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A Randomized Controlled Trial for Chemoprevention of Gastric Cancer in High-risk Japanese Population; Study Design, Feasibility and Protocol Modification

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We have initiated a population-based, double-blind, randomized controlled trial to examine the effects of supplementation of beta-carotene and vitamin C on the incidence of gastric cancer. The subjects were participants in an annual health screening program conducted by four municipalities in Akita prefecture, one of the regions with the highest mortality from gastric cancer in Japan. We measured their serum levels of pepsinogens (PGs) I and II, and asked persons diagnosed with chronic atrophic gastritis (defined as PG I <70 ng/ml and PG I/PG II ratio <3.0) to take diet supplements containing 0 or 15 mg/day beta-carotene and 50 or 500 mg/day vitamin C for 5 years. During the first year of recruitment conducted in one village from June through September, 1995, 52% (635/1214) of screening participants had chronic atrophic gastritis and 73% (439/602) of eligible persons responded. However, in response to a National Cancer Institute press report released on January 18, 1996, indicating that two beta-carotene trials had shown no benefit and potential harm from the supplement, we discontinued the beta-carotene and continued with the trial using only vitamin C. Of 397 participants remaining at this point, 77% (305) consented to stay in the study. The results indicate that a randomized controlled trial for cancer prevention is feasible in the Japanese asymptomatic population.

Key words: Ascorbic acid — Atrophic gastritis — Beta-carotene — Gastric cancer — Randomized Controlled Trial

There has been a growing interest in the possibility that diet supplementation with micronutrients may prevent the occurrence of cancers in an asymptomatic population. Several large-scale intervention trials have been conducted in China,[1)] Finland[2)] and the United States,[3,4)] to test the efficacy of retinol, beta-carotene, alpha-tocopherol and other nutrients, but no studies have been initiated in Japan.

After confirming the feasibility of a population-based chemoprevention study through a pilot investigation,[5)] we started a randomized controlled trial in order to assess the effects of diet supplementation with beta-carotene and vitamin C on the development of gastric cancer using high-risk persons with chronic atrophic gastritis in a district of Akita prefecture in north-eastern Japan, one of the regions with the highest mortality from the disease in the country. However, in response to a National Cancer Institute (NCI) press report[6)] released on January 18, 1996, indicating that two beta-carotene trials showed no benefit and potential harm from the supplement, we modified the study protocol and halted the supplementation of beta-carotene. This paper describes the study designs and response rates for the initial and modified protocols.

MATERIALS AND METHODS

Table I shows a summary of the initial protocol. This protocol was approved by the institutional review boards at the National Cancer Center and Hiraka General Hospital.

Subjects Target subjects were men and women aged 40–69 years living in 4 municipalities (3 towns and 1 village) of Yokote Public Health Center District in Akita prefecture, who participated in annual screening programs for circulatory diseases conducted by each municipality under the National Health and Welfare Services Law for the Aged. After informed consent had been obtained from each subject, serum levels of pepsinogens (PGs) I and II were measured. Those who were diagnosed with chronic atrophic gastritis (defined as PG I <70 ng/ml and PG I/PG II ratio <3.0) were considered for further recruitment.

Persons with any of the following characteristics were ineligible for the study: previous history of gastric cancer or surgery; previous history of liver cancer or cirrhosis;

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Table I. Summary of the Initial Protocol

Subjects
Inclusion criteria
Residents in 4 municipalities in Yokote Public Health Center District
Aged 40–69 years
Participants in annual screening programs for circulatory diseases under the National Health and Welfare Services Law for the Aged
Chronic atrophic gastritis determined by serum pepsinogen (PG) levels (PG I <70 ng/ml and PG I/PG II ratio <3.0)
Exclusion criteria
Past history of gastric cancer or surgery
Past history of liver cancer or cirrhosis
Past history of other cancers within 5 years
Abnormal liver function (GOT >100 IU/liter, GPT >100 IU/liter, or ALP >800 IU/liter)
Use of supplements containing beta-carotene or vitamin C
Unable to follow up for at least 1 year
Recruitment
Description of the study protocol at community centers
Signed informed consent mandatory
Intervention
4-week run-in period
15 mg/day beta-carotene and 500 mg/day vitamin C to all participants
5-year supplementation
Randomized, double-blind, 2×2 factorial design
0 or 15 mg/day beta-carotene, and 50 or 500 mg/day vitamin C
Follow-up
Prescription of the supplements, assessment of compliance, and examination of clinical symptoms
Every 3 months at community centers for the first 5 years
Collection and measurement of blood samples
At the annual screening program for circulatory diseases for the first 5 years
Morbidity and mortality for 10 years
Endpoints
Primary; 10-year cumulative incidence of gastric cancer
Secondary; 5-year change in serum levels of pepsinogens

previous history of other cancers within the last 5 years; abnormal liver function (aspartate aminotransferase >100 IU/liter, alanine aminotransferase >100 IU/liter, or alkaline phosphatase >800 IU/liter); use of diet supplements containing beta-carotene or vitamin C; and expectation of moving outside the study area within 1 year.

Recruitment We first mailed a booklet to eligible participants to describe the objectives and methods of the trial, anticipated efficacy and side effects of beta-carotene and vitamin C supplements, and measures to protect the human rights of the trial participants. We then invited them to community centers to attend a talk about the study. At this point we obtained signed consent forms from persons willing to participate and provided them with supplement capsules.

Intervention At the outset, we prescribed capsules containing full doses of beta-carotene (15 mg/day) and vitamin C (500 mg/day) to all participants for 4 weeks. We conducted this "run-in" procedure in order to identify and exclude at an early stage the subjects who either did not comply or showed side effects. We then randomized the remaining participants into four treatment groups using a 2×2 factorial design, whereby 0 or 15 mg/day beta-carotene and 50 or 500 mg/day vitamin C were supplemented for 5 years in a double-blind manner. One capsule contained half the daily dose of each of the two nutrients, and we instructed the subjects to take two capsules per day after their evening meal.

Follow-up We asked the participants to visit community centers every 3 months, where public health nurses checked them for clinical symptoms, assessed compliance by counting numbers of unconsumed capsules, and provided further capsules. We scheduled collection of blood samples from the participants every year at the annual screening for circulatory diseases in order to measure levels of liver function markers, beta-carotene, vitamin C, and other micronutrients. At baseline and the fifth

year, we scheduled administration of a semiquantitative food frequency questionnaire to assess dietary nutrient intake, and also measurements of serum levels of PGs and *Helicobacter pylori* antibody. Mortality and morbidity of the subjects were to be followed for 10 years using population registry and hospital records. Most of the incident cancer cases were expected to be diagnosed in hospitals and clinics in the study district.

Endpoints The primary endpoint of the trial was the 10-year cumulative incidence rate of gastric cancer. The secondary endpoint was the 5-year change in serum PG levels, which we regarded as a measure of the progression of chronic atrophic gastritis. The endpoints would be compared between the two groups supplemented with 0 mg/day (control group) and 15 mg/day (intervention group) of beta-carotene, and between the groups given 50 mg (control group) and 500 mg/day (intervention group) of vitamin C.

Sample size We estimated that a minimum of 1812 participants would be needed for the trial, 906 allocated to each of the control and intervention groups. The expected 10-year cumulative incidence of gastric cancer in the control group was 7%, and this sample size would permit the detection of a 40% reduction of the incidence in the intervention group (from 7% to 4%) with 5% alpha-error (two-sided) and 20% beta-error. We anticipated that 3,600 (40%) of 9,000 persons who were expected to attend the screening programs for circulatory diseases would be diagnosed with chronic atrophic gastritis, and that 2,340 (65%) of them would eventually participate in the trial, thus exceeding the required sample size. We planned to spend 4 years from 1995 to accrue participants from 4 municipalities, adding one municipality per year.

Modification of the protocol On January 18, 1996, the NCI released a press report[6] indicating that two beta-carotene trials had shown no benefit and potential harm from the supplement (see "Discussion" for the details). In response to this report, we mailed letters to all participants on January 25, 1996, giving a brief summary of the press release and asking them to stop taking the supplement capsules temporarily until we had obtained details of the two trials and had made a final decision on the future of the study.

Subsequently, we decided to modify the initial study protocol as summarized in Table II. First, supplementation of beta-carotene would be stopped, but the prescription of vitamin C would be continued for five years. Second, the study area would be restricted to the village where participants had already been recruited, and no new participants would be recruited from the three other municipalities. Finally, the primary endpoint of the trial would be changed from the 10-year cumulative incidence of gastric cancer to the 5-year change in serum levels of pepsinogens and other biomarkers.

On February 9 or 16, we invited the participants to community centers, described in detail the results of the two US studies, explained the modification of the study protocol, and collected the discontinued capsules from each participant. We obtained signed consent forms again from individuals willing to take part in the modified trial, and provided them with new capsules containing vitamin C only (50 mg/day or 500 mg/day). Participants who did not attend the meetings at community centers were contacted individually to establish whether they wished to remain in the study.

Table II. Modification of the Protocol

Halted the supplementation of beta-carotene and continued the prescription of vitamin C
Restricted the study area to one village where the participants had already been recruited
Altered the primary endpoint to 5-year change in serum levels of pepsinogens and other biomarkers

Table III. Response Rate for the Initial Protocol; the First Year Results in One Village

	Men	Women	Total
A. Participated in the screening program	489	742	1231
B. Provided blood samples for pepsinogen measurements	485	729	1214
C. Diagnosed with chronic atrophic gastritis (C/B)	247 (51%)	388 (53%)	635 (52%)
D. Ineligible by exclusion criteria	26	7	33
Past history of gastric cancer, gastric surgery, liver cancer or liver cirrhosis	11	2	13
Past history of other cancers within 5 years	2	1	3
Abnormal liver function	9	2	11
Use of supplements containing beta-carotene or vitamin C	4	2	6
E. Eligible	221	381	602
F. Responded (F/E)	160 (72%)	279 (73%)	439 (73%)

Table IV. Response Rate for the Modified Protocol

	Men	Women	Total
A. Participated in the initial protocol	160	279	439
B. Dropped out before modification of the initial protocol (B/A)	18 (11%)	24 (9%)	42 (10%)
C. Eligible	142	255	397
D. Responded (D/C)	111 (78%)	194 (76%)	305 (77%)

RESULTS

Response rate for the initial protocol Table III summarizes the first year results for the recruitment of trial participants in one village. Screening for circulatory diseases was conducted from June through September, 1995. Out of 1231 individuals screened, 1214 provided serum for PG measurements, and 635 of these (52%) were diagnosed with chronic atrophic gastritis. Thirty-three people were ineligible since they failed to meet the inclusion criteria. Of the remaining 602 eligible individuals, 439 (73%) consented to take part in the trial. All participants had started taking "run-in" capsules by December 1995.

Response rate for the modified protocol Table IV shows the response rate for the modified protocol. Out of the 439 persons initially participating in the study, 42 had dropped out before the study was altered. Of the 397 remaining participants, 305 (77%) consented to take part in the modified trial.

As reported previously,[5] we had conducted a pilot study using recipients of a health check-up program at the Hiraka General Hospital in order to assess the feasibility of the main population-based trial. Out of the original 55 participants in the pilot study, 54 had completed the 3-month pilot intervention and were subsequently enrolled in the main study. We applied the same procedures to them as to the population-based participants, and 42 decided to remain in the trial.

DISCUSSION

This is the first population-based, randomized controlled trial for cancer chemoprevention initiated in Japan. Despite skepticism that cancer prevention trials may not be possible in this country if informed consent is obtained from participants prior to randomization,[7] the results clearly indicate that such studies are certainly feasible even in asymptomatic populations.

The rationale for the trial has been reviewed elsewhere.[5] Briefly, chronic atrophic gastritis is a precursor for intestinal-type gastric cancer.[8] The serum level of PGs is a good marker for atrophic gastritis.[9] Vitamin C inhibits endogenous nitrosamine formation and also acts as a free radical scavenger.[10] Current per capita intake of vitamin C (117 mg/day) in the Japanese population[11] may not be sufficient for the protective effects of the nutrient, if any, against gastric cancer incidence to be manifested. Chronic atrophic gastritis is associated with a lower level of gastric juice ascorbic acid, and vitamin C supplementation increases its concentration and inhibits gastric *N*-nitrosation.[12] Beta-carotene could prevent the progression of atrophic gastritis through its antioxidant effect.[13] Findings from an ecological study we have conducted in 5 areas of the country including the study district indicate that inhabitants of this region are characterized by higher mortality from gastric cancer, higher prevalence of chronic atrophic gastritis,[14] lower intake of green and yellow vegetables,[15] and lower plasma levels of beta-carotene.[16] Supplementation of the diet with vitamin C and beta-carotene may therefore prevent the development of gastric cancer among persons with chronic atrophic gastritis in this district. We did not plan to eradicate *Helicobacter pylori* in infected subjects, since we considered it difficult to conduct antibiotic therapy at the population (rather than clinical) level.

Soon after we had completed the first year of participant recruitment in December, 1995, the NCI released a press report on two beta-carotene trials on January 18, 1996, prior to the formal publication of the study results.[6] Details of the trials were published subsequently in May.[3,4]

The first study, the Beta Carotene and Retinol Efficacy Trial (CARET), was designed to evaluate the efficacy of the combination of beta-carotene and retinol in preventing lung cancer in high-risk subjects.[3] The study randomized 18,314 participants (men and women who smoked and men with a history of occupational exposure to asbestos) into two groups and provided them with diet supplements consisting of a combination of 30 mg/day beta-carotene and 25,000 IU/day retinol or inactive placebo. Contrary to expectation, the interim results revealed a 28% increase in the incidence of lung cancer and a 17% increase in all-cause mortality in individuals who had taken beta-carotene and retinol for an average of 4 years. The results were similar to the findings of the Alpha-Tocopherol, Beta-carotene (ATBC) Lung Cancer Prevention Trial in Finland[2] which involved 29,133 male

smokers and showed an 18% increase in the incidence of lung cancer and an 8% increase in all-cause mortality in participants who had taken 20 mg/day beta-carotene for 5–8 years. The CARET investigators halted the supplementation 21 months earlier than had been initially planned.

The second study, the Physicians' Health Study (PHS), randomized 22,071 US male physicians into two groups and supplemented 50 mg beta-carotene or placebo every second day.[4] After 12 years of treatment which ended on schedule, the study showed no significant evidence of benefit or harm from beta-carotene in terms of cancer or cardiovascular diseases.

On-going intervention trials using beta-carotene responded in different ways to the NCI press release on CARET and PHS.[17] The Women's Health Study discontinued the supplementation of beta-carotene. In the Age-Related Eye Disease Study, the investigators recommended the smokers (comprising 8% of 5,000 participants) to stop taking the study pills, and many of them did so. On the other hand, the Women's Antioxidant and Cardiovascular Study has continued the use of beta-carotene supplements for all allocated participants.

In the present trial, we discontinued the supplementation of beta-carotene based on ethical considerations. We had originally expected that about 8 cases of lung cancer would have occurred in the planned 2,000 participants during the 10-year study period (6 from 600 smoking men, 1 from 400 nonsmoking men, and 1 from 1,000 women, most of whom were nonsmokers), so that about 4 cases would have occurred in 1,000 subjects allocated to beta-carotene supplementation. The daily supplement dose we used (15 mg of beta-carotene, equivalent to 25,000 IU of vitamin A) is only one third of the dose used in CARET (30 mg of beta-carotene and 25,000 IU of vitamin A, equivalent to 75,000 IU of vitamin A). If we assume that our dose of supplementation caused the same increase in lung cancer incidence among smokers as observed in CARET (28%), we would expect one more case of lung cancer to occur among the 300 smoking men assigned to beta-carotene supplementation (3 cases originally expected $\times 1.28 = 3.84$ cases). We therefore decided to remove beta-carotene, given that the two large-scale trials[2,3] have indicated a possibility that long-term supplementation with beta-carotene may cause serious adverse effects in smokers.

We discontinued the recruitment of new participants from other municipalities, since we considered it logistically impossible to carry out all the procedures necessary to modify the protocol and at the same time to increase the number of study districts. The limited sample size due to this restriction of study area will inevitably hinder evaluation of the effect of vitamin C supplementation on gastric cancer incidence, and the new protocol will only allow us to assess the effect on progression of chronic atrophic gastritis as indicated by the change in the serum PG levels.

Several characteristics of our study may explain the unexpectedly high response rates for both initial and modified protocols. First, we collaborated closely with local organizations including the health care department of the study village, a Public Health Center, hospitals and clinics, and regional medical associations, all of which are well respected by the local people. Second, based on this collaboration, we were able to use the administrative mechanisms that already exist for providing annual screening programs to the target population. Third, eligible subjects diagnosed with chronic atrophic gastritis were informed of their high risk status for gastric cancer, so they would presumably be eager to take any actions that might help prevent the disease. Fourth, the fact that an inactive placebo was not used might further motivate them to take part in the trial (participants were provided with capsules containing at least 50 mg/day of vitamin C). Our experience therefore indicates that a population-based randomized controlled trial for cancer prevention is feasible in other settings in Japan if investigators expect a similar situation and can establish the necessary logistics.

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