

Role of Vitamin K₂ in the Development of Hepatocellular Carcinoma in Women With Viral Cirrhosis of the Liver

Daiki Habu, MD, PhD

Susumu Shiomi, MD, PhD

Akihiro Tamori, MD, PhD

Tadashi Takeda, MD, PhD

Takashi Tanaka, MD, PhD

Shoji Kubo, MD, PhD

Shuhei Nishiguchi, MD, PhD

WE PREVIOUSLY REPORTED a 2-year study showing that vitamin K₂ (menaquinone) helps to prevent bone loss in women with viral cirrhosis of the liver.¹ Most of the women agreed to participate in a longer study to clarify the long-term effects of vitamin K₂ on bone loss associated with cirrhosis. The incidence of hepatocellular carcinoma was found to differ between women who received vitamin K₂ and those who did not.

METHODS

The participants in this study were 50 women with viral liver cirrhosis who were admitted to a university hospital between 1996 and 1998. When the results of abdominal dynamic computed tomography and abdominal ultrasonography suggested the presence of hepatocellular carcinoma, abdominal angiography or needle biopsy was performed to confirm the diagnosis. The diagnosis of cirrhosis was based on histological examination of liver specimens obtained by laparoscopy or needle biopsy performed under ultrasonic guidance.

Context Previous findings indicate that vitamin K₂ (menaquinone) may play a role in controlling cell growth.

Objective To determine whether vitamin K₂ has preventive effects on the development of hepatocellular carcinoma in women with viral cirrhosis of the liver.

Design, Setting, and Participants Forty women diagnosed as having viral liver cirrhosis were admitted to a university hospital between 1996 and 1998 and were randomly assigned to the treatment or control group. The original goal of the trial was to assess the long-term effects of vitamin K₂ on bone loss in women with viral liver cirrhosis. However, study participants also satisfied criteria required for examination of the effects of such treatment on the development of hepatocellular carcinoma.

Interventions The treatment group received 45 mg/d of vitamin K₂ (n=21). Participants in the treatment and control groups received symptomatic therapy to treat ascites, if necessary, and dietary advice.

Main Outcome Measure Cumulative proportion of patients with hepatocellular carcinoma.

Results Hepatocellular carcinoma was detected in 2 of the 21 women given vitamin K₂ and 9 of the 19 women in the control group. The cumulative proportion of patients with hepatocellular carcinoma was smaller in the treatment group (log-rank test, $P=.02$). On univariate analysis, the risk ratio for the development of hepatocellular carcinoma in the treatment group compared with the control group was 0.20 (95% confidence interval [CI], 0.04-0.91; $P=.04$). On multivariate analysis with adjustment for age, alanine aminotransferase activity, serum albumin, total bilirubin, platelet count, α -fetoprotein, and history of treatment with interferon alfa, the risk ratio for the development of hepatocellular carcinoma in patients given vitamin K₂ was 0.13 (95% CI, 0.02-0.99; $P=.05$).

Conclusion There is a possible role for vitamin K₂ in the prevention of hepatocellular carcinoma in women with viral cirrhosis.

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Hepatocellular carcinoma was confirmed in 3 women in the treatment group and 4 in the control group. These 7 women were excluded from further study. The remaining 43 women were randomly assigned using sealed envelopes to a treatment or control group. The treatment group received 45 mg/d of vitamin K₂ (Glakay, Eisai Co, Tokyo, Japan). At the end of the first study, 21 women in the treatment group and 19

in the control group consented to participate in a longer trial. All but 1 woman in each group had hepatitis C

Author Affiliations: Departments of Hepatology (Drs Habu, Tamori, Takeda, and Nishiguchi), Nuclear Medicine (Dr Shiomi), Public Health (Dr Tanaka), and Surgery (Dr Kubo), Graduate School of Medicine, Osaka City University, Osaka, Japan.

Corresponding Author: Susumu Shiomi, MD, PhD, Department of Nuclear Medicine, Graduate School of Medicine, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan (shiomis@med.osaka-cu.ac.jp).

virus infection; the other 2 women had hepatitis B infection. Seven women, 4 in the control group and 3 in the treated group, had previously received interferon alfa for their hepatitis C virus infections, but hepatitis C virus was not eradicated. No one was given interferon therapy after study entry.

Surveillance for hepatocellular carcinoma was performed according to detailed procedures recommended for follow-up of patients with liver cirrhosis in Japan.² Abdominal ultrasonography or abdominal dynamic computed tomography was performed and serum α -fetoprotein levels measured tumors at 3-month intervals. Any abnormality was followed up by tumor biopsy or abdominal angiography to confirm a diagnosis of hepatocellular carcinoma.

Diagnosed cases of hepatocellular carcinoma were classified according to the primary tumor, regional lymph nodes, and distant metastasis (TNM) system of the International Union Against Cancer.³ Histopathologic diagnosis of hepatocellular carcinoma was performed according to the criteria proposed by Edmondson and Steiner.⁴ Compliance with vitamin K₂ in the treatment group was good; no patient had adverse reactions or dropped out of the study. This trial was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee at the Osaka City University Medical School. Written informed consent was obtained from each participant.

Statistical analysis was performed using SAS statistical software (version 8.12, SAS Institute Inc, Cary, NC). The χ^2 test was used to assess homogeneity between the groups. Cumulative incidences were plotted using the Kaplan-Meier method and the statistical significance of differences was analyzed using the log-rank test. Cox regression analysis was used for univariate and multivariate analyses. $P < .05$ was considered significant.

RESULTS

The 2 groups were similar with respect to age, virus type, platelets, ala-

Table 1. Baseline Characteristics*

	Treatment Group (n = 21)	Control Group (n = 19)	P Value
Age, y	59.8 (8.7)	61.4 (7.1)	.54†
Hepatitis virus, No. (%)			
B	1 (4)	1 (5)	.94‡
C	20 (95)	18 (95)	
Albumin, g/dL	3.9 (0.3)	3.9 (0.3)	.87†
Platelets, $\times 10^9$ /mL	147 (54)	121 (52)	.13†
Total bilirubin, mg/dL	0.8 (0.2)	0.9 (0.4)	.47†
Alanine aminotransferase, U/L	81.7 (42.7)	70.4 (33.4)	.36†
α -Fetoprotein, mg/mL	13.4 (17.7)	13.3 (8.7)	.99†
Interferon, No. (%)			
Prior to enrollment§	4 (19)	3 (16)	.79‡
At enrollment	17 (81)	16 (84)	

SI conversion units: To convert bilirubin to μ mol/L, multiply by 17.1.

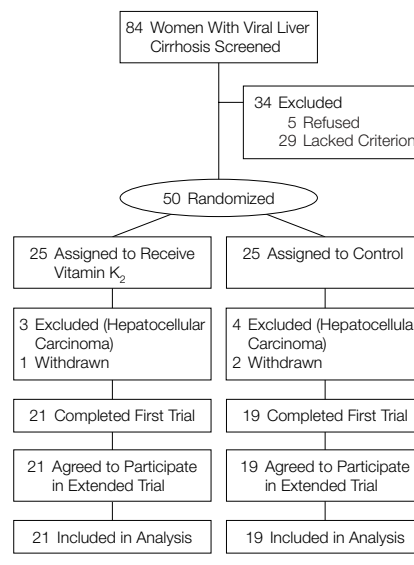
*Values expressed as mean (SD) unless otherwise indicated.

†Values calculated using the Mann-Whitney U test.

‡Values calculated using the χ^2 test.

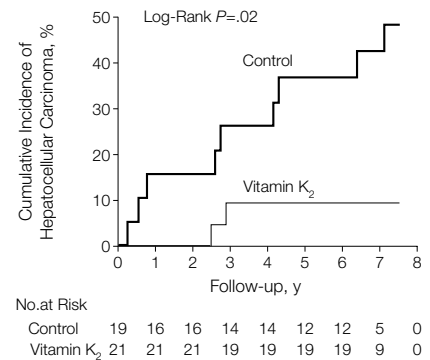
§Hepatocellular carcinoma developed in 1 of 4 patients in the treatment group and 1 of 3 patients in the control group who received interferon prior to enrollment.

Figure 1. Flow of Participants Through Trial



nine aminotransferase, α -fetoprotein, and other clinical findings (TABLE 1). Risk factors for hepatocellular carcinoma^{5,6} were also similar between the groups. After the first study commenced, hepatocellular carcinoma was detected in 2 of the 21 patients given vitamin K₂ and in 9 of the 19 patients in the control group (FIGURE 1). The cumulative proportion of women with hepatocellular carcinoma was smaller in the treatment group compared with the control group (log-rank test, $P = .02$; FIGURE 2). The clinical characteristics

Figure 2. Cumulative Incidence of Hepatocellular Carcinoma Diagnosed in Women Treated With Vitamin K₂



of the women in whom hepatocellular carcinoma was detected during surveillance are shown in TABLE 2. All newly diagnosed cases of hepatocellular carcinoma were stage I or II according to the International Union Against Cancer classification and were given aggressive anticancer therapy. On univariate analysis, the risk ratio for the development of hepatocellular carcinoma in the treatment group compared with the control group was 0.20 (95% confidence interval, 0.04-0.91; $P = .04$; TABLE 3). On multivariate analysis with adjustment for age, alanine aminotransferase activity, serum albumin, total bilirubin, platelet count, α -fetoprotein, and history of treat-

Table 2. Profiles of Women With Hepatocellular Carcinoma

Case No./ Age, y	Group	Type of Hepatitis Virus	No. of Days Diagnosis Occurred After Enrollment	No. of Tumors	Diameter of Largest Tumor, mm	UICC Cancer Stage	Method of Diagnosis	Histological Grade	Type of Therapy
1/70	Control	C	200	1	15	1	Biopsy	1*	PEIT
5/62	Control	C	2333	2	20	2	AAG	Unknown	TAE
6/59	Control	C	282	1	9	1	Biopsy	1*	PEIT
9/70	Control	C	91	1	13	1	Biopsy	1*	PEIT
21/43	Control	C	1516	1	30	2	Biopsy	2*	MCT
25/59	Control	C	1569	2	18	2	AAG	Unknown	TAE
26/67	Control	B	949	1	32	2	AAG	Unknown	TAE
33/57	Control	C	2600	1	30	2	AAG	Unknown	TAE
40/68	Control	C	1002	1	21	2	Biopsy	3*	Operation
4/64	Vitamin K ₂	C	907	1	30	2	AAG	Unknown	TAE
27/68	Vitamin K ₂	C	1054	1	11	1	Biopsy	1*	PEIT

Abbreviations: AAG, abdominal angiography; MCT, microwave coagulation therapy; PEIT, percutaneous ethanol injection therapy; TAE, transcatheter hepatic arterial embolization; UICC, International Union Against Cancer.

*Based on Edmondson and Steiner classification system (scale of 1-4).⁴

Table 3. Crude Odds Ratios for Development of Hepatocellular Carcinoma

	Odds Ratio (95% Confidence Interval)	P Value
Group*	0.20 (0.04-0.91)	.04
Total bilirubin	1.23 (0.33-4.64)	.76
Albumin	3.80 (0.80-18.06)	.09
Platelets	1.55 (0.46-5.19)	.48
Alanine aminotransferase	0.62 (0.17-2.36)	.49
α -Fetoprotein	1.67 (0.36-7.79)	.51
Interferon	1.00 (0.22-4.65)	>.99

*Vitamin K₂ group compared with control group.

Table 4. Adjusted Odds Ratios for Development of Hepatocellular Carcinoma*

	Odds Ratio (95% Confidence Interval)	P Value
Group†	0.13 (0.02-0.99)	.05
Total bilirubin	0.29 (0.04-2.04)	.22
Albumin	33.43 (2.36-473.35)	.009
Platelets	2.24 (0.46-10.90)	.32
Alanine aminotransferase	0.39 (0.07-2.16)	.28
α -Fetoprotein	1.69 (0.31-9.34)	.55
Interferon	1.26 (0.20-7.90)	.81

*Values are adjusted for age and all other variables in this table.

†Vitamin K₂ group compared with control group.

ment with interferon alfa, the risk ratio for the development of hepatocellular carcinoma in women given vitamin K₂ was 0.13 (95% confidence interval, 0.02-0.99; $P = .05$; TABLE 4).

COMMENT

Vitamin K is a cofactor for the enzyme γ -glutamyl-carboxylase, which con-

verts glutamate residues into γ -carboxy-glutamate. Vitamin K-dependent proteins include prothrombin II and the coagulation factors VII, IX, X, proteins C and S, osteocalcin, surfactant-associated proteins, and bone matrix protein. The vitamin K family of molecules comprises the natural forms vitamin K₁ (phylloquinone) and vitamin K₂ (menaquinones) and the synthetic form of vitamin K₃ (menadiolone). These naphthoquinone-containing molecules inhibit tumor cell growth in culture, with vitamin K₃ being more potent than either vitamin K₁ or K₂. Vitamin K₂ inhibits growth of human cancer cell lines and induction of differentiation in various human myeloid leukemia cell lines.^{7,8} Clinically, vitamin K₂ has successfully treated myelodysplastic syndrome.⁹

A number of findings indicate that vitamin K may play a role in controlling cell growth. Underlying mechanisms possibly involve (1) cycling of oxidation and reduction (as known for vitamin K₃), (2) proteins with growth-inhibitory properties induced by vitamin K, such as prothrombin,¹⁰ (3) previously unidentified pathways involving arylation,¹¹ (4) or growth arrest genes such as gas 6.¹² Geranylgeraniol, which is a side chain of vitamin K₂, strongly induces apoptosis of tumor cells, suggesting that geranylgeraniol might play an important role in inhibiting cell growth.¹³ The mechanisms respon-

sible for the inhibition of cell growth mediated by vitamin K₂ remain unexplained. These or other hypothetical mechanisms may have contributed to the reduced hepatocellular carcinoma incidence among patients receiving vitamin K₂. Indeed, the annual incidence of hepatocellular carcinoma in the control group was 8.8%, which is similar to the incidence of hepatocellular carcinoma (7.9%; 32/107) in cirrhotic patients in Japan⁵ compared with 1.6% in the treatment group.

As shown in Table 4, the albumin level showed the highest odds ratio for the development of hepatocellular carcinoma. The serum albumin level is considered an important prognostic factor in liver cirrhosis.¹⁴⁻¹⁷ Low serum albumin levels in patients with liver cirrhosis are associated with disease progression, poor nutritional status, and compromised immunity, which increases the risk of carcinogenesis. The importance of low serum albumin levels as a risk factor for hepatocellular carcinoma should be confirmed in larger studies.

The original goal of our trial was to assess the long-term effects of vitamin K₂ on bone loss in women with viral liver cirrhosis. Our trial had several important limitations when the data were used to assess the value of vitamin K₂ for the primary prevention of hepatocellular carcinoma in patients with liver cirrhosis, resulting from the small study group,

the inclusion of only women, and the participation of only 1 center. However, similar to previously reported randomized controlled studies of cirrhosis in which the primary end point was the development of hepatocellular carcinoma, patients with evidence of hepatocellular carcinoma on highly sensitive imaging studies were excluded, and the 2 study groups were similar with respect to risk factors for hepatocellular carcinoma, such as age, severity of cirrhosis, history of interferon therapy, and type of hepatitis virus infection. The procedures used for the surveillance and diagnosis of hepatocellular carcinoma were also similar to those used in our study. The sensitivity of these procedures for the detection of hepatocellular carcinoma was underscored by the fact that all of the detected cases of hepatocellular

carcinoma were stage I or stage II. Our results must also be tempered by the fact that 3 cases of hepatocellular carcinoma were diagnosed in the control group within a year of enrollment. These patients may have harbored occult disease at the time of enrollment. Nonetheless, despite its small size, our study indicates that vitamin K₂ decreases the risk of hepatocellular carcinoma to about 20% compared with the control group, suggesting that vitamin K₂ may delay the onset of hepatocarcinogenesis. Moreover, the safety, relatively low cost, and ease of use of vitamin K₂ led to good compliance with treatment. The results of this preliminary trial are intriguing and suggest that a potential role for vitamin K₂ to prevent hepatocarcinogenesis in patients with liver cirrhosis. These results must be confirmed by mul-

ticenter randomized controlled studies with the prevention of hepatocellular carcinoma by vitamin K₂ as the primary end point.

Author Contributions: Dr Shiomi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Habu, Shiomi, Kubo, Nishiguchi.

Acquisition of data: Habu, Shiomi, Tamori, Takeda, Nishiguchi.

Analysis and interpretation of data: Habu, Shiomi, Tamaka, Kubo, Nishiguchi.

Drafting of the manuscript: Habu, Shiomi, Tamori, Takeda, Kubo, Nishiguchi.

Critical revision of the manuscript for important intellectual content: Habu, Shiomi, Tamaka, Nishiguchi.

Statistical Analysis: Habu, Tamaka.

Obtained funding: Habu, Shiomi, Tamori, Takeda, Nishiguchi.

Administrative, technical, or material support: Habu, Shiomi, Tamori, Nishiguchi, Kubo.

Supervision: Shiomi, Nishiguchi, Kubo.

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