Rationale and Study Design of Dietary Intervention in Patients Polypectomized for Tumors of the Colorectum

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We have implemented a randomized controlled dietary intervention in patients polypectomized for tumors of the colorectum to elucidate potential beneficial effects of n-3 polyunsaturated fatty acids (PUFAs) on the development of colorectal tumors. Those individuals in the experimental group were advised not only to decrease their consumption of fats/oils as a whole and foods supplying n-6 PUFAs but also to increase intake of foods and supplements containing n-3 PUFAs, while those in the comparison group were cautioned to reduce intake of fats/oils as a whole. Patients’ compliance/adherence was monitored with a semi-quantitative food frequency questionnaire and by assessment of fatty acid concentrations in plasma, membranes of red blood cells and sigmoid colon samples. As for endpoints to assess tumor suppressive effects of n-3 PUFAs, the number/multiplicity, sizes and incidence rates of colorectal tumors were compared between the experimental and comparison groups after 12 and 24 months of the dietary intervention. On the specified assumption, the number of pairs needed for achieving statistical significance was calculated to be approximately 60–80. A randomized controlled trial is under way to secure enough patients, sustain compliance/adherence and minimize dropouts.

Key words: colorectal tumors – dietary intervention – n-3 polyunsaturated fatty acids – polypectomized patients – randomized controlled trial

INTRODUCTION

The rationale for this randomized controlled trial (RCT) was that an adenoma–carcinoma sequence with a long latency period appears to be involved in development of most sporadic colorectal cancers (1). Thus, prevention of tumors of the colorectum would be beneficial for reducing the onset of/mortality from cancers. Furthermore, not only epidemiological/ecological studies (2,3) but also experimentation in animals (4), in cell lines (5) and in humans (6–8) suggests that n-3 PUFAs [(ω3 PUFAs, including α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] may act as anti-promoters in colonic carcinogenesis like NSAIDs (9), which inhibit the synthesis of arachidonic acid in the upper eicosanoid/arthachidonic acid cascade.

On the other hand, n-6 PUFAs (or ω6 PUFAs, mainly linoleic acid) may act as tumor promoters, because they are upstream chemicals for the arachidonic acid cascade and substrates for prostaglandin E2 (PGE2), which is catalyzed by cyclooxygenase isoenzymes (COX) (3,10,11). The underlying mechanisms, however, may be explained not only by COX-mediated cell proliferation but also with reference to cell differentiation (6–8), apoptosis/programmed cell death (5) and angiogenesis (3), which are partly dependent on genetic polymorphisms, including examples affecting the peroxisome proliferation-activated receptor γ (PPARγ) (1). Thus, elevated intake of n-6 PUFAs and low intake of n-3 PUFAs, as well as increased consumption of fats/oils as a whole, may elevate the risk of colorectal cancer (12).

Because RCTs are considered the most appropriate approach to clarify hypothesized associations between factors and dis-
cases precisely, we are now executing a dietary intervention for patients polypectomized for tumors of the colorectum (DIPP study) to elucidate whether n-3 PUFAs might indeed have a tumor-suppressive role. Although several trials have provided evidence of beneficial effects of n-3 PUFAs regarding the onset of aberrant crypt foci of the colon (6–8), as mentioned, dietary interventions directly examining the effects of n-3 PUFAs on colorectal tumors, to our knowledge, have hitherto not been performed. Here we present the rationale and study design of our DIPP study.

**SUBJECTS AND METHODS**

**STUDY SUBJECTS**

Patients polypectomized for tumors of the colorectum were recruited. The lesions included grade 3 adenomas with light/moderate atypia, grade 4 adenomas with severe atypia and grade 5 adenocarcinomas according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus (13), and individuals with FAP or HNPCC, bleeding diathesis or a past history of colorectal cancer, gastrectomy or cholecystectomy were excluded. The subjects were first randomly allocated to the experimental or comparison group according to the modified Zelen’s method (14). Under a fixed schedule, 7 ml of overnight fasting venous blood was sampled and divided into plasma, buffy coat [layer of white blood cells (WBCs)] and red blood cells (RBCs) (Fig. 1). WBCs are to be used for the analysis of genetic polymorphisms relevant to fat metabolism, including PPARγ, to detect whether gene–environment interactions might exist which could impact on the onset of colorectal tumors.

**REGIMEN**

For the experimental group we recommended (1) reduction of intake of fats/oils as a whole; (2) decreased consumption of n-6 PUFAs, including cooked/deep-fried foods; (3) increased intake of n-3 PUFAs, (3-a) from fish/marine foods and (3-b) from perilla oil rich in α-linolenic acid, instead of other vegetable oils, and (3-c) from eight capsules of fish oil/day (equivalent to 100 mg/day of EPA and 400 mg/day of DHA) for 2 years (Table 1). For the comparison group we advised a decreased intake of fats/oils as a whole.

**SAFETY**

The safety of perilla oil is well established and it is used as a cooking oil in Japan, Korea and China. Fish oil is also generally accepted to be safe and the amount recommended is free from side effects, that is, the concentrations of EPA and DHA are approximately the same as contained in half a sardine or one-third of a horse mackerel/saury. The recruited patients are periodically checked and monitored according to prothrombin time and lipid peroxide concentrations every 6 months after the intervention. If any severe adverse effects are observed, our RCT would be discontinued.

**INFORMED CONSENT**

Information on the protocol, blood sampling and collection of three biopsies of normal sigmoid colon membranes is being

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Table 1. Regimen of the DIPP study

<table>
<thead>
<tr>
<th>Diet Intervention</th>
<th>Experimental group</th>
<th>Comparison group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake of fats/oils, as a whole</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Intake of n-6 PUFAs</td>
<td>↓</td>
<td>NA*</td>
</tr>
<tr>
<td>Intake of n-3 PUFAs</td>
<td>↑</td>
<td>NA</td>
</tr>
<tr>
<td>Functional foods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake of perilla oil†</td>
<td>↑</td>
<td>NA</td>
</tr>
<tr>
<td>Intake of fish oil‡</td>
<td>↑</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA, not applicable. †Intake of perilla oil (rich in α-linolenic acid) is ad libitum. ‡Intake of fish oil (EPA = 12 mg, DHA = 45 mg/capsule) is eight capsules/day.
given to both the experimental and comparison groups. Descriptions of possible side effects to the experimental group, especially for intake of perilla and fish oils in terms of bowel symptoms, if any, are also provided. Informed consent is secured after the respective interventions. There is no discrimination in regular checkups according to participation/refusal to take part in the study or any drawbacks ensuing on withdrawal from the regimen. Only patients who give informed consent are included in the study.

**Compliance/Adherence**

For patients’ compliance/adherence we monitor dietary modifications, including intake of cooked/deep-fried foods and fish/marine foods, and consumption of perilla and fish oils by a semi-quantitative food frequency questionnaire (SQFFQ). We also measure concentrations/compositions of fatty acids in plasma, membranes of RBCs and intact sigmoid colon samples.

**Intermediate Biomarkers**

Cell-cycle and cell growth markers (including Ki67 and cyclin D1), expression of COX2 and apoptosis markers (including TUNEL and expression of Bax, Bcl-2 and Bax/Bcl-2) are to be examined by immunohistochemical staining. For future monoclonal enzyme immunoassay, PGE2 formation in fresh biopsy specimens is halted by adding indomethacin with storage at −80°C after rapid freezing.

**Endpoints**

We intend to assess any tumor-suppressive effects of n-3 PUFAs by comparing the number/multiplicity, sizes and incidence rates of colorectal tumors between the experimental and comparison groups after 12 and 24 months of the dietary intervention.

**Follow-up**

The patients are being traced carefully for the 2-year period according to the schedule. Every 6 months during the intervention, an SQFFQ survey and sampling of overnight fasting venous blood are conducted. After 12 and 24 months of the intervention, all the participants will undergo total colonoscopy. Intermediate markers and endpoints are to be scrutinized and the differences between the experimental and comparison groups will be tested according to univariate and multivariate analyses. Intention-to-treat procedure will be adopted for the subjects dropping out during the follow-up period.

**Sample Size**

Assuming the incidence of colorectal tumors after cleaning the colon to be 40–50% in the comparison group and a relative risk of 2 (or 0.5), \( \alpha = 0.05 \) (two-sided) and \( \beta = 0.20 \) (one-sided), the number of pairs in the experimental and comparison groups necessary for achieving statistical significance was calculated to be ~60–80.

**Research Team**

Random allocation to the experimental or comparison group was made. The information for assignment was naturally blind to both the attending physicians and the patients. Trained colonoscopists conduct polypectomy, clean the colorectums and diagnose the sizes, number and onset of colorectal tumors before and during the intervention. Pathologists diagnose the tumors polypectomized according to the standardized criteria (13).

**Ethical Issues**

Our protocol was, *a priori*, reviewed and approved by the Ethical Committee of our Institute.

**Discussion**

Our RCT is conducted according to the revised Zelen’s method (14) and allocation is made prior to the intervention. The perspective of RCT is not fully communicated to the patients and may not be sufficient for his/her autonomy whether to participate in the RCT or not. We could not set the comparison group with placebo foods because placebo oil and dummy fish capsules were not available, partly because a Production Liability (PL) Law had just been issued in 1999 when the present study was launched. Under this law, companies can be fined if their products turn out to be unhealthy or harmful. Hence this law may restrain companies from producing any new drugs/products and seems to be one of the greatest obstacles in executing RCTs, including those targeting chemoprevention, in Japan.

When compared with the lifetime of experimental rodents, including rats and mice, the human life span is much longer, as is the latent period for intestinal tumors. The intervention and follow-up periods set in the present trial may be insufficient and observations of short duration may be secured through a small ‘window’ (15), although 2 years of intervention and follow-up, in fact, seems generally longer than the period in RCTs executed so far. To maintain/support patients’ compliance/adherence for 2 years is laborious, even in people having precancerous lesions for cancer.

In summary, our DIPP study appears valid and robust in design. However, care is primarily needed to sustain compliance/adherence and minimize dropouts. We conclude that such a study can be best carried out in countries, including Japan, where fish and marine foods are routinely eaten, allowing assessment of modification effects of n-3 PUFAs intake on the risk of tumors of the colorectum by way of analysis of biomarkers and tumor endpoints, with recruitment of sufficient numbers of participants, which will hopefully be achieved within 1 year.

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