REPORTS

Serum α-Tocopherol Concentration in Relation to Subsequent Colorectal Cancer: Pooled Data From Five Cohorts

Matthew P. Longnecker, Jose-Maria Martin-Moreno, Paul Knekt, Abraham M. Y. Nomura, Susan E. Schober, Hannes B. Stähelin, Nicholas J. Wald, K. Fred Gey, Walter C. Willett*

Background: **Vitamin E is an antioxidant that inhibits mutagenesis and cell transformation. Previous findings in five prospective epidemiologic studies suggested that the level of serum α-tocopherol, the predominant form of vitamin E in the blood, was lower in subjects who subsequently developed colorectal cancer than in control subjects. However, the difference was neither obvious nor statistically significant in any one of these five studies.** ***Purpose:*** **To evaluate in greater detail the association between serum α-tocopherol concentration and risk of colorectal cancer, we pooled and analyzed the original data from the five studies. Our analyses were designed to (*a*) test the hypothesis with greater statistical power, (*b*) examine the association after adjustment for serum cholesterol levels, and (*c*) evaluate the association after uniform exclusion of cases diagnosed shortly after blood specimens were drawn.** ***Methods:*** **Data for individual subjects were analyzed. To make the design of the component investigations uniformly nested case-control studies with individual matching, we matched controls to cases in two of the cohorts. Subjects were categorized according to study-specific quartile of serum α-tocopherol level within the study. The pooled analysis included 289 cases of colorectal cancer and 1267 matched controls.** ***Results:*** **For cancers of the colon and rectum combined, the matched odds ratio (OR) for the highest quartile of serum α-tocopherol concentration compared with the lowest was 0.6 (95% confidence interval [CI] = 0.4-1.0). Adjustment for serum cholesterol level attenuated the OR to 0.7 (95% CI = 0.4-1.1).** ***Conclusion:*** **The results suggest that serum α-tocopherol concentration may be inversely related to risk of colorectal cancer. It is unclear whether an association exists, however, because the association between serum α-tocopherol level and decreased risk of colorectal cancer was modest, the CIs were wide, and, overall, the tests for trend in effect were not significant.** ***Implications:*** **Larger observational studies with concurrent dietary data are needed to determine whether vitamin E has a modest but potentially important protective effect against colorectal cancer. [J Natl Cancer Inst 84:430–435, 1992]**

Vitamin E inhibits mutagenesis and cell transformation, probably by blocking peroxidation of lipids in membranes (*1*). In some animal models, vitamin E is anticarcinogenic, but the study results are not entirely consistent (*1,2*).

Although few epidemiologic studies have examined vitamin E intake in relation to risk of cancer (*3*), serum α-tocopherol concentration, which reflects intake of vitamin E, has been examined in relation to risk of cancer in several prospective studies (*4-10*). In reviewing these studies, we noted results suggesting that the level of serum α-tocopherol was lower in subjects who subsequently developed colorectal cancer, compared with that in control subjects. However, the difference in α-tocopherol level was neither obvious nor statistically significant in any one of the studies. While the prospective design in these observational studies was methodologically strong, the statistical power in any given study was somewhat limited. Further, the interpretation of the findings in some investigations was unclear because the inverse association might have been due to confounding by serum cholesterol level, which is correlated with serum α-tocopherol, or to an effect of preclinical disease on the concentration of α-tocopherol in the blood.

To evaluate the association between serum α-tocopherol concentration and

Received July 17, 1991; revised November 4, 1991; accepted December 13, 1991.

Supported in part by the American Cancer Society and the Medical Foundation, Inc. (M. P. Longnecker).

M. P. Longnecker, Department of Epidemiology, University of California, Los Angeles, School of Public Health.

J.-M. Martin-Moreno, Escuela Nacional de Sanidad, Madrid, Spain.

P. Knekt, Research Institute for Social Security, The Social Insurance Institution, Helsinki, Finland.

A. M. Y. Nomura, Japan-Hawaii Cancer Study, Kuakini Medical Center, Honolulu, Hawaii.

S. E. Schober, Division of Epidemiology and Prevention Research, National Institute on Drug Abuse, Rockville, Md.

H. B. Stähelin, Medizinisch-geriatrische Klinik, Kantonsspital Basel, Basel, Switzerland.

N. J. Wald, Department of Environmental and Preventive Medicine, Medical College of St. Bartholomew, London, England.

K. F. Gey, Vitamin Unit, Institute of Biochemistry and Molecular Biology, University of Berne, Switzerland.

W. C. Willett, Departments of Epidemiology and Nutrition, Harvard School of Public Health, and the Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, Mass.

We thank Fran Fullerton, Roger Newson, Chris Frost, and Jean de Traversay. We especially thank the investigators who collected the data that were analyzed.

**Correspondence to:* Matthew P. Longnecker, M.D., Sc.D., Department of Epidemiology, UCLA School of Public Health, 10833 Le Conte Ave., Los Angeles, CA 90024-1772.

risk of colorectal cancer in greater detail, we pooled and analyzed the original data from the five prospective, nested case-control studies of this relationship. Analysis of the combined data allowed us to (*a*) test the hypothesis with greater statistical power, (*b*) examine the association after adjustment for serum cholesterol levels, and (*c*) evaluate the association after uniform exclusion of cases diagnosed shortly after blood specimens were drawn.

Subjects and Methods

By 1988, authors of five prospective studies had presented data on serum α-tocopherol concentration in relation to risk of colorectal cancer (*5-9*); no new studies of this type have been published since.

In the two other prospective studies of serum α-tocopherol level in relation to subsequent cancer, the colorectal cancer cases were grouped either with all other cancers (*4*) or with other gastrointestinal cancers (*10*). We estimated that there were fewer than 10 cases of colorectal cancer in each of these studies and excluded them because little additional information would have been obtained. In these two studies, serum α-tocopherol levels for cases were slightly lower than those for controls.

Table 1 describes the five original studies included in the analysis. The follow-up of subjects was virtually complete in four studies, but the completeness of follow-up was not determined in one study (*7*). Incident cases of colorectal cancer were used as the end point in all but one of the studies (*8*), in which death from colorectal cancer was the end point. In addition, the duration and temperature for storage of blood specimens varied considerably from study to study.

For the present analysis, the authors of the five studies provided data on serum α-tocopherol and cholesterol concentration, cigarette smoking, the date the blood was drawn, and the date of diagnosis for each subject in the original reports.

The method of selecting controls varied among studies. In three of the studies (*5,7,9*), controls had been individually matched to cases and the matched data were supplied for the current analysis. In the study by Nomura et al. (*6*), controls had originally been frequency matched to cases. Stähelin et al. (*8*) supplied data on their entire cohort before selection of controls. To facilitate analysis of the combined data, the data from Nomura et al. (*6*) and Stähelin et al. (*8*) were reorganized so that each control in these two studies was individually matched to a case. The resulting matched sets each had one case and one or more controls. The matching factors were 5-year-age category, gender, and 3-month period since blood was drawn. Because no match was available, five cases and 41 controls were excluded from the study by Nomura et al. (*6*), and 2896 controls were excluded from the study by Stähelin et al. (*8*). Thus, data for 289 cases and 1267 controls matched by age, gender, and period of blood collection were pooled for the present analysis (Table 2).

The SAS statistical software package was used to perform tabular analyses (*11*). To evaluate the average difference between the serum α-tocopherol concentrations for cases and their matched controls, a general linear model was fitted to the data. The dependent variable was serum α-tocopherol concentration, and the following parameters were estimated for the independent variables: grand (overall) mean, a fixed-effect term for case-control status, and a random-effect term representing set membership (*12*). A likelihood ratio test was used to evaluate the difference in fit between models, with and without the term for case-control status. Dose–response and multivariate analyses were conducted with conditional logistic regression models fitted with the EGRET statistical software package (*13*).

The relationship of serum α-tocopherol to risk of colorectal cancer was modeled in two ways. 1) The quartiles of the α-tocopherol distribution in controls were determined for each study, and summary odds ratios (ORs) (across all studies) according to quartile were calculated. This approach assumed that the OR for a given quartile was constant across studies. 2) The OR per 20 μmol/L increase in serum α-tocopherol was calculated. Dietary supplementation with 800 IU/d of α-tocopherol results in an increase in serum α-tocopherol of approximately 20 μmol/L (*14*). This approach assumed that the relative risk per unit of α-tocopherol was linear on the log scale. The study-specific distributions of serum α-tocopherol levels were skewed, long tail to the right. How-

Table 1. Description of the five prospective studies of serum α-tocopherol concentration in relation to risk of colorectal cancer that were included in the pooled analysis

Description	First author (ref. No.)				
	Knekt (*5*)	Nomura (*6*)	Schober (*7*)	Stähelin (*8*)	Wald (*9*)
Place	Finland	Hawaii	Maryland	Basel	London
Year begun*	1968	1971	1974	1971	1975
No. of subjects	36 265	6860	25 802	3620	22 000
Age, y	15-99	52-75	36-79	16-71	35-64
Follow-up period, y*,†	8	10	8	8	7
Gender	Male and female	Male	Male and female	Male and female	Male
Matching criteria‡	Age, sex, place of residence§	Age‖	Age, sex, period of blood collection¶	Age, sex	Age, smoking status, duration of blood storage§
Blood specimen storage					
No. of years†	14	10	9	0	6
Temperature, °C	–20	–75	–73	NA#	–40

*Approximate.
†Average.
‡Refers to original authors' analyses; all were nested case-control studies.
§Effectively matched on period of blood collection.
‖In the original analysis done by Nomura et al. (*6*), subjects were frequency matched by age; all other studies were individually matched by age. *See* "Subjects and Methods" section for description of matching procedure used in pooled analysis.
¶Subjects were also matched according to whether they had been included in a private census of county residents.
#Not applicable.

Table 2. Serum α-tocopherol level for cases of colon cancer or rectal cancer and controls, by study*

Source of data, first author (ref. No.)	No. of subjects				α-Tocopherol, μmol/L†			
	Colon cancer		Rectal cancer		Colon cancer		Rectal cancer	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Knekt (5)	21	37	37	71	21.3 ± 8.2	22.9 ± 8.3	22.7 ± 6.2	23.1 ± 10.2
Nomura (6)	78	188	30	72	32.0 ± 14.0	32.0 ± 12.7	27.8 ± 7.1	30.1± 10.8
Schober (7)	72	143	0	0	27.1± 8.5	29.5 ± 12.4	—	—
Stähelin (8)	18	536	5	165	36.2 ± 10.2	36.4 ± 10.4	31.4 ± 7.9	36.8 ± 11.6
Wald (9)	17	34	11	21	25.4 ± 11.0	25.3 ± 9.0	21.8 ± 7.8	24.0 ± 14.4
Total‡	206	938	83	329	29.0 ± 11.8	30.0 ± 11.7	24.9 ± 7.4	25.9 ± 12.7

*Results based on data from five prospective case-control studies of colorectal cancer. Nos. of subjects in original papers of Stähelin et al. (8) and Nomura et al. (6) differ from those included in present analysis. *See* "Subjects and Methods" section for explanation. The current study includes females from the Stähelin et al. cohort, whereas the original report did not.

†Values = means ± SD.

‡The overall mean of the values for serum α-tocopherol levels in controls was calculated as the arithmetic mean of values for cases plus the mean case-control difference estimated from the generalized linear model described in the "Subjects and Methods" section. The crude analysis indicated that for colon cancer the case mean was significantly different from the control mean ($P<.03$). However, the difference after adjustment for serum cholesterol was not significant ($P = .13$). All other case-control differences in the table were not statistically significant at the $P = .05$ level.

ever, analyses performed with log-transformed serum α-tocopherol levels led to the same conclusions as those conducted with the untransformed values. Results based on untransformed values were presented to ease interpretation.

Results

The results of the individual studies were relatively homogeneous. Within each cohort, the concentration of α-tocopherol tended to be similar among cases and controls, although in a few instances mean levels for cases were 1 μmol/L or more less than those for controls (Table 2). The standard deviations for the serum α-tocopherol concentrations were large in each study; i.e., the distributions overlapped substantially among the cohorts. For colon cancer, the overall average difference between case and control serum α-tocopherol levels was of borderline significance ($P = .03$); for rectal cancer, the difference was not significant ($P = .17$). When the average difference was examined after adjustment for serum cholesterol level, there were no statistically significant differences between levels for cases and controls (colon, $P = .13$; rectum, $P = .18$).

The mean concentration for the highest quartile of the serum α-tocopherol distribution in each study was approximately 25 μmol/L greater than that for the lowest quartile (Table 3). The values for crude OR according to quartile of serum α-tocopherol concentration were generally similar for cancer of the colon and for cancer of the rectum (Table 4). For cancers of the colon and rectum combined, the crude OR for the highest quartile compared with the lowest was 0.6 (95% confidence interval [CI] = 0.4-1.0). However, serum α-tocopherol and cholesterol levels were correlated (Pearson $r = .27$, $P = .0001$; calculated with log-transformed α-tocopherol), and serum cholesterol level was inversely associated with risk of colorectal cancer. Thus, when the ORs were adjusted for serum cholesterol level, the association between serum α-tocopherol concentration and colorectal cancer risk was reduced (OR = 0.7; 95% CI = 0.4-1.1). Adjustment for cigarette smoking status (current, past, or never) did not affect the results.

To check for evidence of a threshold effect for cancers of the colon and rectum combined, a quadratic term was added to the model, with α-tocopherol as a continuous variable. The additional term did not improve the fit ($P>.80$). Using a similar model, a test for heterogeneity in effect across studies was performed, and the results were consistent with homo-

Table 3. Ranges and means for quartiles of serum α-tocopherol concentration (in μmol/L), by study*

First author (ref. No.)	Quartile, μmol/L							
	1		2		3		4	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Knekt (5)	9.3–<17.3	14.0	17.3–<21.7	19.6	21.7–<27.5	24.2	27.5–80.1	33.8
Nomura (6)	13.7–<24.0	20.7	24.0–<28.7	26.1	28.7–<35.1	31.4	35.1–96.7	48.5
Schober (7)	14.2–<22.8	19.7	22.8–<26.8	24.9	26.8–<32.3	29.5	32.3–100.8	44.1
Stähelin (8)	15.6–<30.2	26.4	30.2–<35.3	32.8	35.3–<41.1	38.2	41.1–122.4	50.0
Wald (9)	4.9–<17.4	12.8	17.4–<24.6	21.8	24.6–<29.3	26.8	29.3–74.5	38.3

*Results based on data from five prospective case-control studies of colorectal cancer.

geneity (P = .90). The association between serum α-tocopherol level and risk of colorectal cancer was not appreciably modified by cigarette smoking, age, or gender. In the first 2.5 years of follow-up, the relative risks of colorectal cancer in subjects with serum α-tocopherol levels in quartiles 2, 3, and 4 were greater than 1 (Table 5). In later periods, the association was almost uniformly inverse.

As noted above, serum cholesterol concentration was inversely associated with risk of colorectal cancer. The OR per mmol/L increase was 0.9 (95% CI = 0.8-1.0). The OR for colorectal cancer among those in the highest quartile of serum cholesterol level relative to those in the lowest was 0.6 (95% CI = 0.4-0.9). The association did not vary with the number of years between blood collection and diagnosis. The ORs per mmol/L cholesterol according to number of years were the following: 0.9 for 0-2.4 years, 0.9 for 2.5-5.0 years, 0.9 for 5.0-7.5 years, and 0.8 for 7.5 years or more.

Discussion

The results suggest that serum α-tocopherol concentration may be inversely related to risk of colorectal cancer. The laboratory procedures and the matching procedures employed in the five studies we used, together with the procedures used in our current analysis, ensured that differences between case and control values, where present, were not due to unequal length of specimen storage or to variation over time in laboratory measurement of serum α-tocopherol. However, it is unclear whether an association exists between serum α-tocopherol level and decreased risk of colorectal cancer because, in our study, the association was modest, the CIs were wide, and, overall, the tests for trend in effect were not significant.

Because colorectal cancer is common, even a modest protective effect could have important public health implications. Dietary intake of vitamin E is a determinant of serum α-tocopherol level (*15*). If an increase in daily vitamin E intake of 800 IU truly reduces the relative risk of colorectal cancer by 20% or 30% (within the CI of our estimate), this information could be useful for preventive health care.

One strength of the pooled analysis lies in overcoming the problem of lack of power in any individual study, especially when subgroups are analyzed (*16*). Another advantage is that uniform adjustment for confounding factors is facilitated. If we had combined data from well-conducted, randomized clinical trials, we would have no serious concern about bias in the summary findings (*16*). However, because the results of the present analysis reflect findings in observational studies, biases, if any, in the component investigations will be reflected in the summary.

The apparent association between α-tocopherol level and decreased risk of colorectal cancer may be due to uncontrolled confounding in our data from the

Table 4. ORs for risk of colorectal cancer according to quartile of serum α-tocopherol level and per 20-μmol/L increase in level, by cancer site*

Cancer site	Type of OR	Quartile 1	2	3	4	*P* value for trend across quartiles	Per 20 μmol/L increase	*P* value for increase
Colon	Crude	1.0	0.9 (0.5–1.4)	0.7 (0.4–1.2)	0.6 (0.4–1.1)	.06	0.82 (0.61–1.11)	.21
	Adjusted†	1.0	0.9 (0.6–1.5)	0.8 (0.5–1.3)	0.7 (0.4–1.3)	.26	0.90 (0.63–1.27)	.55
Rectum	Crude	1.0	0.8 (0.4–1.8)	1.0 (0.4–2.2)	0.6 (0.2–1.6)	.32	0.59 (0.30–1.16)	.13
	Adjusted†	1.0	0.8 (0.4–1.8)	1.0 (0.4–2.2)	0.6 (0.2–1.5)	.41	0.59 (0.28–1.20)	.15
Colon and rectum	Crude	1.0	0.9 (0.6–1.3)	0.8 (0.5–1.2)	0.6 (0.4–1.0)	.03	0.77 (0.58–1.02)	.07
	Adjusted†	1.0	0.9 (0.6–1.3)	0.8 (0.5–1.3)	0.7 (0.4–1.1)	.15	0.82 (0.60–1.11)	.20

*Results based on pooled data from five prospective case-control studies of colorectal cancer. Estimates were from conditional logistic regression models. Values in parentheses = 95% CIs. Subjects with the lowest serum α-tocopherol concentrations are in quartile 1.
†Adjusted for serum cholesterol level.

Table 5. ORs for risk of colorectal cancer according to quartile for serum α-tocopherol level, by No. of years between blood collection and diagnosis*

No. of years	Type of OR	Quartile† 1	2	3	4	*P* value for trend across quartiles	No. of cases	No. of controls
<2.5	Crude	1.0	2.7 (1.0–7.3)	1.5 (0.6–4.3)	1.2 (0.4–3.6)	.75	66	180
	Adjusted‡	1.0	2.9 (1.0–8.1)	1.7 (0.6–5.2)	1.6 (0.5–5.4)	.90		
2.5–<5.0	Crude	1.0	0.5 (0.2–1.0)	0.8 (0.4–1.6)	0.6 (0.3–1.2)	.31	89	344
	Adjusted‡	1.0	0.4 (0.2–1.0)	0.7 (0.3–1.6)	0.6 (0.3–1.3)	.44		
5.0–<7.5	Crude	1.0	0.9 (0.4–1.9)	0.8 (0.4–1.8)	0.3 (0.1–0.8)	.02	77	219
	Adjusted‡	1.0	0.9 (0.4–2.0)	0.8 (0.3–1.8)	0.3 (0.1–0.8)	.03		
≥7.5	Crude	1.0	0.5 (0.2–1.3)	0.6 (0.2–1.4)	0.8 (0.3–1.9)	.64	55	524
	Adjusted‡	1.0	0.6 (0.2–1.5)	0.7 (0.3–2.0)	1.2 (0.4–3.3)	.70		

*Estimates were from conditional logistic regression models.
†Subjects with the lowest serum α-tocopherol concentrations are in quartile 1. Study-specific quartiles are specified in Table 3.
‡Adjusted for serum cholesterol level.

five studies we used. In the United States, fats, oils, and vegetables are the major sources of dietary vitamin E (*17*); an observed protective association could be due to constituents of these foods other than vitamin E or to behaviors associated with eating such foods.

Serum α-tocopherol level is determined, in part, by serum lipoprotein concentration (*18*). Serum cholesterol level can be reduced in persons with cancer that is not yet clinically detectable, including colorectal cancer (*19-22*). The effect is usually greatest within 2 years before diagnosis, although Törnberg et al. (*21*) found that, for all cancers combined, persons with a low baseline cholesterol level still had a slightly greater risk of cancer even 10 years later. In the current data, the length of time after blood collection did not modify the relationship between cholesterol level and cancer risk, even though it did in one of the constituent studies (*9*). Although the precise reasons for the inverse relationship between serum cholesterol level and risk of colorectal cancer are not entirely clear, the association of serum α-tocopherol concentration with risk of colorectal cancer was adjusted for serum cholesterol concentration to avoid confounding.

The serum specimens collected by Stähelin et al. (*8*) were analyzed immediately after the blood was drawn; vitamin E levels among those subjects were the highest in the five cohorts. The specimens collected by Knekt et al. (*5*) were stored for a longer period than in any of the other studies, and vitamin E levels among the subjects were the lowest in the five cohorts. The rate of decay of serum α-tocopherol concentration in sera frozen at –40 °C was observed by Wald et al. (*9*) to be 1.2 μmol $L^{-1}y^{-1}$. If one assumes that the decay of α-tocopherol in our specimens was proportional to storage temperature and length of storage, it is possible to estimate, based on Wald's decay rate at –40 °C, the mean serum α-tocopherol level in each cohort at the time the blood was drawn. Such estimates suggest that the mean α-tocopherol levels in the specimens from the cohorts were more similar at the time the blood was drawn than in the stored specimens. However, Gey et al. (*23*) found that the α-tocopherol concentration in freshly analyzed sera from subjects from Finland was remarkably similar to the levels Knekt et al. (*5*) observed in sera after storage. Thus, the differences in serum α-tocopherol levels observed among cohorts may have been largely due to true population differences rather than to storage conditions.

If the rates of degradation of α-tocopherol were equivalent for the corresponding cases and controls within case-control sets, degradation would have had no effect on the observed ORs. Any departure from equality of case-control degradation rates would have resulted in misclassification of relative levels and, thus, less precise and possibly biased results. For example, serum cholesterol and, by inference, serum lipoprotein levels were associated with serum α-tocopherol levels. If lipoprotein concentration in the stored specimens was associated with rate of loss of α-tocopherol, the results might be biased toward the null. Although the observed association might have been more precise, and possibly stronger, in the absence of degradation, the fact that the findings did not vary markedly across studies suggests that our findings were not seriously distorted by differences in specimen preservation.

Although vitamin E inhibits mutagenesis and cell transformation (*1*), its anticarcinogenic effects, if any, may be through other mechanisms. For example, because vitamin E is excreted in bile (*24*), it might influence large-bowel carcinogenesis in the lumen of the gut. Dion et al. (*25*) showed that dietary supplementation with vitamins E and C reduces the mutagenicity of feces. Nonetheless, animal data regarding large-bowel cancer and vitamin E are contradictory (*1*). Results of experimental studies of polyp recurrence in humans are compatible with a modest inhibitory effect of vitamin E (*26,27*), but the effects have not been statistically significant and could be due to concurrently administered vitamin C. In the present analysis, the evidence supporting a causal relationship between vitamin E level and colorectal cancer is equivocal. If vitamin E truly has a subtle protective effect against colorectal cancer, one might expect results like that observed—a monotonic modest decrease in risk with increasing quartile of serum α-tocopherol concentration and little statistical precision.

The evidence for a protective effect of vitamin E is weak—certainly not strong enough to justify an intervention study powerful enough to detect an effect, if indeed one exists. In the meantime, larger observational studies with control for confounding by other dietary factors will be needed to determine whether vitamin E has a modest but potentially important protective effect against colorectal cancer.

References

(*1*) Chen LH, Boissonneault GA, Glauert HP: Vitamin C, vitamin E, and cancer. Anticancer Res 8:739–748, 1988

(*2*) Wang YM, Purewal M, Nixon B, et al: Vitamin E and cancer prevention in an animal model. Ann N Y Acad Sci 570:383–390, 1989

(*3*) Rogers AE, Longnecker MP: Dietary and nutritional influences on cancer: A review of epidemiologic and experimental data. Lab Invest 59:729–759, 1988

(*4*) Salonen JT, Salonen R, Lappeteläinen R, et al: Risk of cancer in relation to serum concentrations of selenium and vitamins A and E: Matched case-control analysis of prospective data. Br Med J 290:417–420, 1985

(*5*) Knekt P, Aromaa A, Maatela J, et al: Serum vitamin E, serum selenium and the risk of gastrointestinal cancer. Int J Cancer 42:846–850, 1988

(*6*) Nomura AM, Stemmermann GN, Heilbrun LK, et al: Serum vitamin levels and the risk of cancer of specific sites in men of Japanese ancestry in Hawaii. Cancer Res 45:2369–2372, 1985

(*7*) Schober SE, Comstock GW, Helsing KJ, et al: Serologic precursors of cancer. I. Prediagnostic serum nutrients and colon cancer risk. Am J Epidemiol 126:1033–1041, 1987

(*8*) Stähelin HB, Rösel F, Buess E, et al: Cancer, vitamins, and plasma lipids: Prospective Basel study. JNCI 73:1463–1468, 1984

(*9*) Wald NJ, Thompson SG, Densem JW, et al: Serum vitamin E and subsequent risk of cancer. Br J Cancer 56:69–72, 1987

(*10*) Willett WC, Polk BF, Underwood BA, et al: Relation of serum vitamins A and E and carotenoids to the risk of cancer. N Engl J Med 310:430–434, 1984

(*11*) SAS Institute, Inc. Statistical Analysis System. Cary, NC, 1987

(*12*) BMDP Statistical Software, Inc. Los Angeles, Calif, 1990

(*13*) Statistics and Epidemiology Research Corporation. Epidemiological Graphics, Estimation, and Testing Package. Seattle, Wash, 1987

(*14*) Willett WC, Stampfer MJ, Underwood BA, et al: Vitamins A, E, and carotene: Effects of supplementation on their plasma levels. Am J Clin Nutr 38:559–566, 1983

(*15*) Knekt P, Seppänen R, Aaran RK: Determinants of serum α-tocopherol in Finnish adults. Prev Med 17:725–735, 1988

(16) SPECTOR TD, THOMPSON SG: The potential and limitations of meta-analysis. J Epidemiol Community Health 45:89–92, 1991
(17) MURPHY SP, SUBAR AF, BLOCK G: Vitamin E intakes and sources in the United States. Am J Clin Nutr 52:361–367, 1990
(18) BURTON GW, TRABER MG: Vitamin E: Antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 10:357–382, 1990
(19) ROSE G, BLACKBURN H, KEYS A, ET AL: Colon cancer and blood-cholesterol. Lancet 1:181–183, 1974
(20) WALD NJ, THOMPSON SG, LOW MR, ET AL: Serum cholesterol and subsequent risk of cancer: Results from the BUPA study. Br J Cancer 59:936–938, 1989
(21) TÖRNBERG SA, HOLM LE, CARSTENSEN JM, ET AL: Cancer incidence and cancer mortality in relation to serum cholesterol. J Natl Cancer Inst 81:1917–1921, 1989
(22) KNEKT P, REUNANEN A, AROMAA A, ET AL: Serum cholesterol and risk of cancer in a cohort of 39,000 men and women. J Clin Epidemiol 41:519–530, 1988
(23) GEY KF, BRUBACHER GB, STÄHELIN HB: Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. Am J Clin Nutr 45:1368–1377, 1987
(24) TRABER MG, KAYDEN HJ: Preferential incorporation of α-tocopherol vs γ-tocopherol in human lipoproteins. Am J Clin Nutr 49:517–526, 1989
(25) DION PW, BRIGHT-SEE EB, SMITH CC, ET AL: The effect of dietary ascorbic acid and α-tocopherol on fecal mutagenicity. Mutat Res 102:27–37, 1982
(26) MCKEOWN-EYSSEN G, HOLLOWAY C, JAZMAJI V, ET AL: A randomized trial of vitamins C and E in the prevention of recurrence of colorectal polyps. Cancer Res 48:4701–4705, 1988
(27) DECOSSE JJ, MILLER HH, LESSER ML: Effect of wheat fiber and vitamins C and E on rectal polyps in patients with familial adenomatous polyposis. J Natl Cancer Inst 81:1290–1297, 1989

Phase II Study: Treatment of Non-Hodgkin's Lymphoma With an Oral Antitumor Derivative of Bis(2,6-dioxopiperazine)

Ryuzo Ohno, Kazumasa Yamada, Masami Hirano, Shigeru Shirakawa, Masao Tanaka, Takashi Oguri, Yoshihisa Kodera, Yasuharu Mitomo, Yasushi Ikeda, Shozo Yokomaku, Osamu Kamiya, Masahide Kobayashi, Hidehiko Saito, Kiyoji Kimura, The Tokai Blood Cancer Study Group*

Background: **Although razoxane (ICRF-159), a derivative of bis(2,6-dioxopiperazine), has shown significant antitumor activity in several murine tumors, inadequate bioavailability has limited its clinical efficacy. Sobuzoxane (MST-16), another derivative of bis(2,6-dioxopiperazine), has shown activity against a broad spectrum of murine tumors and human tumor xenografts in nude mice and a lack of cross-resistance to vincristine, doxorubicin, cyclophosphamide, fluorouracil, etoposide, and mitomycin C. These findings suggest that MST-16 has a mode of cytocidal activity different from that of other antitumor agents.** ***Purpose:*** **The present late phase II study was conducted to evaluate the clinical efficacy and toxicity of MST-16 in non-Hodgkin's lymphoma (NHL).** ***Methods:*** **As part of a multi-institutional cooperative study, we conducted a study of MST-16 in 27 patients with NHL who were assessable for drug efficacy and toxicity. MST-16, a bis(2,6-dioxopiperazine) analogue and an inhibitor of topoisomerase II, was administered orally at a dose of 1600 mg/m^2 a day for 5-7 days at intervals of 2-3 weeks.** ***Results:*** **Response consisted of one complete remission and seven partial remissions in 27 assessable patients. Response was achieved at a median of 13 days (range, 9-62 days) after initiation of therapy and lasted a median of 46 days (range, 29-155 days). Major toxic effects were leukopenia in 70% of the patients, thrombocytopenia in 44%, and gastrointestinal disorders in 37%.** ***Conclusions:*** **MST-16 was shown to be effective in NHL and deserves further clinical trial, since the drug shows little cross-resistance to available antitumor drugs.** ***Implications:*** **Phase II clinical studies of MST-16 in treatment of breast cancer, gastric cancer, and adult T-cell leukemia and lymphoma are also being conducted in Japan. Future trials of combination chemotherapy using MST-16 with other antitumor drugs are warranted in view of the additive effects observed in studies of MOLT-3 cells and studies of L1210 leukemia in mice. [J Natl Cancer Inst 84:435–438, 1992]**

4,4′-(1,2-Ethanediyl)bis(1-isobutoxycarbonyloxymethyl-2,6-piperazinedione) (MST-16, sobuzoxane) (Fig. 1) is an antitumor agent that can be administered orally. It is a derivative of bis(2,6-dioxopiperazine) (*1*). Although ICRF-159 (razoxane), another derivative of bis(2,6-dioxopiperazine), showed significant antitumor activity in several murine tumors, its inadequate bioavailability has limited its clinical efficacy (*2-5*). MST-16 has been selected from a number of synthetic derivatives of bis(2,6-dioxopiperazine) as the most promising compound for clinical use (*6,7*). It has shown a broad spectrum of activity against murine tumors including L1210 leukemia, P388 leukemia, B16 melanoma, Colon26 carcinoma, and Lewis lung carcinoma, as well as against human tumor xenografts such as MX-1 breast carcinoma, CO-4 colon carcinoma, and LX-1 lung carcinoma in nude mice. Results of these studies demonstrate a higher therapeutic ratio than that for ICRF-159 (*7*). MST-16 demonstrated no cross-resistance to vincristine, doxorubicin, cyclophosphamide, fluorouracil, etoposide, or mitomycin-C,

Received August 5, 1991; revised December 16, 1991; accepted December 23, 1991.

R. Ohno, K. Yamada, Department of Medicine, Nagoya University School of Medicine, The Branch Hospital, Nagoya, Japan.
M. Hirano, Department of Medicine, Fujita Health University Hospital, Toyoake, Japan.
S. Shirakawa, Department of Medicine, Mie University Hospital, Tsu, Japan.
M. Tanaka, K. Kimura, Department of Medicine, Nagoya National Hospital, Nagoya.
T. Oguri, Department of Medicine, Aichi Medical University Hospital, Nagakutecho, Japan.
Y. Kodera, Department of Medicine, Nagoya First Red Cross Hospital, Nagoya.
Y. Mitomo, Department of Medicine, Nagoya City University Hospital, Nagoya.
Y. Ikeda, Department of Medicine, Hamamatsu University Hospital, Hamamatsu, Japan.
S. Yokomaku, Department of Medicine, Aichi Shokuin Hospital, Nagoya.
O. Kamiya, Department of Medicine, Anjo Kosei Hospital, Anjo, Japan.
M. Kobayashi, Department of Hematology, Hamamatsu Iryo Center, Hamamatsu.
H. Saito, Department of Medicine, Nagoya University Hospital, Nagoya.
The Tokai Blood Cancer Study Group, Nagoya University School of Medicine, The Branch Hospital.

We thank participating physicians from 12 institutions for their cooperation, T. Narita and M. Takeyama for the analysis of data, and T. Ushiroku for the preparation of the manuscript.

**Correspondence to:* Ryuzo Ohno, M.D., Department of Medicine, Nagoya University School of Medicine, The Branch Hospital, 1-1-20 Daikominami, Higashiku, Nagoya 461, Japan.