Prophylaxis of Hemodialysis Graft Thrombosis with Fish Oil: Double-Blind, Randomized, Prospective Trial

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Abstract. Diets enriched with fish oil may favorably affect the vascular perturbations underlying synthetic graft thrombosis. Therefore, these studies were designed to test the hypothesis that diets enriched with fish oil would decrease the incidence of thrombosis in newly constructed polytetrafluorethylene grafts. A double-blind, randomized trial was conducted. Twenty-four patients were randomized to receive 4000 mg of fish oil or 4000 mg of control oil. Both preparations were enriched with antioxidants and deodorized with peppermint. Patients began therapy within 2 wk after graft placement and were monitored for 12 mo or until thrombosis developed. With a permuted-block randomization schedule, 12 patients received fish oil and 12 patients received control oil. The primary patency rates at 365 d were 14.9% for the control group and 75.6% for the fish oil-treated group. Survival analysis revealed a significant difference between fish oil-treated and untreated patients (P < 0.03, Mantel-Cox test), with a power of 90%. Moreover, analysis of covariables, including age of ≥50 yr, gender, race, body weight, diabetes mellitus, bleeding times, and lipid profiles, indicated that this effect occurred principally as a result of fish oil administration. Importantly, fish oil treatment also decreased venous outflow resistance and systemic BP, compared with control values. Fish oils possess unique biologic properties that favorably affect the incidence of polytetrafluoroethylene graft thrombosis, and they thus represent a potential treatment strategy for the prevention of access thrombosis.

Despite impressive advances in the treatment of patients with end-stage renal disease (ESRD), vascular access thrombosis has remained a persistent problem since the development of dialysis. The annual cost of maintaining dialysis access has been estimated to be >1 billion dollars. Recent studies have indicated that >50% of all access grafts experience thrombosis within 1 yr after placement and >75% require a salvage procedure to maintain patency (1). Although a number of strategies have been used to limit the incidence of vascular access thrombosis, including the use of pharmacologic agents such as warfarin, aspirin, and dipyridamole, the relative frequency of side effects (gastrointestinal hemorrhage), coupled with inconsistent clinical results, has dampened enthusiasm for these approaches (2,3). Therefore, novel strategies to prevent dialysis access thrombosis are necessitated by the requirement to reduce the cost and morbidity of maintenance hemodialysis. Importantly, the use of diets enriched with ω-3 fatty acids derived from fish oil concentrates may offer such an approach. ω-3 fatty acids are well established as being essential nutrients for developing humans; however, their roles as therapeutic agents in the management of progressive renal disease, atherosclerosis, and hypertension have only recently received attention (4,5). Dietary administration of ω-3 fatty acids was demonstrated to competitively inhibit cyclooxygenase and to prolong bleeding times; however, unlike aspirin, ω-3 fatty acids decreased gastric erosions and ulcers caused by alcohol or aspirin (6). Furthermore, recent evidence suggests that ω-3 fatty acids inhibit intimal hyperplasia in autogenous vein grafts (7). Diets enriched with ω-3 fatty acids may also improve blood rheologic features and flow (decreasing turbulence), favorably modify membrane fluidity in endothelial cells (decreasing shear stress), and reduce polypeptide growth factor and cytokine release from platelets and inflammatory cells (8–11). Collectively, these biochemical and physiologic effects would be expected to have beneficial effects on the incidence of graft thrombosis. Therefore, these studies were designed to investigate the efficacy of orally administered fish oil concentrates in the prevention of polytetrafluoroethylene (PTFE) graft thrombosis.

Materials and Methods

Patients

Patients eligible for enrollment in the study included patients close to the initiation of chronic hemodialysis who required placement of a new PTFE graft (Gore-Tex stretch vascular grafts with an internal diameter of 7 mm and an average length of 12.5 cm; W. L. Gore & Associates, Flagstaff, AZ) and patients who were undergoing chronic hemodialysis but required placement of a new PTFE graft at a different site. Patients requiring surgical revision (e.g., a “jump graft”) were not eligible for enrollment in the study. Additional exclusion criteria included patients with a history of gastrointestinal bleeding, patients already receiving chronic anticoagulation therapy (antiplalet agents or warfarin), and patients with terminal or life-threatening disease, pregnancy, or malignant hypertension. All patients were recruited from the outpatient hemodialysis program at St. Louis University, and...
the same surgical team placed all grafts. Patients were randomized to receive one of the following two regimens: daily administration of four 1-g capsules of fish oil concentrate or daily administration of four 1-g capsules of control oil. The treatment was initiated within 2 wk after graft placement, with full approval by the St. Louis University Institutional Review Board. Patients were monitored for 1 yr or until thrombosis developed.

Fish Oil Test Materials

The Biomedical Test Materials Program (United States Department of Commerce, National Marine Fisheries Service) provided quality-assured/quality-controlled fish oil and control oil for human consumption. The fish oil preparation used in our study contained 80% ω-3 fatty acid ethyl esters (44% eicosapentaenoic acid, 24% docosahexaenoic acid, and 10 to 12% other oils), packaged in 1-g soft-gel capsules. The control oil contained 80% ω-6 ethyl esters (corn oil), packaged in 1-g soft-gel capsules. Both preparations contained 0.2 mg/g t-butylhydroquinone and 2 mg/g α-tocopherols (as antioxidants) and were deodorized with peppermint.

Primary and Secondary Objectives

The primary null hypothesis of the study was that fish oil would have no effect on the incidence of graft thrombosis. Organization of data included the times to events (in days), with appropriate censoring. Additional covariables that were thought to affect the incidence of thrombosis in vascular access were introduced for determination of potential interactions between these measurements and the occurrence of thrombosis (12).

Because fish oil has been demonstrated to inhibit smooth muscle cell growth and to facilitate endothelial cell migration (13,14), we also explored the effects of these agents on access stenosis by analyzing physiologic correlates of venous stenosis, including dialysis venous outflow pressure and urea kinetics. All patients enrolled in the study underwent dialysis with a Fresenius Polysulfone hemodialysis membrane (F-80; Fresenius, Lexington, MA), at a dialysate flow rate of 800 ml/min. The blood flow rate (mean, 389 ± 27 ml/min) and dialysis duration (mean, 3.79 ± 0.28 h) were varied to achieve Kt/V values of 1.2 to 1.4 and were similar for the two groups.

To ensure compliance with the medication regimen and to determine the fatty acid compositional changes induced by ω-3 fatty acid supplements, we analyzed platelet membrane phospholipid fatty acid contents at baseline and at 3 mo. Recent studies suggest that platelet fatty acid composition is a surrogate index of fatty acid incorporation at the tissue and/or organ level (15). Moreover, platelet hemostatic function was measured monthly, as whole-blood bleeding time. The latter measurements were performed by the same individual (Ms. McCloud).

In addition, we monitored serum lipid profiles, because fish oil-enriched diets are well established to be antilipemic. Complete lipid profiles were obtained at baseline and at 3 mo. LDL cholesterol levels were calculated as follows: LDL cholesterol level = total cholesterol level – (HDL cholesterol level + 0.46 × triglyceride level). Serum cholesterol, HDL cholesterol, and triglyceride levels were analyzed in whole plasma with enzymatic reagents, by using a commercially available kit (Boehringer Mannheim, Indianapolis, IN). Lipoprotein(a) (Lp(a)) levels were determined by using a sandwich enzyme-linked immunosorbent assay (Terumo, Inc.). In particular, we were interested in evaluating the relationship of Lp(a) levels to vascular access thrombosis, because recent studies suggested that increases in Lp(a) levels among patients with ESRD contribute to access thrombosis (16). Routine blood chemistry profiles, dialysis prescriptions, body weights, medications, and complications (hypotension) for all patients were recorded in a computerized database and thus were available for inclusion in the final data analysis.

Venous Outflow Pressure and Systemic BP Determinations

Venous pressures were recorded directly from the digital display of the dialysis delivery system (Fresenius 2000 H). Measurements were made after the first 30 min of dialysis, at a blood flow rate of 200 ml/min (15-gauge needles were used for serial assessments, and 16-gauge needles were used for the initial two to four treatments with new grafts). Urea kinetics were determined for each study patient on a monthly basis, using a computer program (Pack-H urea kinetic analysis for the PC, version 6.01, Detroit, MI) based on a single-pool, variable-volume, pharmacokinetic model of urea distribution.

Systemic BP determinations were performed at the midweek predialysis session, using a standard sphygmomanometer, with the patient resting quietly in the seated position for a period of 10 min. A mean of three determinations was obtained at each visit.

Fatty Acid Composition Determinations

The fatty acid composition of platelet membranes was analyzed as previously described by our group (17). Briefly, the major lipid classes were extracted from platelet membranes with chloroform/methanol. The triacylglycerol and phospholipid fractions were isolated by thin layer chromatography using silica gel H glass plates. The plates were developed in a solvent system of petroleum ether/dimethyl ether/acetic acid (100:25:1). The methyl esters of the phospholipid and triacylglycerol fractions were prepared by transesterification using hexane and were analyzed with a Varian model 3500 capillary gas chromatograph (Varian, Palo Alto, CA) equipped with a flame ionization detector and a 30-m capillary column (Supelcowax 10; Supelco, Bellefonte, PA). The column temperature was programmed from 175°C to 212°C, with a ramp of 1.5°C/min and a final holding period of 17 min.

Statistical Analyses

All results are reported as mean ± SEM. Statistical analyses were performed on an intention-to-treat basis. A sample size of 25 was targeted to ensure a power of >80%, according to the following assumptions: α = 0.05 (one-tailed test), the average primary patency rate for untreated PTFE grafts in a 12-mo follow-up period is 30% (P1 = 0.3), and treatment improves the survival rate to 75% (P2 = P1 = 0.45) (18). These assumptions were derived from preliminary data and recent studies establishing the natural graft survival history among a large cohort of patients who required new synthetic grafts (1). Survival analyses were based on Kaplan-Meier estimates, rank tests, and a regression model (Cox proportional-hazards model). The t test was used when the means of two groups were compared. Statistical data analyses were performed with Stat-View software (SAS Institute Inc., Cary, NC).

Results

Between December 1996 and January 1998, a total of 24 patients met the study criteria for enrollment. A permuted-block randomization schedule was used to ensure equal numbers of patients in both groups. The demographic and baseline laboratory findings for each group are summarized in Table 1. There were no significant differences in age, body weight, gender, race, or incidence of diabetes mellitus between the fish
oil-treated and control groups. Bleeding times and serum cholesterol levels were similar at baseline and remained stable throughout the study period. No overt bleeding episodes were observed in either group. In addition, there were no differences between baseline and follow-up hematocrit values or changes in recombinant human erythropoietin doses during the study period for either group. Triglyceride levels were moderately increased at baseline in the fish oil-treated group, compared with the control group. After 3 mo of treatment, circulating triglyceride levels had decreased to 98 ± 22 mg/dl in the fish oil-treated group (P = 0.07) but remained unchanged (120 ± 29 mg/dl) in the control group. Levels of small dense lipoproteins [LDL cholesterol and Lp(a)] were similar at baseline (Table 1) and at 3 mo in both treatment groups. In addition, logistic regression analysis of the relationship between dense lipoprotein levels and the development of graft thrombosis at baseline and monthly thereafter was unrevealing. Graft locations and configurations had no effects on the development of thrombosis in either group, although the numbers in each subgroup were small (Table 1). Four patients in each group were enrolled after placement of a second graft, whereas a third graft was placed in one fish oil-treated patient. The latter patients, regardless of treatment, underwent a mean of 1.2 salvage procedures/graft-yr, indicating no special tendency toward graft thrombosis in either group.

Importantly, only two patients developed graft thrombosis in the fish oil-treated group, compared with nine patients in the placebo-treated group. One patient in the fish oil-treated group was lost to follow-up monitoring at 7 mo. Therefore, the primary patency rate at the end of the study period was 14.9% among patients who received placebo versus 75.6% among patients who received fish oil supplements (Figure 1). The cumulative graft survival rate for the control cohort was similar to those observed for historical control subjects (Figure 1, 1996) and new patients monitored for 1 yr after completion of the study (Figure 1, 1999).

Regardless of the weighting method used to detect differences between the fish oil-treated patients and untreated patients, all rank tests, including the Mantel-Cox, Breslow-Gehan-Wilcoxon, Tarone-Ware, Peto-Peto-Wilcoxon, and Harrington-Fleming tests, revealed P values of <0.05. In addition, when the Mantel-Cox rank test was applied to data for the fish oil-treated patients versus historical control subjects (Figure 1, 1996) or recently studied patients (Figure 1, 1999), a significant benefit of treatment was observed.

The Cox proportional-hazards regression model was used to analyze the association of several covariables with the development of thrombosis. Covariables thought to influence the risk of graft thrombosis included age of ≥50 y, gender, diabetes mellitus, venous outflow resistance (venous pressure), urea kinetics, lipid profile, platelet hemostatic function (bleeding time), race, and BP (12). None of the covariables measured at baseline or follow-up times was correlated with the development of graft thrombosis. However, two interesting trends were observed with the follow-up venous outflow pressure measurements and systemic BP determinations (Figure 2). A trend toward an increase in venous pressure was observed for patients who received the control supplement (96 ± 12 mmHg at baseline versus 112 ± 10 mmHg at study termination), whereas patients who received fish oil exhibited no change in venous pressure (85 ± 9 mmHg at baseline versus 88 ± 7 mmHg at study termination). Indeed, the mean venous pressure at the end of the study was significantly less among patients who received fish oil, compared with control

**Table 1.** Demographic and laboratory characteristics at baselinea

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fish Oil (n = 12)</th>
<th>Placebo Oil (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52 ± 6</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>79 ± 5</td>
<td>79 ± 11</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>5 (42%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>female</td>
<td>7 (58%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>African-American, n (%)</td>
<td>10 (83%)</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>7 (58%)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
<td>8.6 ± 2.0</td>
<td>8.5 ± 1.7</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>184 ± 20</td>
<td>176 ± 15</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>209 ± 113</td>
<td>134 ± 78</td>
</tr>
<tr>
<td>Lipoprotein(a) (mg/dl)</td>
<td>37 ± 11</td>
<td>57 ± 10</td>
</tr>
<tr>
<td>Graft location, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>upper arm, straight</td>
<td>3 (25%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>forearm, loop</td>
<td>5 (42%)</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>forearm, straight</td>
<td>4 (33%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Urea kinetics, Kt/V</td>
<td>1.2 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>32 ± 2</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>rhEPO (U/wk)</td>
<td>13,000 ± 1,100</td>
<td>12,700 ± 900</td>
</tr>
</tbody>
</table>

*a* Values represent mean ± SEM. rhEPO, recombinant human erythropoietin (EPOGEN; Amgen, Thousand Oaks, CA).
patients (88 ± 7 versus 112 ± 10 mmHg). Systolic and diastolic BP were reduced by an average of 30 and 15 mmHg, respectively, among patients who received fish oil, compared with control patients (Figure 2). The change in BP could not be attributed to specific modifications in the antihypertensive regimen for either group. Therefore, patients who received fish oil experienced control with a mean of 2.8 medications (62% were receiving a calcium channel blocker and 75% were receiving an angiotensin-converting enzyme inhibitor), compared with 3.0 for the control group (66% were receiving a calcium channel blocker and 73% were receiving an angiotensin-converting enzyme inhibitor). Body weights were similar at baseline and study termination in both groups, suggesting that volume manipulations were not likely to account for the disparity in BP.

Table 2 summarizes the fatty acid composition at 3 mo for each group. Importantly, the concentration of the ω-3 fatty acids in the phospholipid fraction of the platelet membrane reflected the composition of the ω-3 fatty acid ethyl esters in the concentrate. In addition, a decrease in the membrane content of arachidonic acid was observed among patients who received fish oil. There were no significant changes in fatty acid composition among patients who received the control oil. These observations provided an additional index of patient compliance, as well as insights into the mechanisms responsible for these benefits (17).

**Discussion**

There are >350,000 patients undergoing dialysis in the United States (19). Although autologous arteriovenous fistulae are the preferred access for hemodialysis, synthetic PTFE grafts represent the most common hemodialysis access type placed in this country. Although most dialysis centers perform surveillance monitoring (serial measurements of access flow and venous outflow resistance) during dialysis, to detect access stenosis in its earliest stages, thrombosis rates remain unacceptably high. Indeed, in our own center, the incidence of thrombosis at 1 yr for newly placed PTFE grafts was 60 to 80% among patients who were analyzed during three different time periods (Figure 1). These studies were undertaken to determine the effects of
dietary administration of fish oil concentrates on the incidence of graft thrombosis among newly placed PTFE grafts. The results of our analysis suggest that daily administration of 4000 mg of fish oil, beginning within 2 wk after graft placement, was successful in preventing the development of access thrombosis. Importantly, the 12-mo primary patency rate (cumulative survival rate) for the control group was statistically similar to rates for historical control subjects, as well as the rate for a recent group of patients who were studied after the end of the enrollment period. To ensure consistency in patient treatments and surgical approaches, all patients were recruited from a single center and underwent access placement by the same surgical team. A striking finding in our analysis was the incidence of thrombosis in the control group (75%), compared with the fish oil-treated group (16.6%). Although the results for the control group seem relatively poor, this likely reflects the strict criteria used to select patients for study inclusion, i.e., no patient in the control or treatment group was receiving any form of anticoagulation therapy. Moreover, the natural history of graft failure among our untreated subjects paralleled the results of a recent prospective study of a large cohort of patients with newly placed synthetic grafts who were monitored for 24 mo (1). Overall, patients tolerated the fish oil preparation well, although three patients complained of gas, bloating, or a “fishy” aftertaste.

Using a proportional-hazards regression model, we were unable to account for the differences observed in our clinical trial on the basis of age, gender, diabetes mellitus, race, baseline or follow-up lipid profiles, bleeding times, hematocrit levels, or weekly doses of recombinant human erythropoietin. Interestingly, follow-up systemic BP and venous outflow pressure (an index of venous resistance) measurements were favorably affected by the administration of fish oil. We observed consistent increases in venous pressure among the control subjects, whereas fish oil-treated patients demonstrated little change or decreases in venous pressure. We infer from these observations that the chronic administration of fish oil reduces intimal hyperplasia at or near the graft anastomosis, thus attenuating the development of venous stenosis. Importantly, the latter invariably precedes or accompanies the development of graft thrombosis (2). We recognize that additional anatomic data are necessary to ultimately support or refute this plausible hypothesis. Additional consequences of fish oil treatment included decreases in systolic and diastolic BP of 30 and 15 mmHg, respectively. Although the beneficial effects of fish oil supplements on BP were previously reported, we are unaware of such data in the setting of ESRD (20). The mechanisms responsible for eliciting decreases in systemic BP cannot be established by this study; however, alterations in endothelial cell function, nitric oxide synthesis, and prostaglandin generation could produce decreases in arterial pressure (4,5,8,21). Moreover, we cannot exclude a beneficial effect of these hemodynamic changes on graft thrombosis development or venous outflow pressure.

Finally, daily administration of 4 g of our fish oil preparation yielded significant changes in the fatty acid composition of the platelet membrane. Increases in levels of the major ω-3 fatty acids (Table 2), coupled with decreases in arachidonic acid levels, were observed. We observed similar changes in cell membrane composition after dietary supplementation with fish oil in vitro and in vivo (22,23). The latter findings are important, because they confirm patient compliance and indicate that the fatty acid content of this formulation is sufficient to modify the lipid composition of endogenous cells. It is reasonable to surmise that such changes are important in mediating the beneficial effects of these agents on the development of graft thrombosis (17).

Several notable findings from the past two decades provide insights into the pathobiologic processes responsible for these beneficial effects (Figure 3). Importantly, ω-3 fatty acids are readily incorporated into the cell lipid bilayer, resulting in changes in the physical characteristics of the membrane (e.g., altered membrane fluidity). As a result, the kinetic properties of receptors embedded in the membrane are altered, leading to changes in ligand-receptor coupling. Indeed, ω-3 fatty acids have been demonstrated to inhibit smooth muscle cell proliferation in response to various mitogens (13,14). Interestingly, recent studies have demonstrated that diets enriched with ω-3 fatty acids inhibit neointima formation after mechanical vascular injury in the carotid arteries of nonhuman primates (24). In addition, ω-3 fatty acids can directly inhibit endothelial synthesis of platelet-derived growth factor (9). Furthermore, ω-3 fatty acid supplementation has been demonstrated to limit the synthesis of a variety of inflammatory cell cytokines, including interleukin-1 and tumor necrosis factor-α. This may explain the beneficial effects of fish oil on the development of graft thrombosis.

### Table 2. Platelet membrane fatty acid composition

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Composition (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish Oil (n = 12)</td>
<td>Control (n = 12)</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>4.2 ± 1.3</td>
<td>5.2 ± 2.3</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>10.4 ± 1.9b</td>
<td>16.8 ± 0.9</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>3.4 ± 0.2b</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Docosapentaenoic acid</td>
<td>2.3 ± 0.01b</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Docosahexanoic acid</td>
<td>4.3 ± 0.04b</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Others</td>
<td>30.2 ± 3.3</td>
<td>40.2 ± 4.1</td>
</tr>
</tbody>
</table>

a Values represent mean ± SEM. NR, not reported.
b < 0.05 versus control value.
including tumor necrosis factor-α and interleukins 1 and 6 (10,11,25). Other consequences of fatty acid remodeling of the cell membrane include changes in lipid-mediated cell signaling. For example, incorporation of ω-3 fatty acids into membrane phospholipids alters the platelet eicosanoid profile, favoring an antiaggregatory state, as reflected by reduced platelet aggregation and altered bleeding times (21). Additional effects of ω-3 fatty acids that may contribute to antithrombotic effects include increases in the deformability of endothelial cells and modulation of their susceptibility to shear-induced stress (8).

In summary, our study supports a novel approach for the prevention of dialysis access thrombosis. Although we recognize that the sample size in this study is relatively small, the impressive results indicate the need for additional investigations to establish the optimal dose and duration of fish oil therapy in this setting. However, given the tolerability and limited risk associated with supplemental fish oil ingestion, we think that this strategy will prove useful for the prevention of access thrombosis.

Acknowledgments

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