

# Ursodiol Use Is Possibly Associated with Lower Incidence of Hepatocellular Carcinoma in Hepatitis C Virus–Associated Liver Cirrhosis

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## Abstract

In a previous study of patients with hepatitis C virus (HCV)–associated liver cirrhosis (HCV-LC), we showed that increased liver inflammation, as assessed by higher serum alanine aminotransferase (ALT), was associated with increased risk for the development of hepatocellular carcinoma (HCC). This suggested that suppression of inflammation might inhibit HCC development in HCV-LC. Several agents have been suggested to possess chemopreventive potential against the development of HCC in chronic HCV-associated liver disease, including herbal medicines, such as Stronger-Neo-Minophagen C (glycyrrhizin) and Sho-saiko-to (TJ-9). Ursodiol [ursodeoxycholic acid (UDCA)], a bile acid widely used to treat cholestatic liver diseases, also possesses anti-inflammatory properties in liver disease. We hypothesized that suppression of liver inflammation, as assessed by decreases in serum ALT, might inhibit HCC occurrence in patients with HCV-LC. In this study, the preventive effect of UDCA on HCC was examined in patients with early-stage HCV-LC. One hundred two patients with HCV-LC (Child stage A) were treated with anti-inflammatory drugs, Stronger-Neo-Minophagen C, Sho-saiko-to, or UDCA, with the goal of lowering the average serum ALT level to <80 IU. If the

average ALT level did not remain <80 IU after treatment with one agent, multiagent therapy was initiated. The patients were followed up for >5 years and were retrospectively subdivided into two groups: 56 UDCA users (group A) and 46 UDCA nonusers (group B). The mean  $\pm$  SD dosage of UDCA administered in group A was 473.7  $\pm$  183.0 mg/d. The average duration of UDCA administration in group A was 37.3  $\pm$  15.9 months over the 5-year study period. The cumulative incidence of HCC was recorded. The 5-year incidence of HCC in group A was 17.9% (10 of 56) and was significantly lower than that in group B (39.1%, 18 of 46;  $P = 0.025$ ). The risk for HCC incidence, calculated by a logistic regression model, showed that the administration of UDCA significantly decreased hepatocarcinogenesis ( $P = 0.036$ ). The herbal medicines used were comparable in dosage and treatment duration in the UDCA and non-UDCA groups. In conclusion, UDCA might prevent HCC development in HCV-LC. Interestingly, because the serum ALT trends over time were nearly the same in both groups, the chemopreventive effectiveness of UDCA was not accompanied by greater reductions in ALT compared with the UDCA nonusers. (Cancer Epidemiol Biomarkers Prev 2005;14(1):164–9)

## Introduction

In a previous study of patients with hepatitis C virus (HCV) and liver cirrhosis, we showed the possibility that increased liver inflammation, as assessed by higher serum alanine aminotransferase (ALT), was associated with increased risk for the development of hepatocellular carcinoma (HCC; refs. 1, 2). This suggested to us that suppression of inflammation might inhibit HCC development in HCV-LC. Stronger-Neo-Minophagen C (SNMC, glycyrrhizin; ref. 3) and Sho-saiko-to (TJ-9; ref. 4) are herbal medicines with anti-inflammatory properties. These agents have been suggested to possess chemopreventive potential against the development of HCC in chronic HCV-associated liver disease (5, 6). Ursodiol is used worldwide for the treatment of cholestatic liver diseases (7–9) and is also anti-inflammatory (10). In addition,

recent studies suggest that ursodiol may prevent ulcerative colitis–associated colon cancer (11, 12), another gastrointestinal malignancy arising in an inflammatory background. However, the action of ursodiol in hepatocarcinogenesis remains to be explored.

In addition to the anti-inflammatory properties of SNMC, long-term administration of SNMC in the treatment of chronic hepatitis C seemed to be effective in preventing liver carcinogenesis in a retrospective study by Arase et al. (5). In a prospective study, Oka et al. (6) showed that the cumulative incidence of HCC was significantly decreased in the Sho-saiko-to-administered group compared with the nonadministered group in patients with HCV-associated liver cirrhosis (HCV-LC). Moreover, Yano et al. (13) showed that Sho-saiko-to inhibited proliferation of a human HCC cell line *in vitro* by inducing apoptosis and cell cycle arrest in the G<sub>0</sub>–G<sub>1</sub> phase. Recently, Sakaida et al. (14) showed that Sho-saiko-to prevented liver fibrosis and enzyme-altered lesions in a model of rat liver cirrhosis. Because ursodeoxycholic acid (UDCA; ref. 10), SNMC (3), and Sho-saiko-to (4) exert anti-inflammatory actions in liver disease, we treated patients with HCV liver disease and early cirrhosis with one or more of these agents. Our goal was to suppress liver inflammation by treatment with these agents, escalating from monotherapy to two-drug therapy or, if needed, three-drug therapy to reduce serum ALT levels to <80 IU. In this study, we retrospectively examined the incidence of HCC in these

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patients. To assess the effect of UDCA on the incidence of HCC, we divided these patients into those receiving UDCA and those not receiving UDCA and examined their respective incidences of HCC.

## Materials and Methods

**Study Population.** One hundred and two consecutive posthepatic, cirrhotic patients with Child stage A (15) HCV-LC who were either HCV antibody positive (C-100 antibody or the second-generation tests) or HCV-RNA positive (PCR analysis) were studied. (In 95 patients, the presence of HCV-RNA was confirmed.) The study was confined to patients with Child stage A (15) HCV-LC, because long-term follow-up was required to observe the occurrence of HCC. Habitual drinkers defined as those who drank >40 g ethanol daily or drank >3 days per week were excluded from the study. Patients who had a history of heavy ethanol intake in the past were also excluded from this study. Patients who had been exposed previously to hepatitis B virus and patients who were positive for hepatitis B surface antigen, hepatitis B core antibody, or hepatitis B surface antibody also were excluded from this study, as were patients who had undergone prior hepatic resection for HCC. Patients with other causes of chronic liver disease, including hemochromatosis, Wilson's disease,  $\alpha_1$ -antitrypsin deficiency, and drug injury, were also excluded. All patients were hospitalized at Kanagawa Cancer Center Hospital or Kumamoto University Hospital between July 17, 1985 and January 31, 1997. The diagnosis of cirrhosis was made by liver biopsy in all patients.

The patients were treated with anti-inflammatory drugs, such as SNMC (3), Sho-saiko-to (4), or UDCA (10), aiming at lowering their serum ALT levels to <80 IU on average following the strategy shown in Table 1. If the average ALT level did not remain <80 IU after treatment with one agent, multiagent therapy was also done. The patients were followed up for >5 years and retrospectively subdivided into two groups: 56 UDCA users (group A) and 46 UDCA nonusers (group B). The mean  $\pm$  SD dosage of UDCA administered to the UDCA users was  $473.7 \pm 183.0$  mg/d. The average duration of UDCA administration in group A was  $37.3 \pm 15.9$  months during the 5-year observation period. The cumulative incidence of HCC and ALT trends over time were observed in both groups. Moreover, univariate and multivariate analyses of factors contributing to the development of HCC were analyzed. The characteristics of both groups of patients at the beginning of the study are shown in Table 2. This study was

done in accordance with the Declaration of Helsinki: all patients provided informed consent. Moreover, this study was approved by the institutional review committees of both Kanagawa Cancer Center Hospital and Kumamoto University.

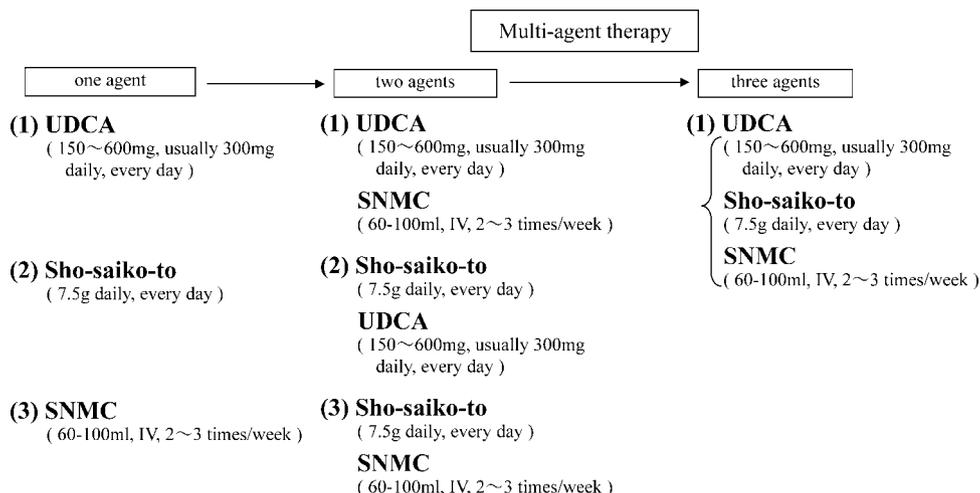
**Follow-up Study.** On admission, a liver biopsy was done, and ultrasonography and either computed tomography scans or magnetic resonance imaging were carried out to exclude any patients who had already developed HCC. After the patients were discharged, ultrasonographic examinations for the purpose of detecting small HCC nodules were conducted every 3 months and computed tomography scans or magnetic resonance imaging every 6 months.

The criteria for a suspicious HCC lesion on ultrasonography, computed tomography scan, or magnetic resonance imaging were as follows: On ultrasonography, a HCC nodule usually appears as a small, solitary, strongly hypoechoic mass lesion measuring 5 to 10 mm in the greatest dimension, although it sometimes appears as a bright loop lesion. On computed tomography scans, a HCC nodule appears as a low-density mass lesion of small size on an unenhanced image and is usually enhanced in the early phase after a bolus injection of contrast medium. On unenhanced magnetic resonance imaging, a HCC nodule appears as a small mass lesion that is hypointense on a T1-weighted image and moderately hyperintense on a T2-weighted image.

Serum  $\alpha$ -fetoprotein levels (RIA, reference range, <15.0 ng/mL) were measured, and biochemical tests, including serum albumin (3.8-5.3 g/dL), serum ALT (5-40 IU), serum aspartate aminotransferase (10-30 IU), thymol turbidity test (0.0-5.0 kIU), zinc turbidity test (4.0-12.0 kIU), and peripheral blood counts were done every month. The patients were followed carefully during the long-term observation period, and an endoscopic examination of esophageal varices was done every 6 months in all patients. If esophageal varices with signs of redness were found, then either sclerotherapy or ligation was carried out to avoid esophageal bleeding.

If the ultrasonography, computed tomography, magnetic resonance imaging, or  $\alpha$ -fetoprotein studies suggested the development of HCC, then further imaging examinations, such as helical dynamic computed tomography scans, lipiodol computed tomography (16), angiography, and biopsy of the tumor, were carried out to confirm the diagnosis of HCC. Ultimately, HCC was confirmed histologically in 25 of 28 cases by examination of biopsy specimens. In particular, angiography and lipiodol infusion into the hepatic artery were done in all patients with suspected development of HCC. All patients were followed for >5 years.

**Table 1. Strategy to maintain average serum ALT level <80 IU**



**Table 2. Characteristics of UDCA users and nonusers in HCV-LC patients (Child A) at the beginning of the study**

Characteristics	Users	Nonusers	P
No. patients	56	46	
Age, y (mean $\pm$ SD)	60.3 $\pm$ 7.6	60.1 $\pm$ 6.1	0.664
Gender (male/female)	27/29	23/23	1.000*
Child classification (A/B)	56/0	46/0	
Liver function (mean $\pm$ SD)			
Albumin (g/dL)	3.92 $\pm$ 0.38	3.85 $\pm$ 0.42	0.096
Thymol turbidity test (units)	9.1 $\pm$ 5.5	8.9 $\pm$ 4.8	0.845
ALT (IU)	113.4 $\pm$ 67.2	118.1 $\pm$ 65.3	0.723
Aspartate aminotransferase (IU)	93.4 $\pm$ 43.8	104.5 $\pm$ 56.5	0.268
$\gamma$ -Glutamyl transpeptidase (IU)	69.3 $\pm$ 42.9	94.7 $\pm$ 83.4	0.056
Prothrombin time (s)	13.4 $\pm$ 0.6	13.3 $\pm$ 1.1	0.704
Platelet counts (per mm <sup>3</sup> $\times$ 10 <sup>4</sup> )	11.3 $\pm$ 3.8	10.4 $\pm$ 3.1	0.381
$\alpha$ -Fetoprotein (ng/mL)	55.8 $\pm$ 76.0	40.8 $\pm$ 44.9	0.807
HCV-RNA level (kIU/mL)	313.4 $\pm$ 293.0	302.1 $\pm$ 261.4	0.812
Body mass index, kg/m <sup>2</sup>	24.1 $\pm$ 3.0	23.6 $\pm$ 3.0	0.389
Brinkman index <sup>†</sup>	191.3 $\pm$ 420.4	159.3 $\pm$ 289.8	0.994

\*Fisher's exact test; all others Student's *t* test.

<sup>†</sup>The sum of the number of cigarettes smoked per day multiplied by years of smoking.

**Therapeutic Procedures.** SNMC (3) and Sho-saiko-to (4) are herbal medicines used throughout Japan to treat chronic viral liver diseases, and they work by reducing inflammatory processes (17, 18) and controlling ALT levels. UDCA (10) and protoporphyrin are also known to suppress elevated ALT levels in some cases. Suzuki et al. (3) showed a significant decrease in serum ALT levels by i.v. injection of SNMC in patients with chronic hepatitis, and Hirayama et al. (4) showed a significant decrease in serum ALT levels by p.o. administration of Sho-saiko-to to patients with chronic active hepatitis. Moreover, Bellentani et al. (10) showed a significant decrease in serum ALT levels by long-term p.o. administration of UDCA treatment of patients with chronic hepatitis. Furthermore, we recently showed a significant decrease in serum ALT levels by combination therapy with these drugs in patients with HCV-associated cirrhosis (19).

In all the patients who were enrolled in this study, we made an intensive effort to maintain an average serum ALT level of <80 IU (Table 1). We did this initially by administering only one agent to every patient (monotherapy): UCDA (150-600 mg, usually 300 mg/d), Sho-saiko-to (7.5 g/d), or SNMC (60-100 mL, twice to thrice a week). When the average ALT level was maintained successfully at <80 IU by one agent, monotherapy of the drug was continued. Each drug was taken for 2 to 3 months. If the average ALT level did not remain <80 IU with treatment by one agent, then the following combinations of two drugs were administered: (a) UDCA (same dosage as in monotherapy, daily) and SNMC (same dosage as in monotherapy, twice to thrice a week), (b) Sho-saiko-to (same dosage as in monotherapy, daily) and UCDA, and (c) Sho-saiko-to and SNMC. The treatment with the two drugs was continued when the ALT level dropped to <80 IU. If the average ALT level could not be maintained at <80 IU with a combination of two agents, then we administered a combination of three agents: Sho-saiko-to (same dosage as in monotherapy, daily), UDCA (same dosage as in monotherapy, daily), and SNMC (same dosage as in monotherapy, twice to thrice a week). Each trial of examining the effectiveness of a drug regimen was done for 2 to 3 months. By using this treatment plan, we tried to find the best therapeutic regimen for each patient. This strategy aiming at an ALT level of <80 IU was done uniformly across all patients. When ALT levels could not be maintained at <80 IU by any means, the regimen that most effectively lowered the ALT level was adopted. This regimen was adopted because the ALT levels in many patients could not be maintained at <80 IU by using only one drug (monotherapy). No other drugs, which were thought to influence HCC development or inflammation, such as IFN- $\alpha$ , ribavirin, or steroids, were used in any of the patients. The number of days from histologic diagnosis

of cirrhosis to detection of HCC by imaging modalities was recorded in all patients who developed HCC.

**Statistical Analysis.** Differences in the means of variables were tested by the unpaired Student's *t* test. Frequency analysis was done with Fisher's exact test. The candidate of a variable was screened by univariate analysis by setting the incidence of HCC during the 5-year study period as the dependent variable.

Candidate variables that showed statistical significance ( $P < 0.05$ ) in the univariate analysis were subsequently included in a multivariate analysis using the rate of likelihood of the forward stepwise logistic regression procedure. Confounding was not observed [tested by the Breslow-Day test and Mantel-Haenszel test (20)].

Longitudinal change in ALT was tested by the repeated-measures ANOVA.  $P < 0.05$  was considered to indicate statistical significance. All tests were two tailed. All statistical analyses were done on a personal computer with the Statistical Package for Windows (version 11.5, Japanese edition).

## Results

The cumulative 5-year incidence of HCC in the patients treated with UDCA was 17.9% (10 of 56) and was significantly lower than that in patients not treated with UDCA (39.1%, 18 of 46;  $P = 0.025$ ; Table 3; Fig. 1). The serum ALT trends over time in both groups of patients are shown in Fig. 2. They are nearly the same in both groups during the 5-year study period. Moreover, the distribution of the drugs administered was nearly the same in both groups, except for the administration of UDCA, as shown in Table 4. The mean  $\pm$  SD dosage of UDCA administered in UDCA users was 473.7  $\pm$  183.0 mg/d. The average duration of UDCA administration over the 5-year observation period in those patients was 37.3  $\pm$  15.9 months.

The mean  $\pm$  SD average duration of SNMC administration was 31.6  $\pm$  25.5 months in UDCA users and 26.2  $\pm$  20.6 months in UDCA nonusers (NS). The mean  $\pm$  SD average duration of Sho-saiko-to was 20.2  $\pm$  22.4 months in UDCA users and 19.0  $\pm$  17.2 months in UDCA nonusers (NS). Moreover, the average durations of combination therapy of UDCA and SNMC, UDCA and Sho-saiko-to, and SNMC and Sho-saiko-to were 31.6  $\pm$  25.5, 20.2  $\pm$  22.4, and 21.2  $\pm$  14.8 months, respectively. The average duration of combination therapy of UDCA, SNMC, and Sho-saiko-to was 34.1  $\pm$  16.7 months.

In the univariate analyses, the following three risk factors significantly affected the cumulative rate of incidence of HCC in all patients: gender ( $P < 0.02$ ), ALT (four or more consecutive annual averages of ALT > 80 IU;  $P < 0.05$ ), and

**Table 3. Number (%) of patients who developed HCC within 5 years in UDCA users and nonusers in HCV-associated LC patients**

	HCC	No HCC	Total
UDCA users	10 (17.9)	46 (82.1)	56
UDCA nonusers	18 (39.1)	28 (60.9)	46

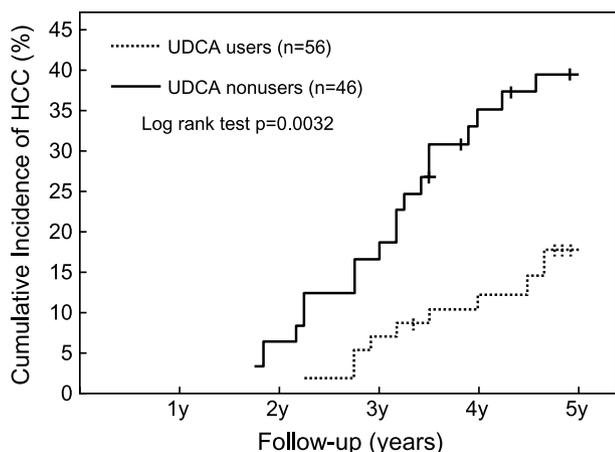
NOTE:  $P = 0.025$ , Fisher's exact test.

UDCA administration ( $P < 0.05$ ; Table 5). A logistic regression model with these three variables was constructed. Two of the factors [i.e., gender ( $P < 0.02$ ) and UDCA ( $P < 0.05$ )] were significant (Table 6). The odds ratio of the non-UDCA users was 2.736 compared with the UDCA users.

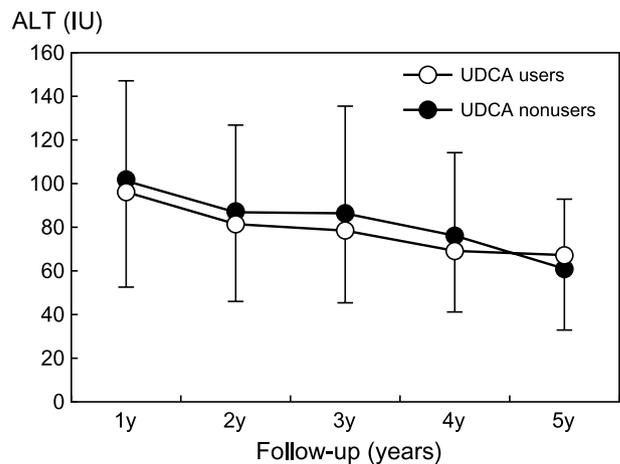
## Discussion

Concerning the preventive effect of UDCA against cancer, the association between the use of UDCA and decreased incidence of colorectal cancer has been well documented. Several studies have suggested that patients with both ulcerative colitis and primary sclerosing cholangitis have an even greater risk of colorectal cancer than patients with ulcerative colitis alone (21-29). UDCA is often used as therapy for primary sclerosing cholangitis. Tung et al. (11) reported evidence that therapy with UDCA might be effective in reducing the risk of colorectal cancer in these patients. Recently, Pardi et al. (12) showed that UDCA significantly decreases the risk of developing colorectal dysplasia or cancer in patients with ulcerative colitis and primary sclerosing cholangitis. Moreover, Larson et al. (30) have reported the efficacy of UDCA in preventing metachronous adenomatous colorectal polyps. Although not significant, there was a 26% decrease in both the number and the size of recurrent polyps in patients who had undergone resection for colorectal cancer and were treated with UDCA. Recently, Serfaty et al. (31) also showed that prolonged administration of UDCA significantly decreased the recurrence of colorectal adenoma following endoscopic resection in the patients with primary biliary cirrhosis.

In animal experiments, Earnest et al. (32) showed that in the azoxymethane model of colonic carcinogenesis in rats dietary supplementation with UDCA (0.4%) significantly decreased tumor incidence by 66% and totally abolished the development of colonic adenocarcinomas.



**Figure 1.** Cumulative incidence of HCC in UDCA users and nonusers in patients with HCV-LC (Child A stage).



**Figure 2.** Serum ALT trends over time in UDCA users and nonusers in patients with HCV-LC (Child A stage). Repeated-measures ANOVA was not significant ( $P = 0.3592$ ) between UDCA users and nonusers.

Thus, it is postulated that UDCA might prevent the development of cancer from a precancerous lesion, such as adenomatous polyp. It is feasible to think that the same mechanisms might be acting in the prevention by UDCA of the development of HCC from HCV-LC, which is thought to be a precancerous state.

With respect to the preventive effect of administration of UDCA on the development of HCC, only one animal study has been reported. Oyama et al. (33) showed that UDCA suppressed hepatocarcinogenesis initiated by diethylnitrosamine in rats. The present study is the first report suggesting a preventive effect of UDCA against HCC in patients with liver disease. All of the clinical studies aimed at demonstrating a preventive effect of UDCA on hepatocarcinogenesis in patients with viral liver disease in Japan have failed.<sup>4</sup> The reason for these failures has been speculated to be the existence of sustained inflammation, which was shown to be a potent promoting factor for HCC development in our previous study (2, 34). Another reason for the failure may have been the selection of the patient group; previous studies examined patients with HCV-associated chronic hepatitis, whereas we examined patients with HCV-LC, who belonged to the highest-risk group for HCC development. In this study, we clearly showed a possible beneficial effect of UDCA administration for the prevention of HCC development in HCV-LC. The 5-year incidence of HCC was only 17.9% in UDCA users, less than half that in the nonuser group (39.1%). The incidence of HCC in UDCA users was only 3.6% per year and was far lower than the generally accepted rate of HCC development from HCV-LC in Japan (6-8% per year; refs. 35, 36). The difference in incidence became prominent 4 years into the study.

Additionally, the mean  $\pm$  SD dosage of UDCA administration in the UDCA users in this study was  $473.7 \pm 183.0$  mg/d and was nearly the same dosage as usually used in Japan in patients with virally induced chronic liver diseases (150-600 mg, usually 300 mg/d) and in those with primary biliary cirrhosis (600 mg/d).

Moreover, it was also shown in this study that the serum ALT trends over time were nearly the same in the patients treated and not treated with UDCA. This was the result of our

<sup>4</sup>Unpublished data.

**Table 4. Drugs administered to UDCA users and nonusers in HCV-LC patients**

	No drug	SNMC	Sho-saiko-to	SNMC + Sho-saiko-to	Total
UDCA users	10	21	8	17	56
UDCA nonusers	6	19	10	11	46

NOTE:  $P = 0.651$ , Fisher's exact test.

efforts to alleviate the inflammation in study patients using anti-inflammatory agents, including multiagent therapy. The data on ALT trends over time suggest that ALT levels did not reflect the effects of UDCA compared with non-UDCA treatment on HCC development.

Our data also showed that the cumulative incidence of HCC was nearly the same in the UDCA user and nonuser groups until 3 years in the study and that the difference became prominent after 4 years and significant at 5 years. These data suggest that UDCA might inhibit the initiation phase of human HCC development or possibly tumor growth by preventing small initiated tumors from growing to detectable sizes. On this point, Wali et al. (37) recently showed that UDCA might inhibit both initiation and postinitiation (promotion/progression) phases in azoxymethane-induced colonic cancer development in rats. They showed that UDCA administration in the initiation phase inhibited the formation of aberrant crypt foci (precancerous lesions) in rats.

In this study, sustained inflammation, which we showed to be a potent accelerating factor in the promotion of HCC development, may have been alleviated by anti-inflammatory therapy, including multiagent therapy, and the inhibitory effects of UDCA on HCC development might have been manifested as a result.

Many reports have proposed mechanisms for the potential of UDCA to prevent cancer development chiefly in the field of colon cancer. The first and most common concept is a change in the bile acid pool by the administration of UDCA; the use of UDCA reduces the fecal concentration of the secondary bile acid, deoxycholic acid, which has numerous adverse effects on the colonic epithelium and has been implicated as a tumor promoter (38-40). The second possible mechanism is that UDCA inhibits cell proliferation. Martinez et al. (41) showed that incubation of tumor cells with UDCA inhibited cell proliferation in several different tumor cell lines. Recently,

**Table 5. Risk for HCC in univariate model**

Items	Risk/control	$P$	Odds ratio (95% confidence interval)
Age, y	$\geq 66$ / $<66$	0.263	1.740 (0.659-4.591)
Gender	Male/female	0.013	3.185 (1.276-7.949)
ALT, y	$\geq 4$ / $<4$	0.034	2.821 (1.080-7.369)
UDCA	Non-UDCA/ UDCA	0.032	2.630 (1.084-6.379)
Body mass index, kg/m <sup>2</sup>	$\geq 25$ / $<25$	0.307	0.612 (0.239-1.569)
Smoking	Smoker/ nonsmoker	0.858	0.919 (0.364-2.319)
Albumin		0.424	0.567 (0.141-2.277)
Platelets		0.247	1.090 (0.942-1.261)

NOTE: Annual average of serum ALT level was calculated every year in all the patients:  $\geq 4$  years, annual average ALT level was persistently high ( $\geq 80$  IU) for  $\geq 4$  years;  $<4$  years, annual average ALT level was persistently high ( $\geq 80$  IU) for  $<4$  years. Non-UDCA, UDCA was not administered; UDCA, UDCA was administered in the study period.

**Table 6. Risk of HCC by logistic regression model**

Items	Risk/control	$P$	Odds ratio (95% confidence interval)
Gender	Male/female	0.018	3.166 (1.216-8.243)
ALT, y	$\geq 4$ / $<4$	0.066	2.600 (0.938-7.209)
UDCA	Non-UDCA/ UDCA	0.036	2.736 (1.070-6.992)

NOTE: Annual average of serum ALT level was calculated every year in all the patients:  $\geq 4$  years, annual average ALT level was persistently high ( $\geq 80$  IU) for  $\geq 4$  years;  $<4$  years, annual average ALT level was persistently high ( $\geq 80$  IU) for  $<4$  years. Non-UDCA, UDCA was not administered; UDCA, UDCA was administered in the study period.

using anti-Ki-67 antibodies on colonic biopsies, Serfaty et al. (31) showed a significant reduction in colon epithelial cell proliferation in patients treated with UDCA. We showed previously (42) the importance of hepatocellular proliferation in the development of HCC in patients with HCV-LC using bromodeoxyuridine uptake by hepatocytes in liver biopsy specimens *in vitro*. If UDCA also inhibits hepatocellular proliferation, the incidence of HCC would be expected to be decreased in the UDCA-treated LC patients.

The third possible mechanism is the inhibitory effect of UDCA on the cyclooxygenase-2 gene expression: Khare et al. (43) showed that UDCA inhibited cyclooxygenase-2 expression, which is an important factor in tumor progression, in azoxymethane-induced colonic tumors. They (44) recently showed in the same model that UDCA inhibited cyclooxygenase-2 induction by *ras*-dependent and *ras*-independent mechanisms. They also showed that UDCA decreased cyclooxygenase-2 protein and mRNA levels in tumors with normal *ras* activity.

Then, what mechanisms are likely to account for the preventive efficacy of UDCA on hepatocarcinogenesis? Oyama et al. (33) examined the effect of UDCA administration on the number of liver tumors and percentages of the glutathione *S*-transferase-positive hepatocytes induced by diethylnitrosamine in rats and showed that both were significantly reduced by UDCA treatment. They also showed that apoptosis was increased in both glutathione *S*-transferase-positive cells and HCC cells. They concluded that UDCA treatment reduces hepatocarcinogenesis by inducing apoptosis of "initiated hepatocytes" as well as inhibiting proliferation. Our present data showed that the difference in the cumulative incidence of HCC becomes evident 4 years after the administration of UDCA, suggesting the possibility that increased apoptosis of initiated hepatocytes might have been elicited by UDCA administration in our UDCA-treated patients, because it would take about 3 years for a HCC to reach 2 cm in diameter from a size of 0 cm (45).

In conclusion, our results suggested a preventive effect of UDCA administration on HCC development in HCV-LC.

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