Yonago Acta medica 2008;51:95–99

Vitamin K2 Has No Preventive Effect on Recurrence of Hepatocellular Carcinoma after Effective Treatment

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Hepatocellular carcinoma (HCC) has a poor prognosis because of its high recurrence rate. Recently, vitamin K2 has been reported to inhibit the growth of HCC cell lines. To clarify the preventive effect of vitamin K2 on HCC recurrence, we studied 72 HCC patients who had been treated with surgical resection, local ablation or transarterial embolization: their etiologies were hepatitis B virus (n = 21), hepatitis C virus (n = 47), both B and C viruses (n = 2) and non-B or non-C virus (n = 2). We divided them into 2 groups: in one group, patients were treated with 45-mg/day vitamin K2 [K2-treated group (n = 23)], and in another, patients were not given vitamin K2 or a placebo [non-treated control group (n = 49)]. The obtained results between the 2 groups were compared. HCC recurred in 12 (52.2%) of the 23 K2-treated patients, and 22 (44.9%) of the 49 control patients. The differences in cumulative recurrence-free rate and cumulative survival rate between both groups were not significant (P = 0.92 and P = 0.08, respectively). As observed, chemopreventive effects of vitamin K2 at a clinically relevant dose on HCC recurrence were ineffective after effective treatment for HCC. Different regimens such as higher doses of vitamin K2 or combination therapy with other drugs may be worth testing to further explore the preventive effect on HCC recurrence.

Key words: chemoprevention; hepatocellular carcinoma; vitamin K2

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. In Japan, primary liver cancer, 95% of which is HCC, is ranked 3rd in men and 5th in women as a cause of death from malignant neoplasm. Chronic viral hepatitis associated with hepatitis B virus and hepatitis C virus (HCV) is a major cause of HCC. In spite of curative treatments, including surgical resection and local ablation therapy, the recurrence rate in the remnant liver is high (Araii et al., 2000). Therefore, chemoprevention for the recurrence of HCC after curative treatment is important.

For chemopreventive therapy in patients with chronic HCV infection, interferon has been administrated and promising results have been obtained (Ikeda et al., 2000; Suou et al., 2001). Acyclic retinoid has been shown to prevent the recurrence of HCC after surgical resection or local ablation therapy (Muto et al., 1996). Vitamin K2 is known to inhibit the growth of a variety of tumor cells including hepatoma cells by inducing apoptosis or differentiation (Miyazaki et al., 2001). Recently, Mizuta et al. reported the benefit of vitamin K2 on the recurrence and survival in patients with HCC after curative therapy (Mizuta et al., 2006). To re-confirm their findings, we conducted a similar study after local ablation therapy or curative resection.

Abbreviations: CT, computed tomography; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; JIS, Japan Integrated Staging; MRI, magnetic resonance imaging; PIVKA II, protein induced by vitamin K absence or antagonist

Materials and Methods

Patients and study protocol

The study included 72 patients with HCC who were admitted to Tottori University Hospital between August 2002 and June 2006. Diagnosis of HCC was confirmed by a combination of histopathological examination from biopsy specimens, serological examination and findings with diagnostic imagings including contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI) and transhepatic arteriography. All patients underwent curative treatment including surgical resection, percutaneous ethanol injection or percutaneous radiofrequency ablation with or without transarterial chemoembolization. After 1 month of treatment, curative treatment was defined as no residual HCC visualized with any of the above described imagings. In total, 72 patients were enrolled in the present study. The patients were assigned to a group treated with or another not treated with vitamin K2 (control group) based on the judgments by their physician in charge. Vitamin K2, Glakay (Eisai, Tokyo, Japan), was given at a fixed dose of 45 mg/day during the follow-up periods. Patients of the control group were not given a placebo. Serum alfa-fetoprotein levels were measured with a commercially available enzyme immunoassay kit (Abbott Japan, Tokyo), and protein induced by vitamin K absence or antagonist (PIVKA II) was tested with an ELISA kit (Picolumi PIVKA II kit, Sanko Junyaku, Tokyo). Liver functions were estimated according to the Japan Integrated Staging (JIS) score (Kudo et al., 2003). The JIS score is a new score system that includes 2 previous classifications, the TNM endorsed by the Union Internationale Contre le Cancer and the Child-Pugh classification (Henderson et al., 2003). The 1st endpoint was the time to recurrence of HCC, which was diagnosed with imagings. The 2nd endpoint was overall survival. Contrast-enhanced CT scans or MRI was performed every 3 months after curative treatment. Examinations were continued until the detection of recurrent HCC. Alfa-fetoprotein and PIVKA II were assayed monthly. The present study conformed to the ethical guideline of the Declaration of Helsinki. Informed consent was verbally obtained from all patients.

Statistical methods

Statistical analysis was performed by standard methods. The cumulative incidence of HCC recurrence and deaths were plotted using the Kaplan-Meier method, and the statistical significance of differences was determined using the log-rank test (Cox regression analysis was used for univariate and multivariate analyses). Differences with a P value < 0.05 were considered significant. The data are shown as the mean ± SD.

Results

Compliance with vitamin K2 in the K2-treated group was good. No adverse effects were observed and no patients dropped out of the study. Clinical characteristics are shown in Table 1. Twenty-three patients were enrolled in the K2-treated group, and 49 patients were in the non-treated control group. The number of patients treated with surgical resection, local ablation therapy and transcatheter arterial chemoembolization was 2, 7 and 14 in the K2-treated group, and 7, 18 and 24 in the control group, respectively. Differences in age, gender, observation periods, etiologies and HCC-related findings were not significant between groups. The JIS score showed no difference between groups. The mean AFP level was 382.6 ± 22.4 ng/dL in the K2-treated group and 2267.5 ± 1747.8 ng/dL in the control group. The mean PIVKA II level was 1182.9 ± 960.9 mAU/mL, and 2421.3 ± 1413.4 mAU/mL, respectively. The mean follow-up period was 23.8 ± 14.1 months (range, 0.5–45.6 months) for the K2-treated group, and 26.9 ± 10.9 months (range, 3.3–43.7 months) for the control group.

Table 1. Characterstics of the patients enrolled in the present study

		Vitamin K2-treated group [23]	Non-treated control group [49]	*P*
Age	(year)	67 ± 9	69 ± 9	NS
Sex	(male/female)	11/12	27/22	NS
Observation period after HCC therapy	(month)	23.8 ± 14.1	26.9 ± 10.9	NS
Etiology				NS
Hepatitis virus B		6	15	
Hepatitis virus C		14	33	
Hepatitis viruses B and C		2	0	
Non-hepatitis virus B or C		1	1	
JIS score				NS
0		1	8	
1		10	18	
2		9	14	
3		3	9	
Characteristics of HCC				
Maximum diameter of tumor	(cm)	2.3 ± 0.9	2.8 ± 1.7	NS
Number of nodules		1.3 ± 0.6	1.4 ± 0.9	NS

[ ], number of subjects.
HCC, hepatocellular carcinoma; mo, months; JIS, Japan Integrated Staging; NS, not significant.

The mean time to tumor recurrence was 16.3 ± 10.1 months in the K2-treated group and 10.4 ± 6.6 months in the control group, respectively. During the follow-up period, recurrent HCC was observed in 22 patients in the control group and in 12 patients in the K2-treated group. The cumulative recurrence-free rates were 73.9% at 12 months, 67.2% at 24 months and 21.9% at 36 months in the K2-treated group, and were 50.6%, 38.3% and 32.4% in the control group, respectively (Fig. 1a). The difference in cumulative recurrence-free rate was not significant between the 2 groups (log-rank test; $P = 0.92$). In HCV patients, the cumulative recurrence-free rates were 77.4% at 12 months, 46.4% at 24 months and 31.0% at 36 months in the K2-treated group, and were 47.9%, 40.5% and 36.0% in the control group, respectively (log-rank test; $P = 0.58$, Fig. 1b).

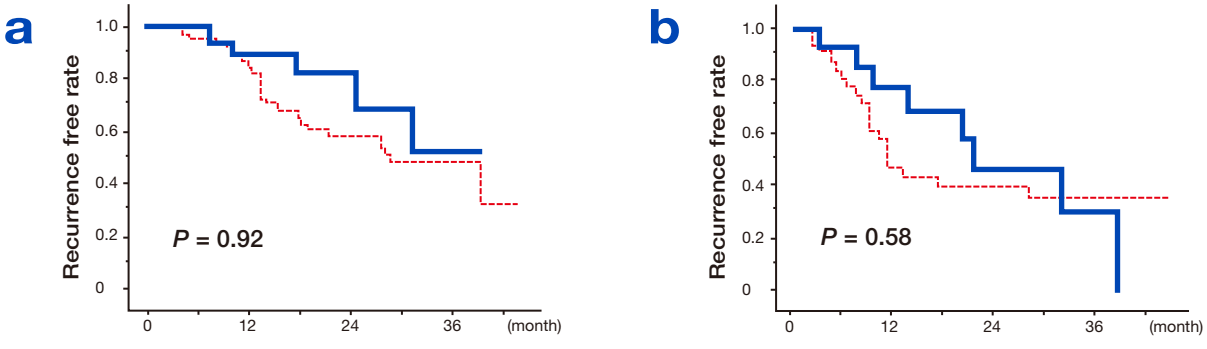

Fig. 1. Cumulative recurrence-free rates in patients.
a: HCC patients after curative treatment: ——, K2-treated group ($n = 23$); ---, control group ($n = 49$).
b: HCV-positive HCC patients after curative treatment: ——, K2-treated group ($n = 15$); ---, control group ($n = 33$).
Differences between groups are not significant both in **a** and **b**. HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

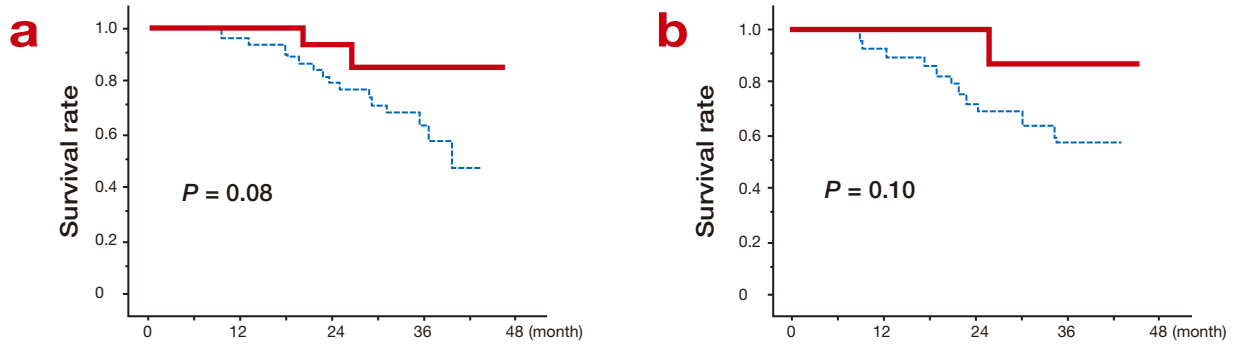

Fig. 2. Cumulative survival rates in patients.
a: HCC patients after curative treatment: ——, K2-treated group (n = 23); - - -, control group (n = 49).
b: HCV-positive HCC patients after curative treatment: ——, K2-treated group (n = 15); - - -, control group (n = 33).
Differences between groups are not significant both in **a** and **b**. HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

During the follow-up period, 2 K2-treated patients and 16 control patients died. The 2 K2-treated patients and 12 of the 16 control patients died of cancer progression: among the rest 4 control patients, 1 died of hepatic failure and 3 died of non-hepatic disease. The cumulative survival rates for the K2-treated group were 100% at 12 months, 93.3% at 24 months and 85.6% at 36 months. The corresponding rates for the control group were 93.3%, 79.1% and 56.5%, respectively. The difference in survival rate was not significant between both groups (log-rank test; P = 0.08, Fig. 2a). The cumulative survival rates of HCV patients were 100% at 12 months, 100% at 24 months and 87.5% at 36 months in the K2-treated group; and 93.3%, 72.6% and 56.9% in the control group, respectively (log-rank test; P = 0.10, Fig. 2b). Vitamin K2 treatment immediately decreased the PIVKA II level, which was kept within normal ranges (data not shown).

Discussion

Vitamin K is a fat-soluble essential vitamin that activates blood coagulation factors by converting glutamic residues to γ-carboxy glutaminic residues. The vitamin K family of molecules comprises the natural forms vitamin K1 (phylloquinone) and vitamin K2 (menaquinones) and the synthetic form of vitamin K3 (menadione). This vitamin K family has tumor inhibition properties in vitro (Lamson et al., 2003). Recently, it has been shown that vitamin K, specifically vitamin K2, may have antiproliferative effects toward a variety of cancers including HCC (Nishikawa et al., 1995; Otsuka et al., 2004), and that it may cause differentiation in various human myeloid leukemia cell lines (Miyazawa et al., 2001), although the mechanism of cell growth inhibitory effects has not been explained. Mastumoto et al. reported that vitamin K2 inhibits the proliferation of HCC cells in vitro through a cell cycle arrest at the G1 phase and apoptosis (Mastumoto et al., 2006).

Clinically, vitamin K2 treatment has been widely used for the treatment of osteoporosis in Japan. Recently, Habu et al. revealed that vitamin K2 exerted a suppressive effect against the development of HCC in women with HCV cirrhosis, who took vitamin K2 for osteoporosis (Habu et al., 2004). Furthermore, Mizuta et al. reported that vitamin K2 treatment significantly decreased the recurrence rate of HCC and achieved a significant improvement in the overall survival rate (Mizuta et al., 2006). In the present study, we did not find preventive effects of vitamin K2 on HCC recurrence or improvement in the survival rate after curative treatment. Although our study was not double-blinded or controlled, there were

no significant differences in age, gender, etiology, observation period and characteristics of HCC between groups with and without vitamin K2 treatment. In addition, the patient number, tumor characteristics and etiology were not different from the previous study (Mizuta et al., 2006). More recently, Hotta et al. also reported that vitamin K2 appeared not to be able to inhibit or delay the recurrence of HCC after curative treatment (Hotta et al., 2007), which our results agreed with. In the future, a large-scale study with a longer observation period is required to clarify the preventive effect of vitamin K2 on recurrence of HCC.

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Received June 30, 2008; accepted September 16, 2008

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