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Effects of Eicosapentaenoic Acid on Biochemical Failure after Radical Prostatectomy for Prostate Cancer

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Abstract. *Aim: To study the effects of eicosapentaenoic acid (EPA) on prostate-specific antigen (PSA) failure in prostate cancer patients who underwent prostatectomy. Patients and Methods: Sixty-two prostate cancer patients whose PSA levels were less than 0.2 ng/ml 3 months after surgery were randomized to either an EPA group (n=32) or a control group (n=30). EPA (2.4 g/day) was administered in the EPA group for 2 years. PSA was measured every two months. Results: The EPA concentration increased but the docosahexaenoic acid concentration decreased significantly (P<0.001) in erythrocytes. The PSA recurrence rates during a mean follow-up of 53.8 months were not different between the two groups (p=0.16). Conclusion: A longer and/or larger intervention or docosahexaenoic acid supplementation might be necessary to identify significant preventive effects of mega-3 polyunsaturated fatty acids on PSA recurrence.*

A several-fold difference in prostate cancer (PC) incidence has been reported from country to country and between racial groups. The rate of PC in Japanese men who move to the United States increases to intermediate between the low risk in Japan and high risk in the United States (1). An inverse relationship between dietary intake of fish and the risk of PC has been reported (2-4), although other studies showed no significant effects regarding fish consumption (5, 6). Most animal and *in vitro* studies suggest the inhibitory effects of ω3 polyunsaturated fatty acids (PUFAs), rich in fish oil, on PC growth (7-9). However, a number of studies have revealed a positive association between dietary, plasma, or red blood cell levels of α-linolenic acid, the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the risk of PC (5, 10-12). It was reported that a reduced PC risk was associated with high erythrocyte phosphatidylcholine levels of EPA and DHA (13), and that an increased risk was associated with decreased plasma levels of ω3 and increased levels of ω6 fatty acids (14). The direct measurement of prostatic tissue levels of PUFAs suggested that ω3 and ω3-to-ω6 fatty acid ratios were inversely related to the invasiveness of PC (15). A prospective study examining the relation between prostatic concentrations of fatty acids and cancer recurrence following radical prostatectomy showed that the percent total PUFA was significantly lower in men who experienced biochemical recurrence compared to those without recurrence (16).

The results of dietary modulation studies are consistent with a role for ω3 PUFAs in the growth inhibition of human prostatic tumor cells in nude mice (7, 8) as well as genetically induced prostate tumor in prostate-specific *Pten*-knockout mice (9). In the animal model, which simulated radical prostatectomy, it was found that a diet

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Table I. *Basic and cancer characteristics of the patients.*

Basic and cancer characteristics	Control (n=30)	EPA (n=32)
Age (years)	68±7	68±5
PSA (ng/ml) at biopsy	10.2±6.6	7.8±4.3
Pathological T-stage		
pT1	2	3
pT2	21	22
pT3≥	7	7
Gleason score	6.2±1.4	6.3±0.9
Follow-up month	54±4	54±4

Data are shown as the mean±SD. There were no significant differences between the two groups regarding any item.

Table II. *Plasma hormone levels.*

Hormone	Control	EPA	*P*-value+
Luteinizing hormone (mIU/ml)			
Baseline	7.4±4.3	9.0±5.2	0.195
6 months	8.7±5.9	7.5±4.1	0.390
24 months	7.5±3.7	7.8±4.9	0.802
At recurrence	9.2±4.6	8.9±3.3	0.948
Total testosterone (ng/dl)			
Baseline	363±219	353±203	0.862
6 months	400±204	395±248	0.931
24 months	307±264	241±265	0.384
At recurrence	461±45	457±220	0.980

Data are shown as the mean±SD. *P*-value for comparison of the two groups by analysis covariance.

rich in ω3 PUFAs promoted apoptosis and decreased the PSA doubling time after prostatectomy compared to one rich in ω6 PUFAs (8).

One pilot study observed the effects of a flaxseed-supplemented, fat-restricted diet on apoptotic indices in prostate tissue and compared them with those of a historical control, and found that these diet modulation may affect prostate cancer biology and associated biomarkers (17). Thus, EPA and DHA are promising dietary agents to reduce PSA recurrence after radical prostatectomy in patients with PC. We, therefore, investigated the effects of EPA on the biochemical recurrence rate of PC in patients who had undergone radical prostatectomy.

Patients and Methods

Patients. Eighty-one PC patients, scheduled for radical surgery in nine hospitals, without metastatic lesions on bone scintigraphy or computerized tomography and without preoperative endocrine therapy were invited to participate in the present study. Seventy-six of them gave preliminary informed consent preoperatively. After excluding those patients who had pathologically positive pelvic lymph nodes or plasma PSA values more than 0.2 ng/ml 3 months after the operation, 68 patients finally entered the trial between August 2002 and December 2003.

Study design. The enrolled patients were randomly assigned to one of two groups (EPA and control groups). Patients in the EPA group (n=34) took 2,400 mg of EPA ethyl ester (Epadel-S, purity >98%; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) per day for two years. The other patients (the control group, n=34) took none. The 2-year trial started 4-5 months after the operation. All participants were asked not to take any EPA-rich supplement in either the pre-trial or trial period. Patients receiving other treatments such as hormonal treatment and chemotherapy during the trial were excluded.

At 0 (baseline), 6, and 24 months (the end) of the trial, plasma luteinizing hormone and testosterone were measured by standard methods. At the same checkpoints, packed erythrocytes were obtained from EDTA-anticoagulated blood from each patient, washed twice with saline, and frozen at –80˚C until analysis. The fatty acid composition of the total phospholipid fraction of washed erythrocytes was determined by gas-chromatography, as described elsewhere (18). The participants were asked to complete a semiquantitative food-frequency questionnaire for the 4 weeks prior to these checkpoints. Food intake was estimated with a calculation program, Eiyokun 3.0 (Kenpakusha Co., Ltd., Tokyo, Japan).

Plasma PSA was measured every two months by the method of two-site immunoradiometric assay. PSA failure was defined when PSA values were more than 0.2 ng/ml on two consecutive measurements. Participation was terminated at PSA failure. For PSA-recurrent patients, measurements of hormones, fatty acids and food intake analysis were completed at termination.

The study was approved by the Ethics Committee of each participating hospital, and written informed consent was obtained from each patient before entering the trial.

Statistical analysis. Deaths due to unrelated causes were treated as cases that could not be followed-up (excluded from calculation). The results are expressed as means±SD. StatView (ver. 5.0; SAS Institute Inc., Cary NC, USA), was used for statistical analysis. The fatty acid composition of erythrocytes, and plasma values of PSA, testosterone, and luteinizing hormone were analyzed parametrically (unpaired *t*-test and analysis of covariance, if there were baseline values, for inter-group comparison, and paired *t*-test for intra-group comparison). Survival rates were analyzed using the log-rank test. $P<0.05$ was considered to be significant.

Results

Characteristics of the patients. In the EPA group, two patients discontinued taking capsules because of nausea one week and six months after the trial respectively. In the control group, four patients were excluded from the study due to taking EPA-rich supplements, starting chemotherapy, death from lung cancer, and moving overseas one week, three months, 12 months and 16 months after the trial, respectively.

Therefore, 62 patients (32 in the EPA group and 30 in the control group) completed the study. Their basic and cancer characteristics are shown in Table I. There was no significant difference in cancer characteristics between the two groups.

Table III. *Unsaturated fatty acids (area %) in the total phospholipid fraction of erythrocytes.*

Fatty acids	Control	EPA	*P*-value+
Arachidonic acid			
Baseline	9.8±1.3	9.7±1.2	0.807
6 months	9.6±1.6	7.6±1.2	<0.001
24 months	9.3±1.3	7.1±1.0	<0.001
At recurrence	9.2±0.7	7.7±0.9	0.080
Eicosapentaenoic acid			
Baseline	2.6±1.2	2.7±1.0	0.783
6 months	2.6±1.0	6.1±1.4	<0.001
24 months	2.3±1.0	5.8±1.2	<0.001
At recurrence	3.0±1.7	6.1±0.9	0.049
Docosapentaenoic acid			
Baseline	2.2±0.3	2.2±0.4	0.983
6 months	2.2±0.4	4.0±0.7	<0.001
24 months	2.1±0.3	4.1±0.5	<0.001
At recurrence	2.5±0.1	3.8±0.2	<0.001
Docosahexaenoic acid			
Baseline	8.0±1.1	8.0±1.1	0.879
6 months	8.1±1.2	6.6±1.3	<0.001
24 months	7.7±1.1	6.3±1.0	<0.001
At recurrence	9.0±0.9	7.3±2.0	0.130
ω3/ω6 Ratio			
Baseline	0.59±0.17	0.60±0.15	0.910
6 months	0.62±0.17	0.96±0.19	<0.001
24 months	0.59±0.17	0.95±0.19	<0.001
EPA/AA ratio			
Baseline	0.28±0.17	0.29±0.14	0.880
6 months	0.28±0.14	0.82±0.21	<0.001
24 months	0.27±0.17	0.85±0.26	<0.001

Data are shown as the mean±SD. +*P*-value for comparison of the two groups by analysis of covariance. EPA, Eicosapentaenoic acid; AA, arachidonic acid.

Plasma hormonal levels were not significantly different between the groups at the baseline, and remained so throughout the study (Table II).

EPA and docosapentaenoic acid (ω3) concentrations in the total phospholipid fraction in erythrocytes significantly increased in the EPA group during the trial, with no significant changes occurring in the control group (Table III). In contrast, arachidonic acid and DHA decreased significantly in the EPA group, without any significant changes occurring in the control group. Food analyses revealed no significant difference in the average intakes of macronutrients, nor of ω3 and ω6 fatty acids between the groups (data not shown).

Recurrence-free survival rate. PSA failure occurred in four patients in the EPA group, whereas it developed in eight patients in the control group. The recurrence-free survival rate is shown in Figure 1. Kaplan-Meier analysis identified no significant difference between the groups (p=0.16).

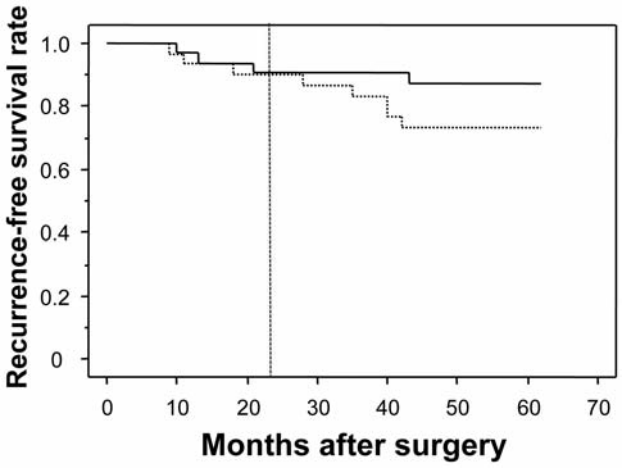

Figure 1. *PSA recurrence-free survival rates after prostatectomy. There was no significant difference (p=0.16) between the two groups. Solid line: EPA group. Broken line: Control group. Vertical broken line: the end of intervention.*

Plasma hormonal levels and fatty acid concentrations in erythrocytes between patients with PSA failure and those with non-failure in each group were not significantly different (data not shown). Major dietary fatty acid intakes between the PSA failure and non-failure subgroups were not significantly different (data not shown).

Discussion

PSA recurrence after radical prostatectomy is thought to stem not from *de novo* PC, but mostly from the remaining cancer cells not eradicated surgically (19, 20). Biochemical recurrence after radical prostatectomy usually occurs rapidly, suggesting the underestimation of preoperative clinical staging (19). The PSA recurrence-free rate of the present study was comparable to that of a reported series of organ-confined cases (21). Two-year intervention by EPA might not be sufficient to suppress the growth of cancer cells not eradicated by surgery because PSA recurrence occurs even after two years.

In the present trial, the DHA concentrations in the total phospholipid fraction in erythrocytes decreased after EPA administration. Similar findings were reported previously (22, 23). These changes may be explained by the following actions of EPA itself: During the conversion of EPA to DHA, Δ6-desaturase is necessary. EPA competitively inhibits this enzyme. DHA incorporation into position *sn-2* of phospholipids probably competes with EPA, which is also preferentially incorporated into position 2.

It was reported that there was a strong correlation between EPA and DHA levels in leukocytes and prostatic tissue (24), and that short-term intervention with a low-fat diet and fish

oil-supplementation caused a parallel increase in the ω3/ω6 fatty acid ratio in plasma and gluteal adipose tissue in men with PC (25). Taking these experimental findings together, EPA and DHA levels in erythrocytes may reflect those in PC tissue not eradicated by surgery in the EPA group. Total EPA and DHA levels and EPA-to-DHA ratios at baseline were similar between two groups. During a two-year intervention with EPA, combined EPA and DHA levels increased from 10.7 to 12.1 area % and EPA-to-DHA ratios decreased from 1:3 to 1:1. EPA and DHA have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids and other components (23, 26). The 'optimal' ratio expected to be effective is currently unknown (27). If DHA, rather than EPA, is important in preventing PSA failure, intervention involving fish oils containing DHA might generate different results.

Prior to the present study, we examined the effect of EPA on the levels of PSA, testosterone, and luteinizing hormone (18). Twenty men were randomly allocated to the EPA group or control. Those in the EPA group were administered the same dose of EPA ethyl ester (2,400 mg/d) as in the present trial for 12 weeks, whereas controls took none. EPA concentrations in erythrocytes increased by 174±96% in the EPA group with no significant changes in the control group (8.5±14.0%). There were no significant differences between the two groups regarding the serum levels of PSA, testosterone, and luteinizing hormone. We therefore concluded that it was appropriate to use PSA as the surrogate marker of recurrence in the present study. The increase in EPA identified in that preliminary study (+174%) was very similar to that the present trial.

Direct anticancer effects against not-surgically-eradicated cancer tissue are required to prevent early cancer recurrence after radical prostatectomy. Fish oil may have preventive effects against the *de novo* occurrence of PC as well as direct anticancer effects on PC (7, 8). A longer term and/or larger number of patients, and supplemental administration of DHA might be required to observe any potential preventive effects of EPA on PSA recurrence after radical prostatectomy.

Conflict of Interest

EPA ethyl ester capsules (Epadel-S®) and research funds were provided by Mochida Pharmaceutical Co., Ltd. (Tokyo, Japan) to each institute.

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