Enhanced Survival to Endotoxin in Guinea Pigs Fed IV Fish Oil Emulsion

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Improved survival to endotoxin has been demonstrated in rats pretreated with cyclooxygenase inhibitors or made essential fatty acid deficient, implying that excessive ω6 fatty acids, possibly through their eicosanoid products, contribute to mortality. Following endotoxin administration, we also have shown improvement in survival with oral diets supplemented with fish oil. This study sought to explore whether parenteral fish oil ameliorates the adverse impact of endotoxin.

Male Hartley-strain guinea pigs were obtained at a body weight of 500 g and fed a normal laboratory diet. Central venous lines through which the animals received either a 10% safflower oil emulsion (n = 11) or a 10% fish oil emulsion (n = 11) during two, 24-hr periods separated by two days were inserted. Two days after the second infusion, endotoxin (0.35 mg/100 g b.w.), was given intraperitoneally, and survival was noted. The animals received a total of 25.4 g of IV fat per kg b.w., including 5.3 g of eicosapentaenoic acid per kg b.w., for the fish oil group.

From six hr after endotoxin through four days, there was better survival in the fish oil group ($p < .006$). Final mortality showed 7/11 fish-fed vs 2/11 safflower-fed animals surviving. We conclude that the administration of parenteral fish oil, even for a brief time, can have a profound effect on subsequent survival to endotoxin.
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Endotoxic shock involves activation of platelets and leukocytes (1). Investigations have shown enhanced survival to endotoxin when the effects of the vasoactive prostanoids thromboxane A_2 and prostacyclin I_2 have been inhibited through either decreased production or blocked action (2,3). Methods used to arrive at this common physiologic end-point include essential fatty acid deficiency, pretreatment with the cyclooxygenase inhibitors indomethacin or ibuprofen, or with the thromboxane synthetase inhibitor imidazole or by the use of 13-azaprostanoic acid, a thromboxane antagonist (2,3).

Fish oils contain eicosapentaenoic acid (EPA), a long chain polyunsaturated fatty acid of the ω3 family. As a substrate for cyclooxygenase in platelets, it forms thromboxane A_3, a vasoconstrictor like thromboxane A_2, but not a platelet aggregator (4,5). Likewise, in endothelial cells, EPA serves as substrate for prostacyclin I_3, which is similar to prostacyclin I_2 as a vasodilator and platelet anti-aggregator (5). This shifts the overall balance towards lessened clotting, manifested by prolonged bleeding times (6) and decreased thromboxane production (7).

Fish oils also have been shown to dampen leukocyte function (8). Lee and colleagues (8) showed decreased chemotaxis and decreased production of leukotriene B4 in neutrophils obtained from human subjects taking supplemental fish oil. This leukocyte inhibitory effect from dietary fish oil may be another mechanism through which survival to endotoxin challenge could be modulated.

We recently have shown (9) enhanced survival to an LD_{50} of endotoxin in animals orally fed fish oils for a six-wk period. We considered the hypothesis that fish oils would be protective against endotoxic shock by utilizing an IV preparation of fish oil given over several days.

METHODS

Animals. Male Hartley strain guinea pigs were obtained at a weight of 500 g from the Elm Hill Breeding Laboratories (Chelmsford, MA). They were housed in plastic cages with pine shavings on a 12-hr light and 12-hr dark cycle with the temperature controlled at 23 C and the relative humidity between 50–55%. This study met with the approval of the Institutional Animal Care and Use Committee of the New England Deaconess Hospital.

Study protocol. The animals were allowed a normal laboratory guinea pig diet (Agway Inc., Syracuse, NY), ad libitum, throughout the study. One wk after arriving, the animals underwent central vein catheterization. While anesthetized, a 0.025 in. (i.d.) × 0.037 in. (o.d.) silastic catheter was inserted into the superior vena cava from the internal jugular vein. The tubing was tunneled subcutaneously where it exited from the midscapular region and connected to a flow-through swivel that permitted free movement.

One day after the catheters were placed, the first of two days of lipid infusion was started. The animals received the respective lipid emulsions (safflower oil or fish oil) for two 24-hr periods separated by two days. Prior pilot studies revealed gross lipemia after one day of infusion, which cleared after two days of saline infusion. After the second day of lipid infusion, two more days were allowed to clear the second lipemia. On the morning of the seventh day, the animals received endotoxin intraperitoneally, 0.35 mg/100 g b.w., and then were followed for survival. Survival was noted at 3, 6, 9, 12, 15, 21, 24, 48, 72 and 96 hr after endotoxin injection. There were 11 animals in each group.

Emulsions. The emulsions used were each 10% emulsions. The fish oil emulsion was obtained from Baxter Healthcare Corp. (Deerfield, IL), and the safflower oil emulsion was from Abbott Laboratories (North Chicago, IL). Liposyn, the original formulation made solely from safflower oil and containing no soybean oil, was used. Table 1 depicts the fatty acid composition of the emulsions.

Endotoxin. Endotoxin was obtained from Difco Laboratories (Detroit, MI). The lipopolysaccharide was derived from *Escherichia coli* (026:B6, Lot #3920-10-9, Control #718687). Using the method of Reed and Muench (10), an LD_{50} of 0.35 mg/100 g b.w. was obtained for this lot of

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Abbreviation: EPA, eicosapentaenoic acid.

TABLE 1

Fatty Acid Composition of the IV Emulsions in Relative Percentages

	Fish oil	Safflower oil
Myristic (14:0)	8.1	
Palmitic (16:0)	12.5	7.0
Palmitoleic (16:1ω7)	7.3	
Stearic (18:0)	1.5	2.5
Oleic (18:1ω9)	6.1	13.9
Linoleic (18:2ω6)	5.9	77.0
α-Linolenic (18:3ω3)	1.2	0.1
Eicosapentaenoic (20:5ω3)	19.7	
Docosahexaenoic (22:6ω3)	8.6	

Fish oil emulsion made by Baxter Healthcare Corporation (Deerfield, IL). Safflower oil emulsion was Liposyn, made by Abbott Laboratories (North Chicago, IL) and contained no soybean oil.

TABLE 2

Lipid Intake

Parameter	Fish oil	Safflower oil
Body weight (b.w.)	500.8 ± 21.0	485.9 ± 24.3
Lipid infused (g/animal)	12.6 ± 0.3	13.0 ± 0.5
Lipid infused (g/kg b.w.)	25.4 ± 1.5	27.5 ± 2.5
Lipid infused (g/kg b.w./dose)	12.7 ± 0.1	13.7 ± 1.2
Lipid infused (g/kg b.w./hr)	0.53 ± 0.3	0.57 ± 0.05
EPA infused (gm/kg b.w.)	5.3 ± 0.3	0

Except for EPA, no weight or lipid differences by Student's t-test. N = 7 for each group. Data expressed as $\bar{x}$ ± SEM.

endotoxin in normal guinea pigs using the intraperitoneal route. The endotoxin was diluted in sterile saline to a maximum volume of 1 ml and injected intraperitoneally.

Anesthesia. A mixture of ketamine (44 mg/ml), acepromazine (0.2 mg/ml) and atropine (40 mcg/ml) was used for anesthesia at a dose of 1 ml/kg body weight, intramuscularly. Lidocaine as a 5% solution was used locally for any dissection.

Statistics. Survival was tested by Kaplan-Meier survival analysis using Surv-Pak-PC, software developed by the Johns Hopkins Oncology Center (Baltimore, MD).

RESULTS

Lipid intake. Table 2 shows the lipid intake for the two groups of animals. Each group received comparable amounts of fat, when analyzed by total amount over the two doses, amount per dose, per kilogram b.w. or rate of infusion. The total amount of EPA the fish oil-fed animals received over the six-day study was 5.3 ± .3 g/kg b.w.

Survival. Figure 1 shows the mortality among the two groups. As shown by Kaplan-Meier analysis, the two curves differ, $p < .006$. By nine hr after endotoxin administration, all nine safflower oil-fed animals that were to die had succumbed. In contrast, two of the four fish oil-fed animals that were to die lived for more than 21 hr.

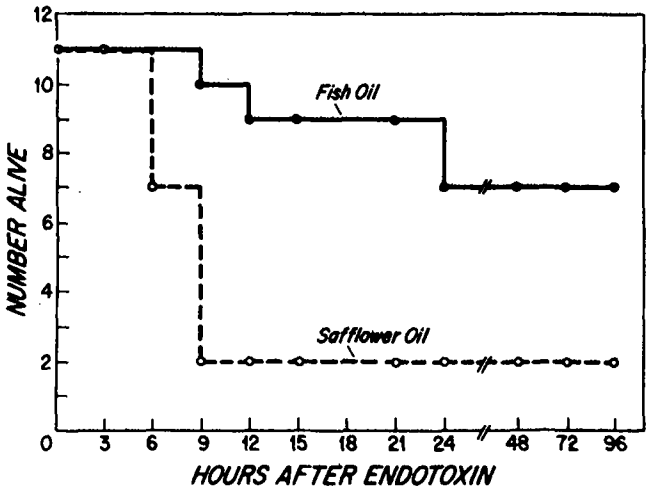

FIG. 1. Survival curves of the two dietary groups. Curves differ by Kaplan-Meier analysis, $p < 0.006$.

DISCUSSION

The beneficial impact of the fish oil may have been mediated through alteration in the metabolism of the prostanoids, thromboxane A_2 and prostacyclin I_2 from their respective tissues of origin, platelets and endothelium, or through leukotriene B_4 from leukocytes. The ability of EPA to antagonize the production of the more vasoconstrictor thromboxane A_2 or to diminish the generation of leukotriene B_4, thereby lessening leukocyte migration and endothelial adherence, could be its primary role. Alternatively, the presence of EPA-derived thromboxane A_3 and prostacyclin I_3 or leukotriene B_5 may have been involved. Because these compounds were not measured in this study, the temporal associations are not known. Further support for this explanation is data recently generated in guinea pigs fed a fish oil-based diet for six wk and then challenged with endotoxin (11). The metabolic acidosis that developed was much less pronounced as compared with those animals fed safflower oil.

Less linoleic acid and therefore less arachidonic acid as a substrate for eicosanoid formation may be another mechanism. This would view the fish oil as merely replacing an oil high in linoleic acid, i.e. safflower oil, leading to less arachidonic acid as substrate. Support for this comes from a recent study in which coconut oil-fed animals, as opposed to corn oil-fed animals, had better survival to endotoxin (12). Determining fatty acid profiles in plasma, platelets and leukocytes could resolve this question.

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