

The Effect of Menatetrenone, a Vitamin K2 Analog, on Disease Recurrence and Survival in Patients with Hepatocellular Carcinoma after Curative Treatment

A Pilot Study

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BACKGROUND. The high recurrence rate of hepatocellular carcinoma (HCC) determines the long-term prognosis for patients with HCC. In the current study, the authors tested the effects of menatetrenone, a vitamin K2 analog, on recurrent HCC and survival after curative treatment.

METHODS. Sixty-one patients who were diagnosed as free of HCC after surgical resection or percutaneous local ablation were assigned randomly assigned to either a menatetrenone group ($n = 32$ patients) or a control group ($n = 29$ patients). Patients in the menatetrenone group received a daily oral dose of 45 mg of menatetrenone. Disease recurrence and survival rates were analyzed in patients with HCC.

RESULTS. The cumulative recurrence rates in the menatetrenone group were 12.5% at 12 months, 39.0% at 24 months, and 64.3% at 36 months; and the corresponding recurrence rates in the control group were 55.2%, 83.2%, and 91.6%, respectively ($P = 0.0002$). Similar results were obtained even for patients who had low baseline levels of serum des- γ -carboxy-prothrombin. Univariate and multivariate Cox proportional hazard analyses showed that the administration of menatetrenone was the only factor related to the recurrence rate of HCC. The cumulative survival rates for the patients who received menatetrenone were 100% at 12 months, 96.6% at 24 months, and 87.0% at 36 months; and the corresponding survival rates for patients in the control group were 96.4%, 80.9%, and 64.0%, respectively ($P = 0.051$).

CONCLUSIONS. The current study findings suggested that menatetrenone may have a suppressive effect on recurrence of HCC and a beneficial effect on survival, although a larger, placebo-controlled trial will be required to prove these effects. *Cancer* 2006;106:867-72. © 2006 American Cancer Society.

KEYWORDS: clinical trial, liver carcinoma, vitamin K, chemoprevention.

Hepatocellular carcinoma (HCC) often develops in individuals who are infected with hepatitis B or C virus, and HCC increasing in frequency worldwide. Despite the multitude of therapeutic approaches available, including surgical resection and local ablation techniques like as percutaneous ethanol injection, percutaneous thermal ablation, and transarterial embolization, the long-term prognosis is poor for patients with HCC.¹ One of the major difficulties for patients with HCC is the high frequency of recurrence in the remnant liver even after curative treatment.^{1,2} Therefore, the development of effective prevention protocols for recurrent HCC after curative treatment is considered important.

Vitamin K (VK) is a fat-soluble vitamin that regulates clotting

factor production by acting as a coenzyme for a VK-dependent carboxylase that catalyzes carboxylation of glutamic acid residues into γ -carboxyglutamic acid.³ VK is involved similarly in bone metabolism by regulating bone matrix metabolism through γ -carboxylation.⁴ VK can be divided into 2 groups: 1) naturally produced VK1 (phytonadione) and VK2 (menaquinone) and 2) chemically synthesized VK3 (menadiolone). VK3 and its derivatives demonstrate potent antiproliferative effects against tumor cell lines in vitro,^{5,6} whereas VK2 and its derivatives demonstrate antiproliferative effects, although they less potent than those of VK3, against leukemia and hepatoma cell lines.⁷⁻⁹ VK2 also has the ability to induce differentiation of leukemic and hepatoma cells,⁷ and it has been used in the treatment of myelodysplastic syndromes.¹⁰

Abnormal, uncarboxylated prothrombin (des- γ -carboxy-prothrombin [DCP]) appears in some patients with HCC¹¹ who have an HCC phenotype that appears more aggressive.^{12,13} It has been shown that administration of VK2 suppresses plasma DCP concentrations in patients with HCC^{13,14}; however, to our knowledge, it is not known whether the administration of VK modulates disease progression. It has been shown that long-term administration of VK2 in the treatment of osteoporosis is safe and effective for improving bone matrix metabolism.¹⁵ Therefore, in this a pilot study, we investigated the effects of VK2 on disease recurrence and survival in patients with HCC after they underwent curative resection or percutaneous local ablation therapy.

MATERIALS AND METHODS

Patients and Trial Profiles

High-density and low-density hepatic nodules were diagnosed as HCC using computed tomography (CT) during hepatic angiography (CTHA) and portography (CTAP), respectively. Nodules that showed other enhanced patterns in CTHA and CTAP were excluded. Patients did not undergo a tumor biopsy before treatment.

The current study included patients with HCC who were admitted to the Department of Internal Medicine at Saga Medical School Hospital between March 1999 and March 2001. All patients underwent curative surgical resection or local ablation therapy, such as percutaneous ethanol injection, percutaneous microcoagulation, or percutaneous radiofrequency ablation, with or without transarterial chemoembolization. Patients who had single tumors that exceeded 3 cm in greatest dimension ($n = 4$ patients) underwent surgical resection, and all other patients underwent local ablation.

Patients were excluded if they were receiving medication, such as warfarin and/or VK analogs, that had to potential to influence the effect of VK metabolism. Patients who had obvious tumor invasion into the portal vein or extrahepatic metastasis or who had uncontrollable ascites or encephalopathy also were excluded.

Selected patients also were required to be free of HCC according to contrast-enhanced CT scans that were obtained within 1 month after treatment. In total, 61 patients met these criteria and were enrolled in the study after their informed consent was obtained. Patients were assigned randomly according to a table of random permutations into 1 of 2 groups: the menatetreneone group ($n = 32$ patients) or the control group ($n = 29$ patients). Patients in the menatetreneone group were given oral menatetreneone (at a dose of 45 mg per day) (Eisai Co, Tokyo, Japan) continuously during the follow-up period. No placebo was used in the control group, because this study was planned as a pilot test. Until the discovery of recurrent HCC, no patient in either group received any type of neoplastic therapy, including chemotherapy or interferon. The study was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Saga Medical School.

Detection of Disease Recurrence

The endpoints of the current study were disease recurrence of HCC and survival. Ultrasonography images and contrast-enhanced CT scans (or magnetic resonance images in patients with iodine allergy) were obtained within 3 months after curative treatment and every 3–4 months thereafter. Examinations were continued until the detection of recurrent HCC. If atypical or nonenhanced nodules were observed, then a tumor biopsy was obtained for histologic examination.

Statistical Analysis

Analyses included all patients who were enrolled in the study according to the intention-to-treat principle. Baseline variables were analyzed by using the Mann-Whitney U test and the Fisher exact probability test. The cumulative incidence of HCC recurrence and deaths were plotted by using the Kaplan-Meier method, and statistically significant differences were determined by using the log-rank test. The hazards ratio was calculated using univariate and multivariate Cox proportional hazard analyses. Differences with a P value < 0.05 were considered statistically significant.

RESULTS

No adverse effects (for example, elevation of hepatic enzymes or thromboembolic events) were observed

TABLE 1
Demographic Characteristics of the Enrolled Patients^a

Characteristic	Mean \pm SD		P value
	Menatetrenone group	Untreated group	
No. of patients	32	29	
Age(range) (yrs)	63.3 \pm 7.5(48-75)	64.5 \pm 6.7(45-74)	0.444 ^b
Gender(males/females)	23/9	18/11	0.586 ^c
Etiology (HCV/HBV/B+C)	28/3/1	26/2/1	0.938 ^c
Daily alcohol consumption(< 40 g/> 40g)	10/22	3/26	0.063 ^c
Tumor stage (Stage I/II/III)	17/11/4	14/8/7	0.487 ^c
Tumor size in mm ² (range)	17.7 \pm 5.1(10-30)	19.4 \pm 6.9(10-38)	0.326 ^c
No. of tumors (range)	1.5 \pm 0.88(1-4)	1.48 \pm 0.74(1-3)	0.754 ^b
AFP (range) (ng/mL)	102.2 \pm 234.4(4-1217)	508 \pm 1528.4(3-7505)	0.406 ^b
DCP (range) (mAU/mL)	41.8 \pm 65.4(8-346)	70.3 \pm 104.1(7-417)	0.049 ^b
ALT (IU/L)	65.8 \pm 34.7(16-147)	66.1 \pm 33.3(22-173)	0.999 ^b
Child-Pugh classification (A/B)	26/6	22/7	0.757 ^c
Treatment (surgical/nonsurgical)	1/31	3/26	0.339 ^c
Months of follow-up (range)	28.9 \pm 8.3(13-42)	27.7 \pm 8.6(15-42)	0.426 ^b

SD: standard deviation; HCV: hepatitis C virus;HBV:hepatitis B+C: hepatitis B virus and hepatitis C virus; AFP: α -fetoprotein, DCP: des- γ -carboxy-prothrombin, ALT: alanine transaminase.

^a Tumor stage was classified according to the general rules for clinical and pathologic studies of primary liver carcinoma of The Liver Cancer Study Group of Japan.

^b Statistical analysis was performed using the Mann-Whitney *U* test.

^c Statistical analysis was performed using the Fisher exact probability test.

as a result of menatetrenone treatment, and no patients were withdrawn during the follow-up period. Patient demographics and clinical characteristics are shown in Table 1. The mean \pm standard deviation follow-up was 28.9 \pm 8.3 months (range, 13–42 mos) for the menatetrenone group and 27.7 \pm 8.6 months (range, 15–42 mos) for the control group. Baseline variables, including age, gender, etiology of liver disease, alcohol intake, and Child-Pugh score, were similar in both groups. In addition, there were no significant differences noted between the groups with regard to tumor stage (tumor size and number), α -fetoprotein levels, and treatment modalities; the only significant difference was noted in DCP levels (41.8 \pm 65.4 mAU/mL vs. 70.3 \pm 104.1 mAU/mL for the menatetrenone group and the control group, respectively; *P* = 0.049).

Menatetrenone decreased the cumulative incidence of HCC recurrence. In the control group, recurrent HCC was detected in 16 patients (55.2%) at 12 months, in 23 patients (83.2%) at 24 months, and in 24 patients (91.6%) at 36 months. In the menatetrenone group, recurrent HCC was detected in 4 patients (12.5%) at 12 months, in 12 patients (39.0%) at 24 months, and in 17 patients (64.3%) at 36 months (log-rank test; *P* = 0.0002) (Fig. 1). During the follow-up period, recurrent HCC was observed in 25 patients in the control group and in 18 patients in the menatetrenone group. There was no difference noted in the

Cumulative recurrence rate (%)

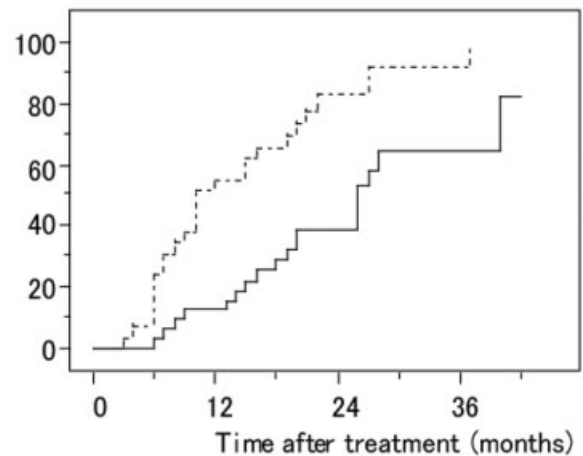


FIGURE 1. Recurrence rates are illustrated for the menatetrenone group (*n* = 32 patients; solid line) and the control group (*n* = 29 patients; dotted line) after curative treatment for hepatocellular carcinoma. Disease recurrence was found to be significantly lower in the menatetrenone group (*P* = 0.0002; log-rank test).

rate of local disease recurrence derived from the original tumor between the 2 groups (7 patients [28%] and 5 patients [28%], respectively), and similar therapeutic effectiveness was observed between the groups. Two patients in the menatetrenone group and one patient in the control group underwent a tumor biopsy for nodules, because these three tumors were

detected with ultrasonography but did not show contrast-enhanced CT scans. Consequently, all three patients were histologically diagnosed with recurrent tumors. The other patients with recurrent HCC were diagnosed only with radiologic findings that demonstrated a typical HCC pattern on ultrasound, CT, and/or magnetic resonance imaging.

Univariate and multivariate Cox proportional hazard models were used to analyze whether each variable was related to the recurrence rate of HCC. Table 2 shows that the administration of menatetrenone was the only factor related to recurrence of HCC in both analyses (hazards ratio, 0.27; 95% confidence interval, 0.124–0.598 in the multivariate analysis).

Because baseline DCP concentrations differed between the 2 groups, we also analyzed patients who had low DCP levels (< 40 mAU/mL) before they received treatment for HCC. Eighteen patients in the control group and 24 patients in the menatetrenone group were found to have low DCP levels. Similar to the overall evaluation, menatetrenone was found to significantly decrease the recurrence rate of HCC (log-rank test; $P = 0.004$) (Fig. 2).

The cumulative survival rates for the menatetrenone group were 100% at 12 month, 96.6% at 24 months, and 87.0% at 36 months; whereas the corresponding rates for control group were 96.4%, 80.9%, and 64%, respectively (log-rank test; $P = 0.051$) (Fig. 3). Eight patients in the control group and three patients in the menatetrenone group died during the observation period, including six patients in the control group and two patients in the menatetrenone group who died as a result of advanced carcinoma, and two patients in the control group and one patient in the menatetrenone group who died because of hepatic failure. No patients died of causes unrelated to liver disease.

DISCUSSION

To our knowledge, the current study is the first clinical trial to examine whether menatetrenone, a VK2 compound, can suppress the recurrence of HCC and improve survival after patients receive curative treatment. The results showed the possible applicability of menatetrenone as a chemopreventive drug for HCC.

However, there were a number of problems with this study. First is the baseline difference in serum DCP levels between the treatment group and the control group. Because patients who are positive for DCP reportedly have a more aggressive HCC phenotype,^{12,13} it is possible that recurrence rates and prognosis are influenced by differences in DCP levels. Therefore, we analyzed what, if any, variables were related to HCC recurrence by using a multivariate Cox

TABLE 2
Univariate and Multivariate Cox Proportional Hazards Analyses of Variables Related to the Disease Recurrence Rate in Patients with Hepatocellular Carcinoma

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age				
> 60 yrs	1.00		1.00	
< 60 yrs	0.52 (0.238-1.121)	0.095	0.47 (0.161-1.396)	0.175
Gender				
Male	1.00		1.00	
Female	0.84 (0.426-1.639)	0.602	0.48 (0.178-1.293)	0.146
Treatment				
Nonsurgical	1.00		1.00	
Surgical	1.34 (0.474-3.764)	0.584	0.43 (0.105-1.748)	0.237
Alcohol				
< 40 g/day	1.00		1.00	
> 40 g/day	0.78 (0.371-1.635)	0.509	1.02 (0.431-2.406)	0.967
Etiology				
Anti-HCV negative	1.00		1.00	
Anti-HCV positive	1.43 (0.529-4.009)	0.499	1.76 (0.464-6.685)	0.405
Tumor size				
> 20 mm	1.00		1.00	
< 20 mm	0.93 (0.498-1.744)	0.825	1.7 (0.705-4.114)	0.237
No. of tumors				
Multiple	1.00		1.00	
Single	1.22 (0.651-2.3)	0.53	0.77 (0.337-1.751)	0.53
AFP				
< 100 ng/mL	1.00		1.00	
> 100 ng/mL	0.65 (0.338-1.234)	0.186	0.46 (0.174-1.188)	0.108
DCP				
< 40 mAU/mL	1.00		1.00	
> 40 mAU/mL	0.9 (0.476-1.685)	0.733	0.59 (0.233-1.494)	0.265
ALT				
< 80 IU/L	1.00		1.00	
> 80 IU/L	0.64 (0.332-1.214)	0.169	1.02 (0.454-2.306)	0.956
Child-Pugh classification:				
Class B	1.00		1.00	
Class A	1.29 (0.591-2.797)	0.526	1.81 (0.679-4.849)	0.235
Vitamin K2 treatment:				
Control group	1.00		1.00	
Treatment group	0.32 (0.171-0.609)	< 0.001	0.27 (0.124-0.598)	0.001

HR: hazards ratio; 95% CI: 95% confidence interval; HCV: hepatitis C virus; AFP: α -fetoprotein; DCP: des- γ -carboxy-prothrombin; ALT: alanine aminotransferase.

proportional hazards model. Consequently, we observed that only the administration of menatetrenone was significant; serum DCP levels were not a risk factor. In addition, menatetrenone suppressed the recurrence of HCC, even in patients with low DCP levels. Judging from these results, the reduced rate of HCC recurrence does not appear to be due to differences in baseline DCP levels.

The second problem with this study is that the treatment methods for HCC were heterogeneous, in-

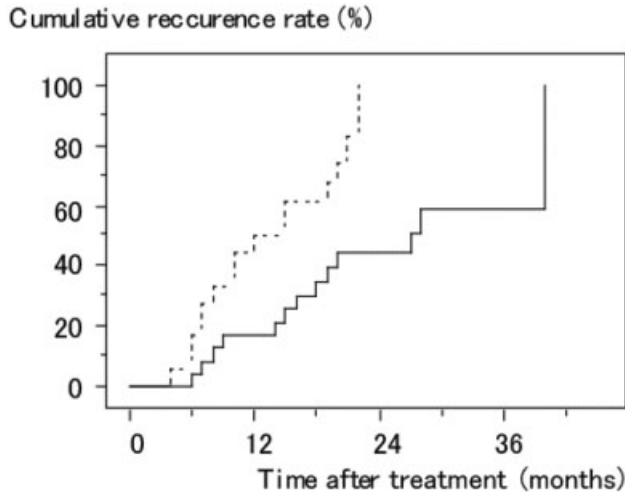


FIGURE 2. Recurrence rates are illustrated for the menatetrenone group ($n = 24$ patients; solid line) and the control group ($n = 18$ patients; dotted line) after curative treatment for hepatocellular carcinoma in patients with low serum des- γ -carboxy-prothrombin levels (< 40 mAU/mL). Disease recurrence was found to be significantly lower in the menatetrenone group ($P = 0.004$; log-rank test).

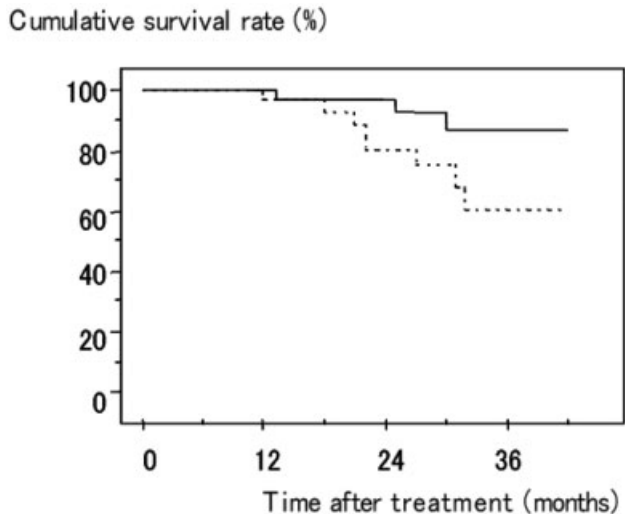


FIGURE 3. Cumulative survival rates are illustrated for the menatetrenone group ($n = 32$ patients; solid line) and the control group ($n = 29$ patients; dotted line) after curative treatment.

cluding surgical resection, percutaneous ethanol injection, percutaneous microcoagulation, and percutaneous radiofrequency ablation. However, because local recurrence rates did not differ between the treatment group and the control group, it was assumed that initial therapeutic methods did not influence the results. Nevertheless, a separate evaluation of each therapeutic method will be required in the future.

The third problem was the method of diagnosing

HCC before entry and at the time of disease recurrence. In patients who have nodules that measure < 2 cm in greatest dimension, the use of fine-needle biopsy is recommended for diagnosis according to a consensus statement from the European Association for the Study of the Liver in 2001¹⁶; however, the current prospective pilot study started in 1999, and we used only radiologic findings for the diagnosis of HCC in most patients. The possibility that nonmalignant tumors, including dysplastic nodules, may exist among the lesions that were diagnosed as HCC in this study should be taken into consideration when interpreting the current study results.

VK inhibits the proliferation of tumor cells in vitro at a potency of $VK3 \rightarrow VK2 \rightarrow VK1$.⁵⁻⁹ To our knowledge, the mechanisms involved in the antiproliferative activity of VKs are not understood fully, although different effects of VK2 and VK3 on hepatoma cells have been reported. Wang et al.⁹ showed that VK3 exerted antiproliferative effects on hepatoma cells in a free radical-dependent manner, whereas the effects of VK2 were not mediated by free radicals. Furthermore, Bouzahazah et al.¹⁷ reported that VK2 increased c-Jun and c-Myc mRNA expression in hepatoma cells, suggesting the induction of apoptosis through VK-dependent proteins. Another report suggested the involvement of geranylgeraniol VK side chains in the induction of apoptosis.¹⁸

Chemoprevention of tumors is an important challenge in the field of medical research, especially with regard to HCC because of the high frequency of recurrence even after patients undergo curative therapy.^{1,2} Interferons reportedly are effective in both primary and secondary prevention of HCC in patients with hepatitis C,^{19,20} and acyclic retinoid has been indicated as effective in preventing the development of secondary HCC.²¹ However, interferon is expensive and has frequent adverse effects that are not tolerated by all patients, whereas to our knowledge, the safety of acyclic retinoids with long-term administration has yet to be established.

Safety is critical when determining the clinical usefulness of chemopreventive drugs. Although VK3 and its derivatives have been shown to be effective in vitro, to our knowledge they have yet to be tested in human studies. Conversely, VK2 is used currently and safely for the treatment of osteoporosis.¹⁵ In the current trial, there were no adverse effects related to daily oral administration of 45 mg menatetrenone, the same dosage used in the treatment of osteoporosis. However, because dose-dependent antiproliferative effects of VKs have been reported,⁵⁻⁹ it may be possible to adjust the dose of menatetrenone administered to

increase its chemopreventive effects on HCC recurrence.

The results from this randomized, controlled, pilot study revealed that menatetrenone possibly may reduce the risk of HCC recurrence after curative surgical resection or local ablation therapy. The effect on survival warrants further research; however, overall, chemoprevention of secondary HCC using menatetrenone appears to be a promising option.

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