

PREVENTION OF SECOND PRIMARY TUMORS BY AN ACYCLIC RETINOID, POLYPRENOIC ACID, IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Abstract Background. In patients with hepatocellular carcinoma (hepatoma), the rate of recurrent and second primary hepatomas is high despite surgical resection and percutaneous ethanol-injection therapy. We developed an acyclic retinoid, polyprenoic acid, that inhibits hepatocarcinogenesis in the laboratory and induces differentiation and apoptosis in cell lines derived from human hepatoma. In a randomized, controlled study, we tested whether the compound reduced the incidence of recurrent and second primary hepatomas after curative treatment.

Methods. We prospectively studied 89 patients who were free of disease after surgical resection of a primary hepatoma or the percutaneous injection of ethanol. We randomly assigned the patients to receive either polyprenoic acid (600 mg daily) or placebo for 12 months. We studied the remnant liver by ultrasonography every three months after randomization. The primary end point of the study was the appearance of a histologically confirmed recurrent or new hepatoma.

Results. Treatment with polyprenoic acid significantly reduced the incidence of recurrent or new hepatomas. After a median follow-up of 38 months, 12 patients in the polyprenoic acid group (27 percent) had recurrent or new hepatomas as compared with 22 patients in the placebo group (49 percent, $P=0.04$). The most striking difference was in the groups that had second primary hepatomas — 7 in the group receiving polyprenoic acid as compared with 20 in the placebo group ($P=0.04$ by the log-rank test). Cox proportional-hazards analysis demonstrated that as an independent factor, polyprenoic acid reduced the occurrence of second primary hepatomas (adjusted relative risk, 0.31; 95 percent confidence interval, 0.12 to 0.78).

Conclusions. Oral polyprenoic acid prevents second primary hepatomas after surgical resection of the original tumor or the percutaneous injection of ethanol. (N Engl J Med 1996;334:1561-7.)

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THE chemoprevention of cancer is one of the most challenging aspects of medical research.¹⁻⁵ Primary chemoprevention addresses a general population or a population at particularly high risk, such as long-term smokers. By contrast, secondary chemoprevention applies to patients with precancerous lesions, such as oral leukoplakia, and patients with treated cancer, in whom the risk of a recurrence or a new primary cancer is high.⁵ Several randomized trials of chemoprevention have yielded inconsistent results.⁶⁻⁹ These trials mainly used micronutrients or antioxidant nutrients, including analogues of vitamin A (retinoids).

In attempts to develop novel synthetic compounds for cancer chemoprevention, we found a 20-carbon polyprenoic acid (3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid) (Fig. 1) that binds to the cellular retinoic acid-binding protein and has relatively low toxicity.¹⁰ This agent inhibits chemically induced hepatocarcinogenesis in rats and spontaneous hepatocel-

lular carcinomas (hepatomas) in mice¹¹⁻¹³ and suppresses cell growth and the production of alpha-fetoprotein in human-hepatoma-derived cell lines.¹⁴ Its action is mediated in part through the retinoic acid receptor and the retinoid X receptor.¹⁵ Hence, this compound can be called an open-chain, or acyclic, retinoid.¹⁶

Phase 1 trials of polyprenoic acid were conducted in 35 healthy subjects at doses of 25, 75, 150, 300, and 600 mg per day. One subject who received 600 mg per day had hyperlipidemia, whereas the others tolerated that dose with no adverse effects. In 12 patients with liver cirrhosis who were given 300 or 600 mg per day, 1 patient receiving the 600-mg dose reported oral dryness, whereas the others had no physical or laboratory abnormalities (Aoyama M, Eisal Co., Ltd.: personal communication).

Since the rates of tumor recurrence and of the occurrence of second primary tumors determine the long-term prognosis in patients with primary hepatoma,¹⁷⁻²³ we conducted a randomized, controlled study to test whether polyprenoic acid prevents recurrences and second primary hepatomas after curative surgical resection or the percutaneous injection of ethanol into the initial hepatoma.

METHODS

Patients

The study involved six clinical centers where patients underwent curative surgical resection of a hepatoma or received a percutaneous injection of ethanol into at least one histologically confirmed hepatoma. The patients selected for the study were considered to be free of further hepatoma by ultrasonography and plain and contrast-

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enhanced computed tomography (CT) of the entire remnant liver and by blood tests, including determinations of alpha-fetoprotein. Intraoperative ultrasonography was performed in every patient who underwent surgical resection. The selection of patients began in September 1990, and follow-up data on all the patients were updated in February 1996.

The evaluation of each patient before entry into the study included a history taking, physical examination, and appropriate laboratory assessment. To be eligible, the patients had to have good performance status and be under 75 years of age. Each patient's tumor was graded on the basis of the clinical criteria defined by the Liver Cancer Study Group of Japan.²⁴ We excluded patients with a serum total bilirubin level higher than 5.0 mg per deciliter (84 μ mol per liter) and those with any severe cardiac, renal, or hematologic disorder that might be worsened by the agent.

The study protocol was approved by the review board for research on human subjects at each institution. Written informed consent, including an agreement not to take vitamin A supplements during the study, was obtained from each patient.

Study Design

At the beginning of the study, we assumed that the incidence of recurrent and second primary hepatomas would be approximately 40 to 50 percent over a two-year period¹⁷⁻²³ and that the active treatment would reduce the incidence to about half that found in our previous studies.¹¹⁻¹³ Using these assumptions and a table proposed by Gehan,²⁵ we calculated that the study sample would require 40 patients in each group, with an alpha error of 5 percent and a beta error of 20 percent.

We reviewed data on 111 potentially eligible patients, 89 of whom were found to be eligible and willing to participate and to provide informed consent. Of the 22 patients who were not randomized, 12 were unwilling to participate in the study, 7 moved to hospitals not involved in the study, 2 did not have serum bilirubin levels in the required range, and 1 was found to have been treated with fluorouracil immediately after surgery.

We randomly assigned the 89 patients to receive active treatment or placebo (peanut oil), with blocking according to study center. The study design called for 14 patients to be randomized at each center, and the actual numbers ranged from 11 to 23 (median, 14). The active treatment was 600 mg of polyphenolic acid daily (two 150-mg capsules taken twice daily) for 12 months. This dose was chosen to correspond to the level of intake that had shown a benefit in laboratory studies and because its safety had been demonstrated in a phase 1 trial. We provided the placebo or polyphenolic acid (Eisai) in soft, opaque gelatin capsules packaged in calendar packs. We recovered the packs every month to confirm patients' compliance with the prescribed regimen. The treatment assignments were not revealed to the patients, their doctors, or the study investigators until the end of the trial.

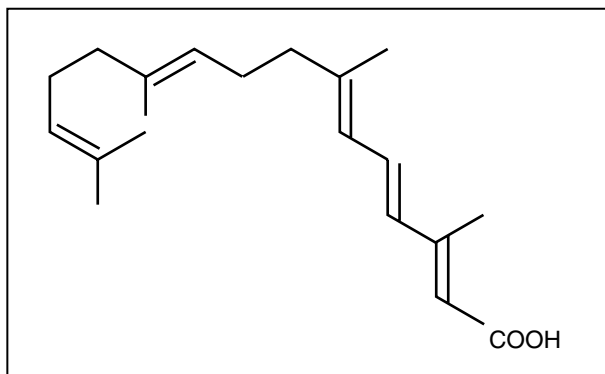


Figure 1. Chemical Structure of Polyphenolic Acid (3,7,11,15-Tetramethyl-2,4,6,10,14-hexadecapentaenoic Acid).

Table 1. Demographic and Clinical Characteristics of the Patients at Entry into the Study.*

CHARACTERISTIC	POLYPHENOLIC ACID GROUP (N = 44)	PLACEBO GROUP (N = 45)
Sex (M/F)	32/12	36/9
Age (yr)	62 \pm 8	60 \pm 8
Cause of hepatoma (no. of patients)		
Hepatitis B virus	5	7
Hepatitis C virus	32	35
Other (including alcoholism)	7	3
Hepatomas treated		
No.	1.5 \pm 0.5	1.4 \pm 0.5
Maximal diameter (cm)	2.9 \pm 1.2	3.0 \pm 1.3
Treatment method (no. of patients)		
Surgical resection	33	36
Ethanol injection	11	9
Clinical stage (no. of patients)		
I	28	33
II	14	9
III	2	3
Plasma alanine aminotransferase (IU/liter)	65 \pm 21	68 \pm 23
Plasma alpha-fetoprotein (ng/ml)	38 \pm 22	37 \pm 21
Plasma retinol (μ g/ml)	8.9 \pm 1.1	8.7 \pm 1.3

*Plus-minus values are means \pm SD.

The study treatment began no later than eight weeks after the surgery or ethanol injection. The protocol called for an ultrasonographic examination every three months and CT every six months during the treatment and follow-up periods. At each visit, CT included plain scanning and helical scanning with rapid intravenous infusion of contrast material. The resulting images were considered satisfactory for the study purposes if all the segments of the remnant liver could be seen. At each visit, we asked the patient about symptoms, performed a physical examination, and noted the patient's general condition. We also obtained a specimen of venous blood for laboratory tests, including the measurement of alpha-fetoprotein levels. An aliquot of plasma was stored at -80°C for the measurement of plasma levels of the study drug by high-performance liquid chromatography after the end of the trial.

End Points

The primary end point was the occurrence of new hepatomas. At each visit for ultrasonography and CT examination, the size and location of all lesions that may have represented treatment failures were recorded; biopsies of all were performed within one week for histologic confirmation of the diagnosis of hepatoma.

Any hepatoma that developed six or more months after the diagnosis of the first tumor was termed a "metachronous," or late, treatment failure; one that developed earlier was termed a "synchronous," or early, treatment failure.²⁶ In analyzing the cancer-preventive effect of polyphenolic acid, we defined any early treatment failure or distant metastasis as representing the progression (or recurrence) of the disease caused by the first tumor. Late treatment failures were considered to involve second primary hepatomas when they met certain strict conditions, as follows. A second primary hepatoma had to be in a liver segment different from the initial hepatoma in patients who received percutaneous injections of ethanol. In patients who had undergone surgery, a second primary hepatoma had to be separated by more than 2 cm of normal liver tissue from the margin of resection. In both patients who received percutaneous injections of ethanol and those who underwent surgery, the histologic grade of cancer²⁴ of the second primary hepatoma had to be at least equal to that of the initial hepatoma. Hepatomas that developed in the metachronous period but did not fulfill these criteria were considered to indicate progression of disease.

Table 2. Incidence of Treatment Failure.

TYPE OF TREATMENT FAILURE	POLYPRENOIC ACID GROUP (N = 44)		PLACEBO GROUP (N = 45)	P VALUE*
	no. of patients (%)			
Disease recurrence	5 (11)	2 (4)	0.23	
Early (<6 mo)	5 (11)	2 (4)	0.23	
Late (≥6 mo)	0	0	—	
Distant metastasis	0	0	—	
Second primary tumor	7 (16)	20 (44)	0.004	
All	12 (27)	22 (49)	0.04	

*By the chi-square test without Yates' correction.

Our criteria for second primary hepatomas incorporated the characteristics of multicentric hepatomas but excluded the features of intrahepatic metastasis from a single clone of hepatoma.²⁷⁻²⁹ These standards agree well with the criteria for multicentric hepatoma recommended by the Liver Cancer Study Group of Japan.^{17,24,30}

The secondary end point of the study was survival. Absolute and disease-free survival were both measured in each patient, beginning with the date of randomization.

Statistical Analysis

Our principal hypothesis concerned the effect of treatment with polyprenoic acid on the proportion of patients with at least one new hepatoma during the study period. We estimated survival curves according to the method of Kaplan and Meier and assessed the differences between the treatment groups by the log-rank test. All P values were two-tailed.

We used the Cox proportional-hazards model to produce adjusted estimates and 95 percent confidence intervals for the relative risk of the development of new hepatomas associated with selected variables at study entry, including the study assignment; the patient's age and sex; the cause of the underlying liver disease; number of earlier hepatomas, their size, and the method of treatment; the clinical stage; and the plasma levels of alanine aminotransferase, alpha-fetoprotein, and retinol.

RESULTS

Patterns of Treatment Failure

Of the 89 patients who enrolled in the study, 44 were randomly assigned to the polyprenoic acid group and 45 to the placebo group. There were no statistically significant differences between the groups with regard to the factors considered to influence treatment failure: age, sex, cause and activity of underlying liver disease, number and size of primary hepatomas, and type of primary treatment (Table 1).

The median follow-up for all patients was 38 months from the date of randomization. During this period, at least one histologically confirmed hepatoma developed in 34 pa-

tients (38 percent) (Table 2). New hepatomas were identified first by ultrasonography in all 34 patients; in 16, they were identified by CT at the same time. The mean (\pm SD) serum alpha-fetoprotein concentrations in the 34 patients who had second hepatomas did not differ significantly from those in the 55 patients who did not have such hepatomas at the end of the follow-up period (45 ± 32 vs. 35 ± 22 ng per milliliter).

The proportion of patients who had second hepatomas was significantly lower in the polyprenoic acid group than in the placebo group (27 percent vs. 49 percent, $P = 0.04$) (Table 2). In particular, polyprenoic acid reduced the rate of second primary hepatomas (16 percent, vs. 44 percent with placebo; $P = 0.004$), whereas the incidence of disease recurrence did not differ between the two groups (Table 2). All the patients in whom second hepatomas developed more than six months after the initial hepatoma met the criteria for having a metachronous tumor. No patient had distant metastasis as an end point.

The characteristics of the 27 patients with second primary hepatomas are shown in Table 3. In all 27, the histologic grade²⁴ of both the initial and the second hepatoma was well-differentiated hepatocellular carcinoma (grade I to II of the classification system of Edmond-

Table 3. Characteristics of the Patients with Second Primary Hepatomas.

PATIENT NO.	STUDY GROUP	AGE (YR)/SEX	SITE OF PRIMARY TUMOR*†	LARGEST INITIAL TUMOR (CM)	CLINICAL STAGE	PRIMARY THERAPY	MONTHS TO SECOND TUMOR	SITE OF SECOND TUMOR*
1	Placebo	62/M	S8 (2)	2.1	I	Surgery	51	S2
2	Active	57/M	S2	3.0	II	Surgery	34	S8
3	Placebo	51/M	S3	1.1	I	Ethanol	31	S8
4	Placebo	59/M	S8	1.7	I	Surgery	47	S6
5	Placebo	74/M	S7	2.5	II	Ethanol	7	S5
6	Placebo	59/M	S8	3.0	I	Ethanol	26	S6
7	Placebo	58/M	S8	1.0	II	Ethanol	7	S6
8	Active	68/F	S4	1.9	II	Ethanol	16	S7
9	Placebo	70/M	S6	2.2	I	Ethanol	13	S3
10	Placebo	60/M	S3 (3)	2.0	I	Surgery	8	S8
11	Placebo	63/F	S3	4.0	I	Surgery	12	S8
12	Active	64/M	S7	5.0	I	Surgery	13	S4
13	Placebo	63/M	S8	4.5	I	Surgery	12	S2
14	Placebo	61/M	S8	4.0	I	Surgery	10	S7
15	Active	73/M	S8	4.0	I	Surgery	8	S3
16	Placebo	66/M	S6 (2)	3.0	I	Surgery	10	S3
17	Placebo	47/M	S2	3.0	I	Surgery	14	S8
18	Placebo	63/F	S3	2.3	I	Surgery	11	S6
19	Placebo	64/F	S5	2.8	II	Surgery	9	S2
20	Placebo	63/M	S6 (2)	1.8	I	Surgery	12	S3
21	Placebo	42/M	S7	6.3	I	Surgery	9	S3
22	Active	69/M	S8 (2)	2.0	II	Surgery	8	S2
23	Placebo	52/M	S4 (2)	3.0	II	Surgery	8	S6
24	Placebo	56/M	S8	4.0	I	Surgery	34	S2
25	Active	72/F	S2 (2)	3.5	I	Surgery	7	S8
26	Active	63/F	S8 (2)	1.5	II	Surgery	10	S5
27	Placebo	60/F	S3 (2)	2.5	II	Surgery	8	S6

*S indicates the liver segment in which the primary tumor was found.

†Numbers in parentheses are the number of tumors at the site shown if more than one was present.

son and Steiner³¹). The characteristics of treatment failure, including the length of time from the first treatment to the second occurrence and the segment, histologic grade, number, and size of the second hepatomas, did not differ significantly between the patients who received percutaneous injections of ethanol and those who underwent surgical resection.

Toxicity and Compliance

Each of the 89 patients took at least one capsule containing poly-prenoic acid or placebo during the study and were included in the analyses of toxicity. One patient given poly-prenoic acid had severe headache on the first day of therapy, and the drug was discontinued. In the placebo group, one patient had a severe skin eruption and one had moderate nausea, necessitating discontinuation of therapy in each. No typical toxic effects of retinoids, such as dry skin, cheilitis, or conjunctivitis, were observed in either group, nor were any abnormal laboratory data possibly related to treatment reported in either group. Mean serum triglyceride levels were 9.6 mg per deciliter (0.11 mmol per liter) at entry and 11.7 mg per deciliter (0.13 mmol per liter) at one year in the active-treatment group and 9.5 and 10.7 mg per deciliter (0.11 and 0.12 mmol per liter), respectively, in the placebo group; these differences were not significant.

Five of the 44 patients in the active-treatment group (11 percent) did not complete the course of treatment: 1 because of toxic effects and 4 because of noncompliance. In the remaining 39 patients, the mean plasma levels of poly-prenoic acid reached 44.9 ± 13.9 ng per milliliter at one year. Six of the 45 patients in the placebo group discontinued therapy because of toxic effects (2 patients) or noncompliance (4).

Survival

Survival curves were estimated for absolute survival, disease-free survival until either the primary tumor progressed or a second primary tumor appeared, and survival until a second primary hepatoma appeared. As compared with placebo, poly-prenoic acid reduced the incidence of treatment failure in all three categories, but only the change in the incidence of second primary hepatomas was significant ($P=0.04$). Kaplan–Meier estimates of the proportion of patients free of second primary hepatomas over time are shown in Figure 2.

When poly-prenoic acid was compared with placebo by the proportional-hazards model, the estimated relative risk of a second primary hepatoma was 0.31 (95 percent confidence interval, 0.12 to 0.78); the relative risk of any type of treatment failure (either a recurrence or the appearance of a second primary hepatoma) was 0.49 (95 percent confidence interval, 0.25 to

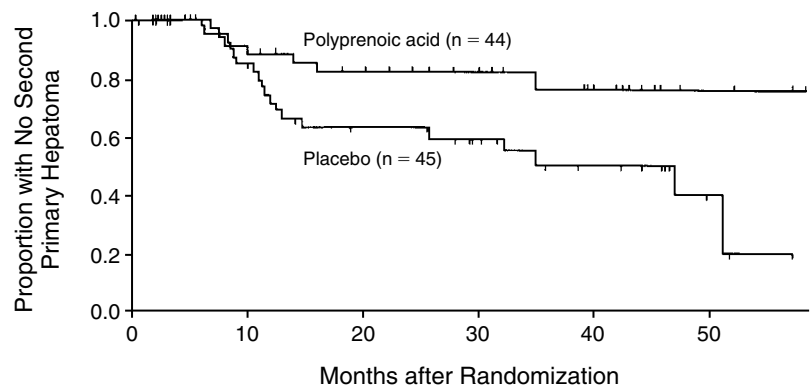


Figure 2. Kaplan–Meier Estimates of the Proportion of Patients without Second Primary Hepatomas in the Two Study Groups.

The treatment period lasted for 12 months, beginning with month 0. $P=0.04$ for the comparison between groups by the log-rank test. Tick marks indicate patients who withdrew or were excluded from the study.

1.1); the relative risk of death was 0.50 (95 percent confidence interval, 0.19 to 1.1). The median duration of absolute survival had not yet been reached in either group as of this writing.

DISCUSSION

We investigated the effect of a derivative of poly-prenoic acid (an acyclic retinoid) on the incidence of new hepatomas after curative treatment of the initial hepatoma. Retinoids are considered chemopreventive but not chemotherapeutic, because they mainly inhibit the promotion of carcinogenesis, but not the later phases of conversion and progression.^{1–4} Accordingly, we hypothesized that poly-prenoic acid would have a greater effect on second primary hepatomas than on recurrent tumors. In fact, it significantly reduced the incidence of second primary hepatomas ($P=0.004$ for the direct comparison with placebo after a median follow-up of 38 months), but not the failure rates associated with the three types of disease progression. These results may explain why poly-prenoic acid did not significantly affect disease-free survival, which included recurrences. Taken together, our data show that poly-prenoic acid can prevent second primary tumors in patients who are clinically free of disease after their primary hepatomas are treated. As for absolute survival, the overall survival rate remains over 80 percent at this writing, and we estimate that at least another 48 months will be required for us to detect a statistically significant difference between groups, assuming that the curves continue to have slopes similar to the current ones.

The patients' clinical characteristics, including age, sex, and the cause of the underlying chronic liver disease, were similar to those reported in the general survey of Japanese patients with hepatoma.^{17,30} The incidence of treatment failure of any type two years after the end of the primary treatment in the placebo group (49 percent) was also in the range previously reported.^{17–23} Hence, the patients in this study were representative patients with hepatoma. Risk factors that may be

related to the occurrence of new tumors after the treatment of small hepatomas — including the size and number of the hepatomas treated, the functional reserve of the liver, and the degree of inflammation of liver parenchyma (as measured by the serum alanine aminotransferase activity)^{17-23,30,32-42} — did not differ between groups. The method of treating the initial hepatoma, whether it was surgical resection or percutaneous injection of ethanol, was also studied in a stratified analysis between the groups. After adjustment for these characteristics, multivariate analysis revealed that the upper bound of the 95 percent confidence interval of the relative risk of a second primary hepatoma was less than 1 in the group treated with polyprenoic acid, giving further evidence of the efficacy of the compound.

Hepatoma associated with chronic viral diseases of the liver is a major health problem in countries such as Japan, where rates of infection with the hepatitis B and C viruses in the general population are as high as 1 to 2 percent.^{43,44} The annual death rate from hepatoma in Japan exceeds 25,000, and such disease is the third leading cause of death due to cancer in Japanese men.⁴⁵ Advances in medical imaging permit the early diagnosis and treatment of hepatoma,^{46,47} but in the most recent reports,^{17,18,30} the five-year survival rate barely reached 40 percent. The low rate of survival after any treatment is due to the high incidence of recurrent tumors and second primary tumors. The incidence of second hepatomas is approximately 25 percent one year after radical surgical resection and about 50 percent after two years.^{17,19-21,30} The percutaneous injection of ethanol can produce survival rates at three to five years that equal or exceed those obtained by surgical resection.^{17,22,23} Surgical resection decreases liver mass and may lead to liver failure, whereas the percutaneous injection of ethanol minimally damages the surrounding noncancerous liver tissue. Hence, such injections are preferred to surgery in patients with hepatoma and chronic viral liver disease,^{17,22,23} who have a high incidence of recurrent hepatoma and may require repeated treatment. However, the rates of second hepatomas in the remnant liver after surgical resection or after the percutaneous injection of ethanol do not differ,¹⁷⁻²³ as we found in this study.

The high incidence of second primary hepatoma in our study is in accord with the concept of field cancerization,⁴⁸ which postulates that cancer arises from a field that is exposed to a continuous carcinogenic insult. The tissue that constitutes the field forms multiple precancerous foci and undergoes multicentric carcinogenesis.⁴⁹ In chronic viral liver disease, a typical example of this concept,⁵⁰ second primary hepatomas frequently develop despite treatment. Chemoprevention of hepatocarcinogenesis may therefore improve the prognosis. Our study is noteworthy in that all new hepatomas were identified first by ultrasonography rather than CT or the determination of serum alpha-fetoprotein levels, a fact that confirms the superior di-

agnostic ability of ultrasonography for small hepatomas.^{46,47}

We found polyprenoic acid to be safe. Plasma drug levels were in the range that was reached in the phase I trial and that showed effects *in vivo*¹¹⁻¹³ and *in vitro*.^{14,15} Only 1 of the 44 patients who took the drug had headache. Other toxic effects of retinoids, such as skin eruptions, musculoskeletal disorders, decreased visual acuity, anemia, and hyperlipidemia,⁵¹ did not appear.

Hepatocarcinogenesis is closely related to the impaired metabolism of retinoid. The retinoid content of hepatoma tissue starts to decline early in carcinogenesis.^{11,52,53} Cellular levels of retinol-binding protein decrease and cellular levels of retinoic acid-binding protein increase in both precancerous and cancerous hepatic conditions.^{11,53,54} A cohort study demonstrated an inverse dose-response relation between the serum retinol level before diagnosis and the development of hepatoma.⁵⁵ Thus, many attempts have been made in the laboratory to test the efficacy of retinoids on hepatocarcinogenesis.^{3,11-13,56-60}

The mechanism of action of polyprenoic acid is unknown. In human-hepatoma-derived cell lines, this compound brought a return of the characteristics of differentiated hepatocytes, such as the synthesis and secretion of albumin, through a signal that began with the binding of the compound to the retinoid X receptor.^{14,15} Polyprenoic acid also induced differentiation in promyelocytic leukemia cells.⁶¹ It caused apoptosis in hepatoma cell lines by blocking the autocrine and paracrine loops of transforming growth factor α .⁶² Deletion of an L3-positive hepatoma clone⁶³⁻⁶⁵ has also been suggested (unpublished data).

Further research is under way to develop potent and safe analogues of polyprenoic acid.⁶⁶ Such compounds may prevent primary hepatomas, since short-term use of polyprenoic acid in low doses inhibited the spontaneous development of mouse hepatomas for more than two years.⁶⁷

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APPENDIX

In addition to the study authors, the investigators in the Hepatoma Prevention Study Group included T. Yamatsu, First Department of Internal Medicine, Gifu University School of Medicine; Y. Nishigaki, Gastrointestinal Disease Center, Gifu Municipal Hospital; H. Okuno, T. Kasai, and K. Takagi, Gihoku Hospital; Y. Ito, Gifu Red Cross Hospital; and H. Koda, Murakami Memorial Hospital, Asahi University.

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CORRECTION

Polyprenoic Acid in Hepatocellular Carcinoma

To the Editor: We believe that more data are needed before polyprenoic acid can be accepted as an effective drug for the adjuvant treatment of hepatocellular carcinoma, as reported by Muto et al. (June 13 issue).¹ We are concerned about several issues that were raised in the report. The authors claimed that tumors recurred in 27 percent of the group given polyprenoic acid (12 of 44) and 49 percent of the placebo group (22 of 45, $P = 0.04$). However, the Kaplan–Meier analysis in Figure 2 of the article shows a total of 24 patients (16 in the polyprenoic acid group and 8 in the placebo group) who were followed up for less than 24 months. Among the 24 patients, 11 in the polyprenoic acid group and 5 in the placebo group had a follow-up of less than seven months. These 11 patients in the polyprenoic acid group either were not followed long enough or had recurrence of tumor within six months and by definition should not be included in the evaluation of the occurrence of a second primary tumor. In addition, the two-year recurrence rate of 49 percent in the placebo group was not substantiated by the data, since not all patients had reached two years of follow-up.

We are also concerned about the selection of six months as the cutoff point to differentiate a recurrence from a second primary tumor. It is important to know whether this time point was preselected, since a P value below 0.05 can easily be reached if multiple cutoff points are examined.² Furthermore, sonography was scheduled every three months, but recurrence was noted at seven, eight, and nine months of follow-up. Systemic bias would easily emerge if the investigators were allowed to make a subjective decision about follow-up time. A much more reliable end point is the overall survival rate.

The authors failed to state clearly some statistical points that are pertinent to a phase 3 clinical trial. These include the time at which enrollment was closed, the point at which the results were disclosed after closure of the study, and the schedule for examining results during the trial. If the results were examined at multiple points, the study should be subject to the rule of multiple interim analyses, and a much lower P value would be required to establish statistical significance.³

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To the Editor: In their excellent article, Muto et al. fail to discuss the importance of the profound vitamin A deficiency that affected most patients enrolled in their chemoprevention trial of the synthetic retinoid polyprenoic acid in hepatocellular cancer. The mean (\pm SD) plasma retinol levels were 8.9 ± 1.1 and 8.7 ± 1.3 μ g per deciliter (not per milliliter, as erroneously reported in Table 1 of their article) in the polyprenoic acid and placebo groups, respectively, values that are below the threshold of severe vitamin A deficiency (i.e., 10 μ g per deciliter).¹ Although these values may be consistent with the expected compromised liver function of this particular cohort, the preventive effect observed in the group receiving the synthetic retinoid may simply have been due to the restoration of normal vitamin A status. It is well known that vitamin A deficiency predisposes patients to alterations in the process of differentiation and to increased cellular proliferation,¹ both of which may have enhanced the underlying process of liver carcinogenesis. The observation by Muto et al. seems to confirm the anticarcinogenic effect of retinoid supplementation in subjects with vitamin A deficiency,² but the broad implication for cancer prevention in subjects with normal vitamin A status remains unclear. Indeed, the recently reported harmful effect of beta carotene supplementation in subjects with normal vitamin A levels would argue against the use of this strategy,³ even though the effects of synthetic retinoids may well be totally different from those of carotenoids.

Another important consequence of vitamin A deficiency is visual problems, particularly night blindness. The authors state that no disorders of visual acuity occurred. However, impaired adaptation to the dark occurs frequently below the threshold of 10 μ g of plasma retinol per deciliter, even though it may be unrecognized in some patients.⁴ Moreover, no mention is made in the article of whether the retinoid modified plasma retinol levels. Other synthetic retinoids significantly decrease plasma retinol levels and induce diminished adaptation to darkness,⁵ a symptom that may affect the subjects' ability to drive.

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The authors reply:

To the Editor: In our study of the prevention of second primary hepatomas by an acyclic retinoid, we included all randomized subjects in the analyses rather than only those who were treated, were eligible, or could be evaluated. This intention-to-treat analysis is the strictest way to evaluate the efficacy of a treatment.¹ For example, the first tick mark in the retinoid-treated group in Figure 2 of our article represents a patient who discontinued the drug on the first day because of headache, but who was followed up for another 61 months. The follow-up after randomization ranged from 28 to 64 months for all surviving patients. Withdrawal of patients due to death occurred in the polyenoic acid group at 12, 13, 21, 25, 30, 32, and 33 months and in the placebo group at 10, 14, 19, 26, 28, 29 (two patients), 31, 32, 37, 39, and 45 months. We are following the remaining patients to determine overall survival rates, since they are the most reliable end points.

The decision to include a cutoff point of six months in the definition of second primary hepatoma was determined before the study. Another definition involving DNA analysis may be possible,² but we believe our definition is advantageous for a multicenter trial. During the entire period before the study was closed to enrollment in August 1994, no interim analyses were either scheduled or performed. Thanks to the strict protocol and definitions of end points, we did not need to examine the data at multiple points after the study was closed. The data were updated in February 1996 solely to provide the latest follow-up data for a final revision of this article. It is very difficult to avoid a one-month deviation from scheduled examinations in the conduct of a clinical trial like ours.

Ingested retinoid is absorbed in the small intestine, stored in the liver, and delivered to target organs by retinol-binding protein, which is synthesized in the liver.³ The plasma retinol level is determined on the basis of either hepatic retinol stores or the synthesis of retinol-binding protein. In vitamin A deficiency, hepatic retinol stores disappear before the plasma retinol level declines, although retinol-binding protein is available. In contrast, impaired synthesis of retinol-binding

protein reduces the plasma retinol level but does not affect hepatic retinol stores in liver cirrhosis. Plasma retinol levels of 8.7 or 8.9 μg per deciliter (not micrograms per milliliter, as was erroneously reported in Table 1 of our article) were consistent with those of patients with hepatoma in our previous study ($18.4 \pm 14.6 \mu\text{g}$ per deciliter).⁴ In such patients, retinoid was not deficient in the noncancerous liver tissue (mean, 318 μg per gram).⁵ Therefore, the mechanism by which acyclic retinoid inhibited second primary hepatomas in the remnant liver is not related to supplementation of a deficient nutrient. In fact, administration of the retinoid for 12 months did not affect the plasma retinol level in the polyenoic acid group ($8.5 \pm 1.2 \mu\text{g}$ per deciliter). However, in organs other than the liver, symptomatic retinoid deficiency may appear as a result of decreased delivery of retinol by retinol-binding protein in such patients. Sensory disturbances such as impaired adaptation to the dark and diminished taste acuity are typical, as we reported previously.⁶ However, no sensory disorders were reported by the patients or observed by the investigators in the present study.

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