Vitamin Analogues in Chemoprevention of Hepatocellular Carcinoma After Resection or Ablation—A Systematic Review and Meta-analysis

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OBJECTIVE: While hepatic resection or local ablative therapy may provide a potentially curative treatment for hepatocellular carcinoma (HCC), more than half of these patients develop recurrent HCC within 5 years after treatment. Thus identification of any therapy which can decrease or delay the incidence of recurrence will improve the results of treatment. However, no chemopreventive agent has been approved for HCC.

METHODS: A MEDLINE database, Embase, Cancerlit (National Cancer Institute), and CBM (Chinese Biomedical Database) search from 1990 to 2009 was performed to identify relevant articles using the keywords “hepatocellular carcinoma,” “vitamin analogue,” and “chemoprevention.” Additional papers were identified by a manual search of the references from the key articles. The fixed effect model was used for a meta-analysis.

RESULTS: Oral administration of acyclic retinoids (vitamin A analogue), and menatetrenone (vitamin K2 analogue) have been tested as chemopreventive agents after hepatic resection or local ablative therapy for HCC. There were one and four randomised, controlled trials (RCTs) which evaluated the efficacy of polypropenoic acid and menatetrenone, respectively. All studies were conducted in Japan. One RCT showed the preventive effect of polypropenoic acid in lowering the incidence of HCC recurrence after hepatic resection or percutaneous ethanol injection, and this effect lasted up to 199 weeks after randomization (or 151 weeks after completion of retinoid administration). Four RCTs evaluated the preventive efficacy of menatetrenone on HCC recurrence after hepatic resection or local ablative therapy. The results of three studies, as well as the meta-analysis of all four studies, showed significantly better tumour recurrence-free survival. The beneficial effect on the overall survival was less definite.

CONCLUSION: There is evidence to suggest that chemopreventive therapy after partial hepatectomy or local ablative therapy is beneficial in prolonging disease-free survival, but the evidence is less for an effect on the overall survival. To confirm the beneficial role of vitamin A or K analogues in the chemoprevention of HCC further and larger randomised trials are now required. [Asian J Surg 2010;33(3):120–6]

Key Words: chemoprevention, hepatectomy, hepatocellular carcinoma, vitamin A, vitamin K
Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. The number of new cases is estimated to be 500,000–1,000,000 per year.1,2 Eighty per cent of HCC can be attributed to chronic hepatitis B and C infection.3 Of the therapies aimed at cure, liver resection and liver transplantation remain the best choices. Surgical resection with complete extirpation of the tumour gives the best chance of a cure for patients with HCC. Unfortunately, only 10–30% of patients with HCC are amenable to “curative” surgical resection at the time of diagnosis. Local ablative therapy may provide a “curative” outcome when indicated. It has been proved that the survival of local ablative therapy is similar to that of surgery when the tumours are small and limited in number.4 In most situations, liver resection or local ablation remains the available “curative” therapy for HCC. However, following “curative” treatment for HCC, 50–90% of postoperative deaths are the result of recurrent disease. Intrahepatic recurrence is frequently the only site of recurrence and it occurs in 68–96% of patients.5,7 Thus any therapy which can decrease or delay the incidence of intrahepatic recurrence will improve the results of liver resection. However, no neoadjuvant therapy/adjuvant therapy/chemopreventive therapy has been approved for HCC.8,9

Cancer chemoprevention is defined as the use of specific natural or synthetic chemical agents to reverse, suppress or prevent carcinogenic progression to invasive cancer. The goal of cancer chemoprevention is either to prevent or reverse the carcinogenic process in the initiation and promotion phases before the clinical development of cancer, and/or to delete premalignant or latent malignant clones from an organ by apoptosis or differentiation induction, in order to reduce the incidence of disease (and thus, ultimately, to reduce mortality). Over the past decade, several substances have been investigated as chemopreventive agents for HCC after “curative” treatments.10–12 Among these agents, vitamin analogues have been shown to be promising.12,13 Some studies have reported that vitamin analogues could reduce HCC recurrence after resection or local ablation and influence survival. Importantly, any new strategy must be tested in randomised, controlled trials (RCTs), including a control group without treatment. However, the results of these RCTs have been inconsistent. To review the current position of vitamin analogues in the chemoprevention of HCC after “curative” treatment, we performed a systematic review and integrated valid information by meta-analysis assessment.

Methods

Search strategy

A MEDLINE database, Embase, Cancerlit (National Cancer Institute), and CBM (Chinese Biomedical Database) search from 1990 to 2009 was performed to identify relevant articles using the keywords “hepatocellular carcinoma,” “vitamin analogue,” and “chemoprevention.” Additional papers were identified by a manual search of the references from the key articles.

Trial selection criteria

RCTs were included that compared HCC patients who were or were not given vitamin analogues as a supplement to curative treatments, including surgical resection, percutaneous ethanol injection, percutaneous thermal ablation and percutaneous radiofrequency ablation. Data were extracted independently by two authors and discrepancies resolved by consensus. Nonrandomised comparative studies, RCTs which were unpublished or published in languages other than English were excluded from the analysis.

Statistical methods

Fixed effect meta-analyses were conducted as described by Fleiss and Gross with the output showing the combined effect estimate [with 95% confidence interval (CI)] from each analysis, together with the results of the \( \chi^2 \) test for homogeneity. Heterogeneity among studies was defined at a \( p \) value less than 0.10. The effect measure of interest was the odds ratio (OR), which was calculated from tumour recurrence rate and cumulative survival in each study. Two independent authors extracted and input the relevant data into Stata/SE 8.0 for Windows (StataCorp, College Station, TX, USA) and performed the analysis.

Results

Oral administration of acyclic retinoids (vitamin A analogue), and menatetrenone (vitamin K2 analogue) have been tested as chemopreventive agents after hepatic resection or local ablative therapy for HCC. There were one and four RCTs which evaluated the efficacy of retinoids and menatetrenone, respectively. All the studies were conducted in Japan.12,13,15–18
Vitamin A
Retinoids are collectively termed as vitamin A (retinol) and its derivatives. Muto et al.12 randomised patients who had undergone curative resection or percutaneous ethanol injection for HCC to receive either polypropenoic acid (a synthetic acyclic retinoid) or placebo for 12 months. After a median follow-up of 38 months, only 12 patients (27%) treated with polypropenoic acid compared with 22 placebo-treated patients (49%) developed recurrence or new HCC. The proportion of patients who had second HCCs was significantly lower in the polypropenoic acid group than in the placebo group. Survival curves were estimated for overall survival and disease-free survival until either the primary tumour progressed or a second primary tumour appeared, and survival until a second primary HCC appeared. Compared with placebo, polypropenoic acid reduced the incidence of treatment failure in all three categories, but only the change in the incidence of second primary HCC was significant. Cox proportional-hazards analysis demonstrated that, as an independent factor, polypropenoic acid reduced the occurrence of second primary HCC (adjusted relative risk: 0.31; 95% CI: 0.12–0.78). Following this RCT, the study group continued to follow-up the patients by imaging and blood chemical analyses, and determined cumulative recurrence rates in the control group were 95.8%, 90.2% and 66.4%, respectively. There was no significant difference between the two groups. In the RCT of Hotta et al.,17 recurrence of HCC occurred in seven patients (33.3%) in the treatment group (n = 21) and 12 patients (50.0%) in the control group (n = 24) during mean observation periods of 19.5 and 16.5 months, respectively. Administration of vitamin K2 analogues was not an independent variable for recurrence on univariate analysis. The cumulative incidence of HCC recurrence did not differ between the two groups, and the cumulative overall survival rate tended to be higher in the treatment group, though this was not significant. In the RCT of Yoshiji et al.,18 a 4-year follow-up revealed that combination treatment with a vitamin K2 analogue (menatetrenone, 45 mg/day) and an angiotensin converting enzyme inhibitor (ACE-I) (perindopril, 4 mg/day) for 36–48 months after curative therapy for HCC markedly inhibited the cumulative recurrence of HCC, in association with suppression of the serum levels of the vascular endothelial growth factor. The cumulative recurrence rates of HCC in the combined treatment group (n = 25) at 1, 2 and 3 years were 12%, 28%, and 32%, respectively. The corresponding cumulative recurrence rates in the control group (n = 25) were 24%, 48% and 68%, respectively. The serum levels of lectin-reactive alpha-fetoprotein were also suppressed almost in parallel with vascular endothelial growth factor.

Vitamin K
Vitamin K is a fat-soluble vitamin that can be divided into naturally produced (vitamin K1 and K2) and chemically synthesized (vitamin K3) forms. Vitamin K inhibits the proliferation of tumour cells in vitro in decreasing potency from vitamin K3 to vitamin K1. Four RCTs evaluated the efficacy of menatetrenone (a vitamin K2 analogue) on HCC recurrence after hepatic resection or local ablative therapy (Table 113,16–18). Three studies showed significantly better tumour recurrence-free survival, while all the studies failed to show any significant effect on overall survival. Menatetrenone was studied in a pilot RCT by Mizuta et al.13 on 61 patients after curative hepatic resection or local ablative therapy. The results suggested that menatetrenone had a suppressive effect on the recurrence of HCC and a beneficial effect on survival.13 The cumulative recurrence rates in the treatment group were 12.5% at 1 year, 39.0% at 2 years, and 64.3% at 3 years; the corresponding recurrence rates in the control group were 55.2%, 83.2%, and 91.6%, respectively. The difference was significant. The 1-year, 2-year and 3-year cumulative overall survival rates for the treatment group were 100%, 96.6% and 87%, respectively, while the 1-year, 2-year, and 3-year cumulative survival rates for the control group were 96.4%, 80.9% and 64%, respectively. The difference was not significant. In the study of Kakizaki et al.,16 60 patients who were diagnosed to be free of HCC after radiofrequency ablation therapy or hepatic resection were randomly assigned to either the vitamin K3 group (n = 30) or the control group (n = 30). The results showed that vitamin K3 had a suppressive effect on the recurrence of HCC, but there was no significant difference in the overall survival rates. The chemopreventive effects of vitamin K2 may not be sufficient to prevent death from recurrent HCC. The cumulative recurrence-free rates in the vitamin K2 group were 92.3% at 1 year, 48.6% at 2 years and 38.8% at 3 years; those in the control group were 71.7%, 35.9% and 9.9%, respectively. The difference was significant. The cumulative overall survival rates in the vitamin K2 group were 100% at 1 year, 95.0% at 2 years and 77.5% at 3 years, and the corresponding rates in the control group were 95.8%, 90.2% and 66.4%, respectively. There was no significant difference between the two groups. In the RCT of Hotta et al.,17 recurrence of HCC occurred in seven patients (33.3%) in the treatment group (n = 21) and 12 patients (50.0%) in the control group (n = 24) during mean observation periods of 19.5 and 16.5 months, respectively. Administration of vitamin K2 analogues was not an independent variable for recurrence on univariate analysis. The cumulative incidence of HCC recurrence did not differ between the two groups, and the cumulative overall survival rate tended to be higher in the treatment group, though this was not significant.
Table 1. Data of the randomised, controlled studies on vitamin K<sub>2</sub>\textsuperscript{13,16–18}

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Viral status (HCV/HBV)</th>
<th>Tumour size (mm)</th>
<th>Tumour number (A/B)</th>
<th>Child-Pugh</th>
<th>Treatment (surgery/ local ablative therapy)</th>
<th>Recurrence rate (%) 1-yr</th>
<th>Recurrence rate (%) 2-yr</th>
<th>Recurrence rate (%) 3-yr</th>
<th>Survival (%) 1-yr</th>
<th>Survival (%) 2-yr</th>
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<td>Mizuta et al\textsuperscript{13}</td>
<td>32</td>
<td>28/3</td>
<td>17.7±5.1</td>
<td>1.5±0.9</td>
<td>26/6</td>
<td>1/31</td>
<td>12.5</td>
<td>39</td>
<td>64.3</td>
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<tr>
<td>Treatment</td>
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<td>26/2</td>
<td>19.4±6.9</td>
<td>1.5±0.7</td>
<td>22/7</td>
<td>3/26</td>
<td>55.2</td>
<td>83.2</td>
<td>91.6</td>
<td>96.4</td>
<td>80.9</td>
<td>64</td>
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<tr>
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<td>26/2</td>
<td>19.4±6.9</td>
<td>1.5±0.7</td>
<td>22/7</td>
<td>3/26</td>
<td>55.2</td>
<td>83.2</td>
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<td>30/0</td>
<td>20.4±11.6</td>
<td>19/11\textsuperscript{†}</td>
<td>22/8</td>
<td>4/26/0</td>
<td>7.7</td>
<td>51.4</td>
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<tr>
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<td>25.0±9.4</td>
<td>22/8\textsuperscript{†}</td>
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<td>7/23/0</td>
<td>28.3</td>
<td>64.1</td>
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<tr>
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<tr>
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<td>16/2</td>
<td>0/18</td>
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<td>44</td>
<td>61</td>
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<td>94.4</td>
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<tr>
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<td>1/3</td>
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<td>Control</td>
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<td>0/25</td>
<td>24</td>
<td>48</td>
<td>68</td>
<td>100</td>
<td>92</td>
<td>88</td>
</tr>
</tbody>
</table>

\textsuperscript{*}≤3 cm/3–5 cm; \textsuperscript{†}single/multiple. HCV = hepatitis C virus; HBV = hepatitis B virus.
These beneficial effects were not observed with single treatment using vitamin K₂ or ACE-I. The 1-year, 2-year and 3-year cumulative recurrence rates in the ACE-I only group (n = 19) were 16%, 37% and 52%, respectively. In the Vitamin K₂ only (n = 18) group, the 1-year, 2-year and 3-year cumulative recurrence rates were 22%, 44% and 61%, respectively. No significant differences in overall survival could be observed among all the groups during the follow-up period.

We conducted a fixed effect meta-analysis on these four studies to assess the effects of vitamin K₂ on tumour recurrence rates and patient survival. Figures 1,13,16–18 213,16–18 and 313,16,18 show the pooled OR of 1-year, 2-year and 3-year tumour recurrence after curative therapy in the vitamin K₂ treatment patients versus no treatment patients. Because of the lack of data on the 3-year recurrence in the study by Hotta et al.,17 the meta-analysis of the 3-year recurrence included only three studies. Our meta-analysis by fixed effect model showed that vitamin K₂ significantly decreased HCC recurrence rates at 1, 2 and 3 years after potentially curative therapy: 1-year tumour recurrence (OR: 0.330; 95% CI: 0.164–0.665, p = 0.002); 2-year tumour recurrence (OR: 0.427; 95% CI: 0.238–0.764, p = 0.004); 3-year tumour recurrence (OR: 0.303; 95% CI: 0.137–0.670, p = 0.003). The heterogeneity tests were not significant (Q = 5.570, p = 0.134; Q = 5.418, p = 0.144; Q = 3.125, p = 0.210, respectively).

Figures 413,16–18 and 513,16–18 show Forrest plots of vitamin K₂ on the 2- and 3-year survivals following curative therapy using the fixed effect model. As indicated, vitamin K₂ significantly increased the 2-year overall survival

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**Figure 1.** Effect of vitamin K₂ on 1-year tumour recurrence after curative therapy for hepatocellular carcinoma.13,16–18 Fixed effect model [total odds ratio (OR) = 0.330; 95% confidence interval: 0.164–0.665]. Test for heterogeneity: Q = 5.570 on 3 degrees of freedom (p = 0.134). Test for overall effect: Z = −3.106, p = 0.002.

**Figure 2.** Effect of vitamin K₂ on 2-year tumour recurrence after curative therapy for hepatocellular carcinoma.13,16–18 Fixed effect model [total odds ratio (OR) = 0.427; 95% confidence interval: 0.238–0.764]. Test for heterogeneity: Q = 5.418 on 3 degrees of freedom (p = 0.144). Test for overall effect: Z = −2.867, p = 0.004.

**Figure 3.** Effect of vitamin K₂ on 3-year tumour recurrence after curative therapy for hepatocellular carcinoma.13,16,18 Fixed effect model [total odds ratio (OR) = 0.303; 95% confidence interval: 0.137–0.672]. Test for heterogeneity: Q = 3.125 on 2 degrees of freedom (p = 0.210). Test for overall effect: Z = −2.937, p = 0.003.

**Figure 4.** Effect of vitamin K₂ on 2-year survival after curative therapy for hepatocellular carcinoma.13,16–18 Fixed effect model [total odds ratio (OR) = 0.286; 95% confidence interval: 0.089–0.916]. Test for heterogeneity: Q = 1.615 on 3 degrees of freedom (p = 0.210). Test for overall effect: Z = −2.108, p = 0.035.
OR: 0.286; 95% CI: 0.089–0.916, \( p = 0.035 \). Although vitamin K2 had no significant effect on the 3-year survival \( (p = 0.055) \), there was a tendency for the 3-year survival to be increased \( (OR: 0.467; 95\% \text{ CI: } 0.214–1.016) \). The heterogeneity tests were not significant \( \left( Q = 1.615, p = 0.656; Q = 1.353, p = 0.508 \right) \).

**Discussion**

Recurrences of HCC after “curative” treatment can be differentiated into two types, early and late recurrences.7,19,20 Early recurrent HCCs are the result of residual hepatic tumours presumably left behind after a R0 resection.20,21 In contrast, late recurrent HCCs (otherwise termed de novo tumour formation) are the results of the underlying procarcinogenic liver diseases or viruses, with the highest risk in patients with hepatitis B or C.22–24 HCC commonly arises from chronic viral hepatitis. The livers in these patients are likely to harbour multiple and independent clones of premalignant cells. When these clones are further exposed to continuous carcinogetic insults, a unicentric or multicentric carcinogenesis follows. Intrahepatic recurrence can either represent de novo tumour formation in a chronic hepatic liver, or intrahepatic metastasis of a clonally identical neoplasm. No matter how the recurrence occurs, it is generally believed that recurrences arise not because of inadequate resection, but because of pre-existing microscopic tumour foci that are undetected by imaging modalities, or because malignant cells have already disseminated during surgical manipulation, or because new tumours form. To prevent tumour recurrence after curative treatments, eradication and/or inhibition of such clones of malignant cells is essential. Thus any therapy which can decrease or delay the incidence of intrahepatic recurrence will improve the results of “curative” treatment.

*In vitro*, vitamin analogues have been shown to inhibit experimental hepatocarcinogenesis and induce differentiation and apoptosis of human HCC-derived cell lines.25,26 The chemopreventive effect of retinoids may be conferred through the deletion of malignant clones before minimal residual disease becomes clinically detectable after liver resection.27–29 However, the mechanism of vitamin K action is not fully understood. Mastumoto et al30 reported that vitamin K2 inhibited the proliferation of HCC cells *in vitro* through cell cycle arrest at the G1 phase and apoptosis. Ozaki et al31 reported that vitamin K2 inhibited the growth of HCC cells via suppression of cyclin D1 expression through the IKK/IkappaB/nuclear factor-kappaB pathway and might therefore be useful for treatment of HCC.

Clinical trials have showed that vitamin analogues had an influence on recurrence and survival of HCC after curative treatments. However, none of them could reach a firm conclusion. Our systematic review found one and four RCTs which evaluated the efficacy of polyprenoic acid and menatetrenone, respectively. All the studies were conducted in Japan. One RCT showed the preventive effect of polyprenoic acid in reducing the incidence of HCC recurrence after hepatic resection or percutaneous ethanol injection, and this effect lasted up to 199 weeks after randomization (or 151 weeks after completion of retinoid administration). Four RCTs evaluated the preventive efficacy of menatetrenone on HCC recurrence after hepatic resection or local ablative therapy. The results of three studies, as well as the meta-analysis of all four studies, showed significantly better tumour recurrence-free survival. The beneficial effect on the overall survival is less definite. Thus there is evidence to suggest that chemopreventive therapy after hepatectomy or local ablative therapy is beneficial to prolong disease-free survival, but the evidence is less in terms of overall survival. To confirm the beneficial role of vitamin A or K analogues in the chemoprevention of HCC further, larger randomised trials are now required.

**Acknowledgements**

This study was supported by the grants from Ministry of Science and Technology Key Program (2008ZX10002-025),
National Natural Science Foundation (No. 30873352), Shanghai Science and Technology Committee (No. 07JC14066), and Shanghai Education Committee of Shuguang Plan (No. 05SG39).

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