hr.

DTH, natural killer cell activity, and leukotriene B_4 production are probably related to acute rejection of grafted organs. Because these parameters can be modified in a few hours by the infusion of n-3 PUFA emulsions, in marked contrast to the case with dietary n-3 PUFA, they may be beneficial for the prevention of acute rejection.

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Dietary supplementation with n-3 polyunsaturated fatty acids (PUFA) is known to reduce plasma lipids (1), platelet aggregability (2), eicosanoid formation (2, 3), and cytokine production. These effects of n-3 PUFA are beneficial for the prevention and treatment of atherothrombotic disease and chronic inflammatory disease. However, it usually takes about 4 weeks for dietary-supplemented n-3 PUFA to take effect. Therefore, n-3 PUFA have not been used therapeutically for acute atherothrombotic or inflammatory disease, including acute rejection of transplanted organs and acute myocardial infarction (which is very similar to acute inflammation in terms of tissue damage). For several years, we have been developing infusible n-3 PUFA emulsions. By infusing them intravenously, we can increase the blood and organ n-3 PUFA concentrations very quickly (4). It may be possible to treat some acute diseases with infusible n-3 PUFA emulsions if the emulsions could take effect in hours instead of days. In the present paper, we report rapid decreases in production of the most proinflammatory eicosanoid, leukotriene (LT) B_4, from polymorphonuclear leukocytes (PMNL) and decreases in immune responses such as delayed-type hypersensitivity (DTH) and natural killer (NK) cell activity.

Materials and Methods

PUFA emulsions were prepared as follows: Trieicosapentaenoyl-glycerol (EPA-TG) (5) and tridocosahexaenoyl-glycerol (DHA-TG) (6) were prepared as described previously. These oils were emulsified with egg yolk lecithin by the method of Geyer *et al.* (7) so that the emulsion contained 10% of either oil, 1.2% egg

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yolk lecithin, and 2.5% glycerol (for maintenance of the physiological osmotic pressure).

Experiment 1. EPA-TG emulsion (8 ml/kg) was infused through ear veins of seven rabbits weighing about 3 kg. Blood samples were taken from ear arteries before the infusion and 6, 24, and 168 hr afterward. After separation of red blood cells by the addition of 6% dextran in saline, PMNL were isolated with Ficoll-Conley solution. The final suspension of PMNL in Hanks' balanced salt solution was incubated at 37°C for 10 min with 10 μM calcium ionophore. Then the mixture (with prostaglandin B_1 as an internal standard) was acidified, and its supernatant was purified through an ODS-silica minicolumn and measured for LTB_4/B_5 by high-pressure liquid chromatography (8). Similar experiments were carried out with DHA-TG emulsion and Intralipid (a commercially available soybean oil emulsion).

Experiment 2. One tenth of a milliliter of a 10% sheep red blood cell suspension was injected subcutaneously into mice. Six days later, 50 μl of a 20% sheep red blood cell suspension were injected into the footpad of the right hind leg of each mouse. Five tenths of a milliliter of a 2.5% glycerol solution or 0.05–0.2 ml of n-3 PUFA emulsions was infused through tail veins just before the second challenge of the antigen. Footpad swelling (ventrodorsal thickness) 24 hr after the second challenge was measured and compared with that of the left hind leg. The difference in the thickness of footpad between the right and left legs were regarded as DTH.

Experiment 3. A volunteer was intravenously infused with 30 ml of EPA-TG emulsion. Blood samples were taken before and 8, 24, 48, and 168 hr after the infusion. Peripheral blood lymphocytes were isolated from blood samples, and NK cell activity was assayed by a standard ^{51}Cr-release test with K562 target cells (9).

Results

Experiment 1. LTB_4 production from rabbit PMNL is shown in Figure 1. The average production was depressed to 60% of baseline ($P < 0.01$) 6 hr after the infusion. It returned to 72% of baseline ($0.05 < P < 0.1$) 24 hr after the infusion. The production of LTB_5 that was synthesized from EPA was mostly under the detection limit at baseline and was increased markedly 6 hr after the infusion to a level slightly more than LTB_4 production at the same time point (data not shown). Infusion of DHA-TG into rabbits also significantly decreased LTB_4 production from PMNL, although the reduction rate was somewhat smaller than in the case of EPA-TG infusion (data not shown). Infusion of Intralipid into rabbits did not change LTB_4 production from PMNL at all (data not shown).

Experiment 2. As shown in Table I, DTH was dose dependently suppressed by the infusion of EPA-TG emulsion. The infusion of DHA-TG (0.1 ml) suppressed DTH as effectively as EPA-TG emulsion, whereas the infusion of Intralipid did not suppresss DTH at all (data not shown).

Experiment 3. NK cell activity of peripheral blood lymphocytes of a volunteer who was infused with 30 ml of EPA-TG emulsion was measured at different effector to target ratios (Fig. 2). The activity was almost completely suppressed at 8 hr after the infusion.

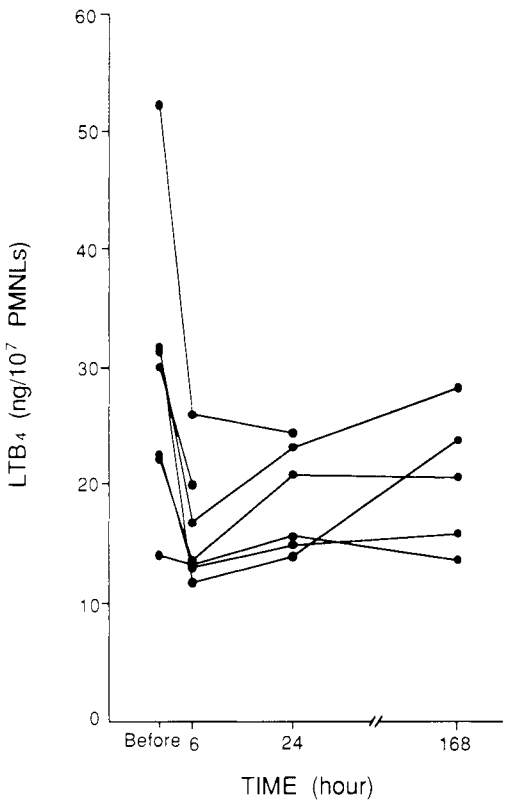

Figure 1. LTB_4 production from rabbit PMNL before and after EPA-TG infusion. An emulsified EPA-TG (10%) was infused into ear veins of rabbits. Blood samples were taken and LTB_4 production from PMNL was measured by high-performance liquid chromatography.

Table I. Effect of EPA-TG Emulsion on DTH (mm) in Mice[a]

2.5% Glycerol 0.5 ml ($n = 5$)	EPA-TG emulsion (ml)		
	0.05 ($n = 4$)	0.1 ($n = 5$)	0.2 ($n = 5$)
1.53 ± 0.31	1.09 ± 0.25	0.43 ± 0.13[b]	0.36 ± 0.26[b]

[a] DTH response was expressed by the difference in footpad thickness (swelling) between sheep red blood cell-injected footpad of the right hind leg and the control footpad of the left hind leg of each mouse 24 hr after sheep red blood cell challenge (means ± SD). EPA-TG emulsion was infused through tail veins just before the sheep red blood cell challenge. Total volume infused through tail veins was adjusted to 0.5 ml by addition of appropriate amounts of a 2.5% glycerol solution.
[b] $P < 0.001$.

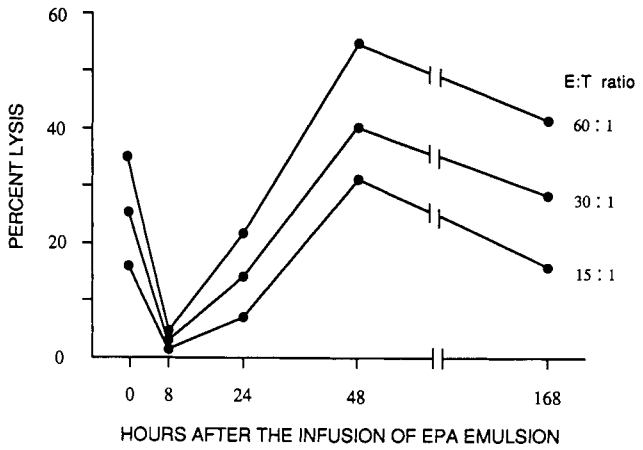

Figure 2. Changes in NK cell activity after the infusion of 30 ml of an EPA-TG emulsion into a volunteer. A 10% emulsion of EPA-TG was infused into a volunteer. Blood samples were obtained before the infusion and at different time points after the infusion. NK cell activity was measured at different effector to target (K562) ratios.

Discussion

LTB_4 which is synthesized from arachidonic acid through lipoxygenase and released from neutrophils and other cells is a very potent inducer of tissue damage because it recruits more neutrophils and stimulates neutrophils to secrete other deleterious mediators, resulting in amplification of inflammatory reaction (10). LTB_4 production may be reduced after the infusion of EPA-TG because of a competition at LTA_4 hydrolase of PMNL between LTA_4 and LTA_5 synthesized from EPA (11). Oral administration of fish oil (3) or pure EPA (8) has already been reported to depress LTB_4 production. It usually takes 4 weeks before fish oil or EPA takes effect. However, the infusion of EPA depressed it in 6 hr. Thus, EPA-TG infusion may be beneficial for treatment of acute disease.

NK cell activity and a DTH-like response may occur during acute rejection of transplanted organs (12). LTB_4 production may also be involved in acute rejection, because rejection is a kind of acute inflammation. In the present paper, we showed that EPA-TG infusion was able to suppress both DTH and NK cell activity. The effect of EPA on DTH is probably explained by a reduction in T cell proliferation in the presence of EPA (13), whereas the suppressive effect of EPA on NK cell activity may not be explained by a reduction in LTB_4 production because exogenously added LTB_4 does not restore NK cell activity (14).

EPA-TG emulsion may be useful for treatment or prevention of acute diseases, such as acute rejection of transplanted organs. By developing infusible n-3 PUFA emulsions, it will become possible to apply n-3 PUFA to clinical areas other than where the oral administration of n-3 PUFA have been thought beneficial before.

1. Goodnight SH Jr, Harris WS, Conner WE, Illingworth DR. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. Arteriosclerosis **2**:87–113, 1982.
2. Siess W, Roth P, Schere B, Kurzman I, Bohlig B, Weber PC. Platelet membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. Lancet **1**:441–444, 1980.
3. Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J, Spur BW, Robinson DR, Corey EJ, Lewis RA, Austen KF. Effect of dietary enrichment with eicosapentaenoic acid and docosahexaenoic acid on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med **312**:1217–1224, 1985.
4. Yamazaki K, Hamazaki T, Yano S, Funada T, Ibuki F. Changes in fatty acid composition in rat blood and organs after infusion of docosahexaenoic acid ethyl ester. Am J Clin Nutr **53**:620–627, 1991.
5. Urakaze M, Hamazaki T, Soda Y, Miyamoto A, Ibuki F, Yano S, Kumagai A. Infusion of emulsified trieicosapentaenoyl-glycerol into rabbits—The effects on platelet aggregation, polymorphonuclear leukocyte adhesion, and fatty acid composition in plasma and platelet phospholipids. Thromb Res **44**:673–682, 1986.
6. Hamazaki T, Urakaze M, Yano S, Soda Y, Miyamoto A, Kubota K, Ibuki F. Injection of tridocosahexaenoyl-glycerol emulsion and fatty acid composition of blood cells. Lipids **22**:1031–1034, 1987.
7. Geyer RP, Olsen FR, Andrus SB, Waddell WR, Stare FJ. Preparation of fat emulsions for intravenous alimentation. J Am Oil Chem Soc **32**:365–370, 1955.
8. Terano T, Salmon JA, Moncada S. Effect of orally administered eicosapentaenoic acid (EPA) on the formation of leukotriene B_5 by rat leukocytes. Biochem Pharmacol **33**:3071–3076, 1984.
9. Yamashita N, Yokoyama A, Hamazaki T, Yano S. Inhibition of natural killer cell activity of human lymphocytes by eicosapentaenoic acid. Biochem Biophys Res Commun **138**:1058–1067, 1986.
10. Parker CW. Lipid mediators produced through the lipoxygenase pathway. Annu Rev Immunol **5**:65–84, 1987.
11. Nathaniel DJ, Evans JF, Leblanc Y, Leveille C, Fitzsimmons BJ, Ford-Hutchinson AW. Leukotriene A_5 is a substrate and an inhibitor of rat and human neutrophil LTA_4 hydrolase. Biochem Biophys Res Commun **131**:827–835, 1985.
12. Bach FH, Sachs DH. Current concepts: Immunology—transplantation immunology. N Engl J Med **317**:489–492, 1987.
13. Hamazaki T, Yamashita N, Yokoyama A, Sugiyama E, Urakaze M, Yano S. Natural killer cells and eicosapentaenoic acid. In: Lands WEM, Ed. Proceedings of the AOCS Short Course on Polyunsaturated Fatty Acids and Eicosanoids. Champaign, IL: American Oil Chemists' Society, pp127–132, 1987.
14. Yamashita N, Maruyama M, Yamazaki K, Hamazaki T, Yano S. Effect of eicosapentaenoic and docosahexaenoic acid on natural killer cell activity in human periferal blood lymphocytes. Clin Immunol Immunopathol **59**:335–345, 1991.