Oral Zinc Supplementation Decreases the Risk of HCC Development in Patients With HCV Eradicated by DAA

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We have reported that the plasma zinc concentration gradually decreases with the progression of fibrosis and is related to hepatocellular carcinoma (HCC) development. The aim of this study was to examine the impact of the zinc concentration on HCC development (study 1) and the relationship between zinc intake and HCC development (study 2) in patients with hepatitis C virus (HCV) eradicated by direct-acting antivirals (DAAs). A total of 599 sustained virological response (SVR) patients treated with DAAs without a history of HCC were retrospectively analyzed in this study. Eighty patients received supplemental zinc (Zn treatment group), and 519 patients did not receive zinc (no Zn treatment group). In study 1, the cumulative incidence rate of HCC was compared between the Zn treatment group and the no Zn treatment group. In study 2, the risk factors for HCC development were examined in the no Zn treatment group. In study 1, in the Zn treatment group, HCC did not develop during follow-up, and the cumulative risk of HCC was significantly lower in the Zn treatment group than in the no Zn treatment group (P = 0.048). In study 2, the 1-year and 3-year cumulative incidence rates of HCC were 1.8% and 5.6%, respectively. The risk factors for HCC identified by multivariate analysis were male sex, cirrhosis, low platelet count before treatment, and low serum zinc concentration 12 weeks after the end of DAA therapy. *Conclusion:* The Zn concentration is related to HCC development in patients with HCV eradicated by DAA therapy. Oral zinc supplementation is recommended as a means of suppressing HCC development in patients who have achieved SVR. (*Hepatology Communications* 2021;5:2001-2008).

Hepatitis C virus (HCV) infection, which affects an estimated 150 million individuals, is a major cause of chronic hepatitis and can lead to cirrhosis and hepatocellular carcinoma (HCC).[1] The recent introduction of direct-acting antivirals (DAAs) has resulted in the achievement of a sustained virological response (SVR) in over 90% of treated patients, with an excellent safety profile.[2,3] This high rate of achieving an SVR suggests the possibility of a significant reduction in the incidence of HCC, as was observed after the introduction of interferon (IFN)–based regimens. The risk of HCC development was also found to be significantly reduced in patients who achieved an SVR after treatment with DAAs.[4] Despite this favorable effect, it is important to investigate the development of HCC after SVR because patients treated with DAA-based regimens are generally older and have more advanced liver disease than those treated with IFN-based regimens, and significant independent predictors of incident HCC

are older age, male sex, low platelet count, high alpha-fetoprotein (AFP) level, and cirrhosis.[5,6] Indeed, these factors are useful for identifying patients at a high risk of developing HCC, but they cannot be modified to further reduce the incidence of HCC.

Zinc, an essential trace element, has been reported to play various pivotal roles in the human body. Notably, in the liver, zinc is required for the activation of many enzymes, such as ornithine transcarbamylase and glutamate dehydrogenase, which are used in the urea cycle or glutamine synthetase cycle. Superoxide dismutase (SOD), which requires zinc for its activation, has strong antioxidant activity. These liver enzymes do not work well when zinc deficiency occurs. SOD is a major antioxidant enzyme and does not function when there is a low level of zinc.[7] The deactivation of SOD increases the formation of reactive oxygen species (ROS), overwhelming the body's antioxidant protection mechanisms and subsequently inducing DNA damage, protein modification and other effects, all of which are characteristics of many different diseases including cancer. A lack of zinc has been speculated to foster cancer development.

In 1956, Vallee et al. first reported the occurrence of marked hypozincemia in patients with severe cirrhosis,[8] which was subsequently confirmed by many investigators.[9,10] However, few clinical trials have evaluated the effects of zinc supplementation on clinical outcomes in patients with chronic liver diseases (CLDs). As zinc helps activate some enzymes in the urea cycle, many investigators have focused on improving hepatic encephalopathy through the administration of supplemental zinc. Reding et al. first reported that oral zinc supplementation improved hepatic encephalopathy in 22 patients with cirrhosis in a double-blind randomized trial. The effect of taking 600 mg zinc acetate per day for 7 days was evaluated in that study.[11] Katayama et al. also showed the importance of zinc supplementation; in that study, 3 months of zinc acetate supplementation was shown to be effective and safe for the treatment of hyperammonemia in patients with cirrhosis.[12] A systematic review and meta-analysis of the use of zinc in patients with hepatic encephalopathy showed a significant improvement in psychological tests in those undergoing zinc therapy.[13] However, many of these studies have focused on short-term zinc administration, and few studies have explored the long-term effects of zinc administration in patients with CLD. We found that zinc administration improves liver function and decreases the cumulative incidence of HCC in patients with CLD over the course of long-term follow-up. We also confirmed that a serum zinc concentration greater than 70 µg/dL must be maintained to obtain good clinical outcomes.[14]

In the present study, we sought to clarify whether the zinc concentration impacted HCC development in patients without a history of HCC who achieved an SVR after treatment with DAAs. We report, for the first time, that zinc administration can decrease the long-term cumulative incidence of HCC in patients who have achieved SVR.

PATIENTS AND METHODS

All patients were evaluated by liver biopsy, and the liver status was diagnosed histologically. A total of 519 patients who had been successfully treated with the following DAAs were enrolled: asnaprevir/daclatasvir (n = 44), sofosbuvir (SOF)/ledipasvir (n = 176), ombitasvir/paritaprevir (n = = 50), elbasvir/grazoprevir (n = 38), SOF/ribavirin (n = 93), and glecaprevir/pibrentasvir (n = 118). Patients who did not have a history of HCC, had achieved an SVR, had been observed for more than 6 months after SVR 12, and had never received supplemental zinc were analyzed

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in this study (no Zn treatment group; Table 1). All laboratory data were examined before DAA treatment (from 3 months before DAA therapy) and after DAA treatment (3 months after the end of treatment [SVR 12]). The no Zn treatment group was divided into three subgroups based on the Zn concentration at 3 months after the end of DAA treatment (low group: <70 µg/dL; intermediate group: 71-90 µg/dL; and high group: >90 µg/dL) (Table 2).

Eighty patients who had achieved SVR had been administered zinc (zinc sulfate or zinc acetate; Zn 50-150 mg/day) for at least 6 months before the start of DAA treatment (Zn treatment group, zinc concentration before zinc treatment: 62.1 µg/dL; mean administration period: 41.2 ± 12.8 months; 6.6 ± 1.2 months before DAA start, 3.1 ± 0.8 months after DAA start, and 31.5 ± 14.2 months after DAA withdrawal) (Table 1). Zinc was administered to patients with hypozincemia (Zn < 70 µg/dL), and informed consent was obtained from patients included in this study. Zinc treatment was continued after SVR unless patients had adverse events, such as copper deficiency or abdominal discomfort. There were no differences in the clinical characteristics between the no Zn treatment group and the Zn treatment group. Each doctor decided whether to administer zinc supplements.

Another 23 patients described in Supporting Table S2 and Supporting Fig. S2 were enrolled in another

TABLE 1. CLINICAL PROFILES OF 599 DAA-TREATED SVR PATIENTS

		Total	No-Zn Treatment	Zn Treatment	
		(n = 599)	(n = 519)	(n = 80)	P Value
Gender (female)		341	293	48	0.52
Age (years)		65.1 ± 13.5	64.7 ± 13.7	67.6 ± 11.7	0.08
Chronic hepatitis/cirrhosis		447/152	396/123	51/29	0.08
DAA therapy (ASV+DCV/SOF+LDV/ OMV+PTV/ EBR+GZR/SOF+RBV/GLE+PIB)		49/217/55/51/102/125	44/176/50/38/93/118	5/41/5/13/9/7	0.32
Previous interferon (absence/presence)		428/171	364/155	64/16	0.054
HCV serotype (1/2/3)		434/164 /1	368/150 /1	66/14/0	0.07
Diabetes (absence/presence/unknown)		423/68/108	365/56/98	58/12/10	0.34
Body mass index (kg/m^2)		24.9 ± 3.2	24.9 ± 3.2	24.8 ± 3.1	0.98
Before DAA treatment	AST (U/L)	49.1 ± 36.1	48.9 ± 37.5	50.5 ± 26.2	0.52
	ALT (U/L)	51.6 ± 41.8	52.1 ± 43.0	48.8 ± 33.2	0.76
	Platelet (104/µL)	17.6± 7.6	17.9 ± 7.6	15.8 ± 6.7	0.06
	Total bilirubin (mg/dL)	0.67 ± 0.29	0.65 ± 0.28	0.78 ± 0.32	0.21
	Albumin (g/dL)	3.88 ± 0.51	3.88 ± 0.51	3.68 ± 0.58	0.07
	PT activity (%)	87.2 ± 16.7	87.7 ± 16.8	81.9 ± 18.2	0.31
	γGTP (IU/L)	69.5 ± 16.9	69.7 ± 16.8	68.9 ± 18.2	0.93
	Creatinine (mg/dL)	0.85 ± 0.29	0.85 ± 0.28	0.86 ± 0.29	0.97
	Hyaluronic acid (ng/mL)	93.1 ± 17.8	92.7 ± 16.9	96.8± 23.7	0.76
	AFP (ng/mL)	13.7 ± 51.6	13.6 ± 54.3	14.5 ± 29.3	0.89
	Zn (µg/dL)	71.7 ± 15.6	71.6 ± 12.8	72.7 ± 26.6	0.78
After DAA treatment (EOT 12 weeks)	AST (U/L)	24.3 ± 9.6	24.5 ± 9.6	22.9 ± 7.4	0.74
	ALT (U/L)	19.8 ± 14.5	19.5 ± 15.2	20.3 ± 10.8	0.45
	Platelet (104/µL)	18.8 ± 7.0	18.9 ± 7.1	16.4 ± 6.2	0.052
	Total bilirubin (mg/dL)	0.75 ± 0.44	0.74 ± 0.46	0.79 ± 0.39	0.81
	Albumin (g/dL)	3.89 ± 0.43	3.91 ± 0.45	3.72 ± 0.38	0.13
	PT activity (%)	79.2 ± 14.7	79.3 ± 14.9	78.3 ± 12.9	0.67
	AFP (ng/mL)	5.4 ± 5.1	5.4 ± 5.3	5.3 ± 3.9	0.59
	Zn (µg/dL)	78.7 ± 11.3	78.7 ± 11.4	78.9 ± 11.3	0.92
Observation period (months)		29.4 ± 15.7	28.9 ± 16.0	34.4 ± 15.0	0.19

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; ASV, asnaprevir; DCV, daclatasvir; EBR, elbasvir; EOT, end of treatment; GLE, glecaprevir; GZR, grazoprevir; LDV, ledipasvir; OMV, ombitasvir; PIB, pibrentasvir; PTV, paritaprevir; RBV, ribavirin; PT, prothrombin time; γGTP, γ glutamyl transpeptidase.

TABLE 2. CLINICAL CHARACTERISTICS OF THE LOW, INTERMEDIATE, AND HIGH ZN CONCENTRATION GROUPS STRATIFIED ACCORDING TO THE ZN CONCENTRATION AT SVR12 (NO-ZN TREATMENT GROUP; n = 519)

Zn Concentration (µg/dL)		Low (≤70) (n = 131): a	Middle (>70-90) (n = 225): b	High (>90) (n = 163): c	P Value a vs. b	b vs. c	c vs. a
Gender (female)		78	124	91	0.18	0.85	0.39
Age (years)		66.9 ± 11.5	64.3 ± 12.7	63.1 ± 15.4	0.26	0.65	0.08
Chronic hepatitis/cirrhosis		97/34	174/51	125/38	0.26	0.84	0.47
Previous interferon (absence/ presence)		98/33	143/82	123/40	0.10	0.11	0.86
HCV serotype (1/2/3)		95/36/0	163/62/0	110/52/1	0.36	0.25	0.18
Before DAA treatment	AST (U/L)	51.7 ± 32.7	45.8 ± 30.4	50.7 ± 45.3	0.25	0.31	0.86
	ALT (U/L)	47.4 ± 33.4	51.3 ± 41.3	56.9 ± 51.2	0.33	0.25	0.06
	Platelet (104/µL)	15.9 ± 6.7	18.0 ± 6.4	19.2 ± 9.4	0.09	0.15	0.09
	Total bilirubin (mg/dL)	0.65 ± 0.33	0.68 ± 0.38	0.63 ± 0.24	0.98	0.23	0.89
	Albumin (g/dL)	3.79 ± 0.48	3.83 ± 0.56	3.92 ± 0.54	0.82	0.89	0.75
	PT activity (%)	86.2 ± 14.2	87.7 ± 16.8	89.1 ± 17.2	0.76	0.65	0.23
	AFP (ng/mL)	12.1 ± 28.8	17.7 ± 27.7	8.9 ± 15.2	0.34	0.10	0.26
	Zn (µg/dL)	64.9 ± 11.5*	73.3 ± 12.1*	75.8 ± 12.6*	<0.001	0.08	<0.001
After DAA treatment (EOT 12 weeks)	AST (U/L)	23.5 ± 12.0	24.2 ± 10.2	24.6 ± 9.5	0.22	0.33	0.19
	ALT (U/L)	19.0 ± 12.5	18.5 ± 15.3	21.9 ± 23.4	0.40	0.21	0.38
	Platelet (104/µL)	18.0 ± 8.3	19.4 ± 6.8	18.9 ± 6.2	0.12	0.51	0.34
	Total bilirubin (mg/dL)	0.73 ± 0.39	0.76 ± 0.42	0.73 ± 0.41	0.38	0.38	0.97
	Albumin (g/dL)	3.84 ± 0.38	3.92 ± 0.43	3.98 ± 0.47	0.21	0.24	0.09
	PT activity (%)	81.2 ± 12.8	77.6 ± 14.6	79.4 ± 13.8	0.18	0.35	0.32
	AFP (ng/mL)	6.7 ± 11.2	6.8 ± 8.3	4.4 ± 3.1	0.54	0.20	0.28
	Zn (µg/dL)	63.9 ± 6.2*	75.6 ± 5.3*	96.9 ± 5.7*	<0.001	<0.001	<0.001
Observation period (months)		31.8 ± 15.6*	33.1 ± 13.5*	22.8 ± 16.5*	0.44	<0.001	<0.001

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; PT, prothrombin time.

double-blind prospective study, and this cohort was completely different, with 599 patients shown in the main text. There were no relationships between the 599 patients and 23 patients.

Imaging (abdominal ultrasonography or computed tomography) and tumor marker (AFP and Des-gamma carboxyprothrombin) evaluations were performed at least every 3 months for high-risk patients, including cirrhosis. The same examinations were performed at least every 6 months for other SVR patients without cirrhosis. This study was retrospectively analyzed and approved by the ethics committee of Osaka-Rosai Hospital.

STATISTICAL ANALYSIS

Data are presented as the mean ± SD. Comparisons between the two groups were performed with unpaired Student t tests. The log-rank test and logistic regression analysis were used to assess the risk factors and the cumulative incidence rate of HCC. $P < 0.05$ was considered statistically significant.

Results

CUMULATIVE HCC INCIDENCE RATES IN THE Zn TREATMENT GROUP AND NO Zn TREATMENT GROUP

We first investigated whether treatment with zinc can reduce the risk of incident HCC. Eighty patients infected with HCV were treated with zinc preparations before DAA therapy because their Zn concentrations were less than 70 µg/dL (Zn treatment group).
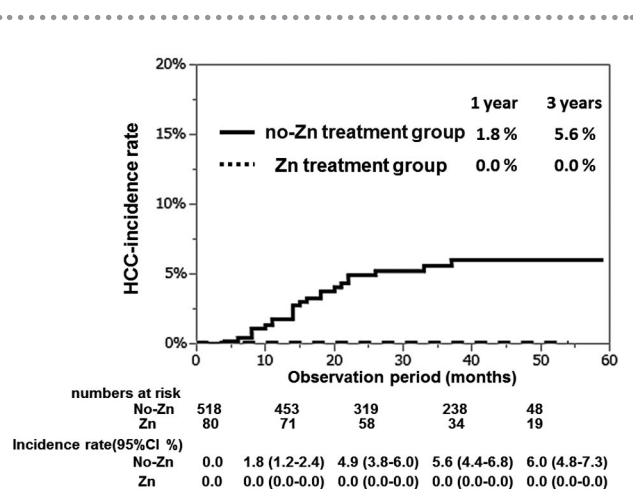
FIG. 1. Cumulative incidence rates of HCC in the no Zn treatment group and Zn treatment group.

These patients had taken zinc preparations for more than 6 months; those who had received supplemental zinc for less than 6 months were excluded from this study. This Zn treatment group was compared with 519 patients who had never been treated with zinc preparations and had achieved an SVR (no Zn treatment group). The clinical characteristics of these two groups are given in Table 1. The Zn treatment group tended to be older and had lower platelet counts than the no Zn treatment group, but no significant differences were observed between these two groups with regard to sex, age, or the results of various blood tests before and after DAA administration. The mean follow-up period in the Zn treatment group was 34.4 ± 15.0 months, and patients had taken zinc preparations for almost the entire duration of follow-up. The mean zinc concentration was 78.9 μg/dL at 12 weeks after DAA treatment (≥70 μg/dL: 25 patients [31.3%]; >70 and ≤90 μg/dL: 32 patients [40.0%]; and >90 μg/dL: 23 patients [28.7%]). The incidence rate of HCC (3 years) was significantly lower in the Zn treatment group (0.0%) than in the no Zn treatment group (5.6%; $P = 0.048$) (Fig. 1). To date, no patients in the Zn treatment group have developed HCC.

RISK FACTORS FOR HCC IN PATIENTS WITHOUT Zn TREATMENT

In the no Zn treatment group, 23 patients developed HCC (HCC group) during follow-up (28.9 ±

16.0 months), and the cumulative incidence rates of HCC at 1 and 3 years were 1.8% and 5.6%, respectively (Fig. 1). In this cohort, we identified risk factors for HCC by multivariate analysis, and sex (male, $P = 0.042$), liver status (cirrhosis, $P = 0.022$), low platelet count before treatment (<15× 10^4/mL, $P = 0.045$), and low serum zinc concentration (<70 μg/dL, $P = 0.021$) at 12 weeks after the end of DAA therapy were independent risk factors for HCC (Table 3). Among these four factors, we focused on the serum zinc concentration in this study, as male sex, cirrhosis, and low platelet count have been previously reported as risk factors for HCC in patients who achieved SVR after treatment with DAAs.[4,15]

Next, the zinc concentration was compared between the HCC group and the no HCC group before and after DAA therapy. As shown in Fig. 2, the zinc concentration was significantly higher in the no HCC group than in the HCC group at all time points except 4 weeks after the start of DAA therapy ($P < 0.01$).

CUMULATIVE HCC INCIDENCE RATES AMONG PATIENTS WITH HIGH, INTERMEDIATE OR LOW ZINC CONCENTRATIONS AFTER DAA THERAPY

The 519 patients in the no Zn treatment group were divided into three subgroups according to their Zn concentrations at 12 weeks after the end of DAA therapy, as follows: ≤70 μg/dL (low group), 71-90 μg/dL (intermediate group), and >90 μg/dL (high group). The HCC incidence rates were compared among these three subgroups to clarify the relationships between the serum zinc concentration and HCC incidence rate. The characteristics of these three groups are given in Table 2. The low group tended to be older and had lower platelet counts than the other two groups, but no significant differences were observed among the three groups with regard to sex, age, or the results of various blood tests except the zinc concentration.

The cumulative incidence rates of HCC recorded at 3 years were 11.7%, 4.4%, and 0.0% in the low, intermediate, and high groups, respectively. The incidence rates were significantly lower in the intermediate and high groups (>70 μg/dL) than in the low group

TABLE 3. RISK FACTORS FOR HCC IN DAA-TREATED PATIENTS WITHOUT A HISTORY OF HCC

|			Univariate Analysis		Multivariate Analysis	
			OR (95% CI)	P Value	OR (95% CI)	P Value
Gender		Male	2.68 (1.18-7.42)	0.028	2.82 (1.04-9.52)	0.042
Liver status		cirrhosis	3.48 (1.89-5.72)	0.027	3.24 (1.72-12.48)	0.022
Before DAA treatment	Albumin (g/dL)	<3.5	1.85 (1.05-3.15)	0.045	1.28 (0.78-2.01)	0.187
	Platelet ($10^4/\mu$L)	<15	3.12 (1.82-3.76)	0.015	2.23 (1.02-7.86)	0.045
	Zn (μg/dL)	<70	4.4 (1.60-15.5)	0.003	2.59 (0.84-9.75)	0.099
After DAA treatment (EOT 12 weeks)	AFP (ng/mL)	≤5	1.82 (1.16-3.82)	0.038	1.28 (0.82-2.97)	0.071
	Zn (μg/dL)	<70	2.92 (1.38-8.20)	0.008	3.28 (1.86-9.28)	0.021
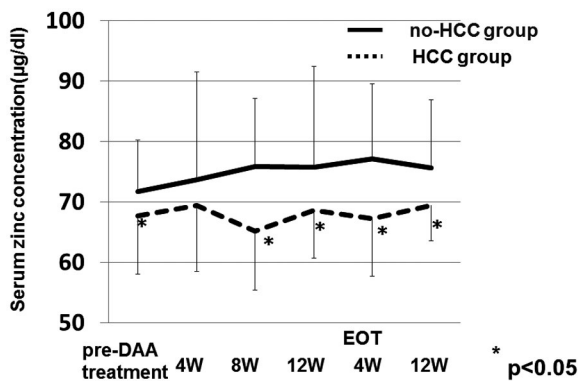
FIG. 2. Serial changes in serum zinc concentration in the HCC group and no HCC group. The asterisks indicate significant differences (*$P < 0.01$).

($\leq$70 μg/dL) ($P = 0.033$ and $P = 0.0007$, respectively), and the incidence rate was significantly lower in the high group than in the intermediate group ($P = 0.029$; Fig. 3).

FIG. 3. Cumulative incidence rate of HCC in the three groups stratified according to the Zn concentration at 12 weeks after the end of DAA therapy, as follows: ≤70 μg/dL (low group), 71-90 μg/dL (intermediate group), and >90 μg/dL (high group). The cumulative incidence rates of HCC were significantly lower in the intermediate and high groups than in the low group ($P = 0.033$ and $P = 0.0007$, respectively), and the cumulative incidence rate of HCC was also significantly lower in the high group than in the intermediate group ($P = 0.029$).

Discussion

In the present study, we identified risk factors for HCC by multivariate analysis, and liver status (cirrhosis) and low zinc concentration were independent risk factors. To ensure this result, all patients were divided into four groups according to liver status and serum zinc concentration (Supporting Table S1). The HCC incidence rate was shown in Supporting Fig. S1, and we found that it was approximately twice as high in the cirrhosis/low zinc group than in the cirrhosis/high zinc group. Thus, a low zinc concentration was a risk factor for HCC development in the same liver status/cirrhosis group. The HCC incidence rate was zero in
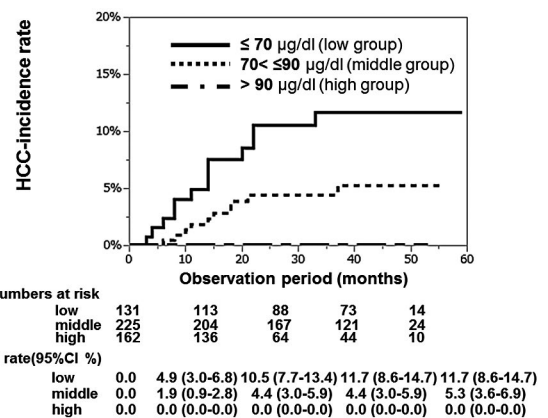
the zinc-treatment group among the cirrhosis/low zinc group, and administration of zinc was thought to be the most promising in this situation. Hypozincemia is not only one of the predictors of hepatocarcinogenesis but also a modifiable condition that could be a therapeutic target to reduce the incidence of HCC after HCV eradication.

The serum zinc concentration usually increases after HCV eradication, and in the present study, it became high in both the no Zn treatment group and Zn treatment group (Table 1). This favorable result (i.e., the elevated zinc concentration) was caused by the reduction in inflammation in the liver. Takeda et al. recently reported that zinc deficiency delays both

extracellular ATP clearance and adenosine generation, which enhances inflammation.[16] They also explained that inflammation leads to the consumption of zinc, and eventually, zinc deficiency. After HCV eradication and the amelioration of inflammation, the serum zinc concentration increased in all groups regardless of the zinc concentration before DAA treatment (Supporting Fig. S2).

In this study, zinc administration was shown to suppress HCC development. The concentration of zinc increased to 78.9 ± 11.3 μg/dL in the Zn treatment group at 12 weeks after the end of DAA treatment. The mechanism by which zinc suppresses cancer development is still unclear, but a possible explanation is as follows. The zinc concentration gradually decreases with the progression of fibrosis, leading to the deactivation of SOD. Importantly, SOD activity is restored to the baseline level after the administration of zinc, making it possible to decrease the concentration of ROS, which can induce epigenetic modulations involved in the process of carcinogenesis. To support this speculation, we examined the value of dROM (Supporting Table S2), which is a possible marker of ROS.[17,18] Another 23 patients were enrolled in a double-blind prospective study, this cohort was completely different from the 599 patients found in Table 1. The value of dROM significantly decreased as the serum zinc concentration increased after zinc administration in some of the zinc-treated patients (Supporting Fig. S3). This result may explain how the administration of zinc suppresses ROS.

In this study, the zinc concentration did not increase above 70 μg/dL in 25 of tithe 80 zinc-treated patients. However, none of these patients developed HCC, although their zinc concentrations were not greater than 70 μg/dL, whereas the cumulative 3-year incidence rate of HCC was 11.7% in the 131 patients who were not treated with zinc and whose zinc concentrations were not greater than 70 μg/dL. This result may suggest that the zinc preparation itself can suppress HCC development. Takahashi reported that the serum levels of type IV collagen and the activity of tissue inhibitors of metalloproteinase-1 (TIMP-1) were significantly reduced by oral supplementation with zinc.[19] Zinc supplementation can directly inhibit the progression of fibrosis, which decreases the risk of HCC development.

Costello et al.[20] already focused on the importance of zinc status in the development of HCC, but regarding

treatment with zinc, we show that zinc administration decreases the long-term cumulative incidence of HCC in DAA-treated patients who do not have a history of HCC and have achieved SVR. We also confirmed that a low zinc concentration after DAA treatment is a risk factor for HCC. Patients were followed after the eradication of HCV, but thus far, there have not been any treatments able to suppress the development of HCC. Based on the evidence provided by this study, it may be possible to prescribe zinc preparations to reduce the incidence of HCC after SVR. Maintaining a zinc concentration greater than 90 μg/dL has been shown to reduce the risk of HCC development, and zinc administration can be a candidate therapy to suppress the development of HCC in patients treated with DAAs who have achieved an SVR and zinc concentrations less than 70 μg/dL. Additional randomized prospective controlled studies with large sample sizes are needed to confirm these results.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1782/suppinfo.