Sustained virological response by direct-acting antivirals reduces the recurrence risk of hepatitis C-related hepatocellular carcinoma after curative treatment

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Abstract. The present study aimed to assess the suppressive effect of direct-acting antivirals (DAAs) on hepatocellular carcinoma (HCC) recurrence following curative treatment, particularly compared with interferon (IFN)-based therapy. Among 117 curative cases of HCV-related initial HCC between 2006 and 2017 at Gifu University Hospital, 13 and 14 cases achieved a sustained virological response (SVR) by DAA- (DAA group) or IFN-based therapies (IFN group), and 64 cases were not treated with any antiviral therapy (non-treatment group). Recurrence-free survival (RFS) following curative treatment in each group was analyzed using the Kaplan-Meier method and log-rank test. A Cox proportional hazards model was used to analyze the factors that affected RFS. Age was significantly lower and serum alanine aminotransferase level was significantly higher in the IFN group than in both the DAA and non-treatment groups. There was a significant difference in RFS between the non-treatment group and antiviral therapy groups, including the DAA (P=0.014) and IFN groups (P=0.009); however, no significant difference was identified in RFS between the DAA and IFN groups (P=0.564). SVR achieved by DAA [P=0.011; hazard ratio (HR), 0.222; 95% CI, 0.069-0.758] or IFN therapy (P=0.007; HR, 0.327; 95% CI, 0.145-0.742) was an independent factor for the prevention of HCC recurrence. SVR by DAA therapy exhibited an anti-liver tumorigenesis effect equal to that of IFN-based therapy and reduced the risk of HCC recurrence.

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Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; DAAs, direct-acting antivirals; SVR, sustained virological response; RFS, recurrence-free survival; CT, computed tomography; MRI, magnetic resonance imaging

Key words: interferon, hepatocellular carcinoma, recurrence risk, hepatitis C virus, direct-acting antivirals

Introduction

Chronic infection with hepatitis C virus (HCV) is a well-recognized risk factor for liver cirrhosis and hepatocellular carcinoma (HCC), which is one of the most common malignancies worldwide (1,2). HCV infection is a leading cause of HCC; the prevalence rate of HCV infection among HCC patients is around 70% in Japan (3,4). The recurrence risk of patients with HCC is extremely high compared to that of other malignancies and this is associated with poor survival (5,6). Therefore, anti-virus therapy for the eradication of HCV is very important to prevent the development and recurrence of HCC and prolong the prognosis of patients with this malignancy (7-10). A meta-analyses including 30 studies analyzing the association between response to HCV interferon (IFN)-based therapy and development of HCC demonstrated that eradication of HCV infection dramatically reduced the risk of HCC (relative risk for all persons 0.24; 95% confidence interval [CI] 0.18 to 0.31) (10).

The recent introduction of IFN-free direct-acting antivirals (DAAs) led to revolutionary progress in HCV treatment because of its higher tolerability and sustained virological response (SVR) rate than conventional IFN-based therapy (11). Therefore, IFN-based therapy has been replaced by DAA therapy and the number of patients who achieved SVR with this therapy is increasing steadily. Several studies suggest that eradication of HCV by DAA therapy is associated with improved liver function and quality of life as well as reduced risk of decompensated liver disease (7,12,13). Moreover, it has gradually been determined that the suppressive effects of DAA therapy on HCC development are similar to those of IFN-based therapy (8,9,14). However, some conflicting data has described unexpected high rates of HCC occurrence and recurrence after successful DAA treatment (15-17). Therefore, it is important to examine whether eradication of HCV by DAA therapy reduces the risk of HCC.

The purpose of the present study was to evaluate the suppressive effect of DAA therapy on the recurrence of HCV-related

HCC after curative treatment. The anti-liver tumorigenesis effects in the DAA therapy group were compared not only to a non-antiviral therapy group but also to an IFN-based therapy group.

Patients and methods

Patients, treatment and determination of HCC recurrence. This retrospective study included 218 patients with HCV-related initial HCC who were treated in Gifu University Hospital (Gifu, Japan) between May 2006 and December 2017. Of these patients, 117 patients who received curative treatment, including surgical resection or radiofrequency ablation, were evaluated.

HCC nodules were detected using imaging modalities, including dynamic computed tomography (CT), dynamic magnetic resonance imaging (MRI), and abdominal arteriography. HCC was diagnosed based on a typical hypervascular tumor stain on angiography and typical dynamic study findings of enhanced staining in the early phase and attenuation in the delayed phase. All patients were followed on an outpatient basis and underwent dynamic CT, MRI, or ultrasound scans every 3 months after the initial treatment. Recurrent HCC was diagnosed when the typical findings of HCC were observed in segments different from where the initial lesions arose. Recurrence-free survival (RFS) time was defined as the interval from the date of the initial treatment to the date of the recurrence or December 2017 for recurrence-free survivors.

All study participants provided verbal informed consent, which was considered sufficient as this study followed an observational research design that did not require new human specimens and instead relied only on preexisting samples. The study design, including this consent procedure, was approved by the Ethics Committee of the Gifu University School of Medicine.

Statistical analysis. Baseline characteristics among the groups were compared using one-way analysis of variance for continuous variables or the χ^2 test for categorical variables. Univariate analysis was performed using the Cox proportional hazards model to identify the factors that affected RFS. RFS was estimated using the Kaplan-Meier method, and differences between curves were evaluated using the log-rank test. The Holm method was used as a post hoc test to counteract the problem of multiple comparisons (18). Statistical significance was defined as P<0.05. All statistical analyses were performed using R version 3.3.2 (The R Project for Statistical Computing, Vienna, Austria; http://www.R-project.org/).

Results

Baseline characteristics of the enrolled patients. Among the 117 patients who received curative treatment for initial HCC, 13 patients received DAA therapy and all of them achieved SVR (DAA group; asunaprevir/daclatasvir [n=6], sofosbuvir/ledipasvir [n=4], and ombitasvir/paritaprevir/ritonavir [n=3]). IFN-based therapy was administered to 34 patients and, among them, 14 achieved SVR (IFN group; pegylated-IFN alone [n=7], pegylated-IFN/ribavirin [n=5], and pegylated-IFN/ribavirin plus simeprevir [n=2]). In order to examine whether HCV

eradication by DAA or IFN therapies can suppress the recurrence of HCC after curative treatment, IFN failure cases (n=20) were excluded in the present study. Antiviral therapies were started as soon as possible after initial HCC was completely cured. Among 70 patients who had not been treated with any antiviral therapy, 6 patients with portal vein invasion or Child-Pugh class C were excluded because such patients are known to have high risk for HCC recurrence and poor prognosis, and the remaining 64 patients were set as the control group (non-treatment group). No patient had such high risk conditions in the DAA or IFN groups. The patient flow in this study is shown in Fig. 1.

The baseline characteristics and laboratory data of the DAA, IFN, and non-treatment groups are shown in Table I. When compared to the DAA (74.1 years; P=0.007) and non-treatment (73.2 years; P=0.002) groups, the patients in the IFN group (65.5 years) were significantly younger. The serum concentration of alanine aminotransferase (ALT) was significantly lower both in the DAA (40.4 IU/L; P=0.003) and non-treatment (44.7 IU/L; P<0.001) groups than in the IFN group (79.5 IU/L). No significant differences were observed in HCC-related factors, including tumor size, tumor numbers, portal vein invasion, stage, and initial treatment among the three groups.

Comparison of RFS among the DAA, IFN and non-treatment groups. The 1-, 2-, and 3-year RFS rates of the DAA, IFN, and non-treatment groups were 84.6, 76.2 and 76.2%; 100, 69.2 and 69.2%; and 76.8, 45.0 and 22.4%, respectively (Fig. 2). The shortest intervals for RFS of the DAA, IFN, and non-treatment groups were 204, 245 and 125 days, respectively. In comparison to the non-treatment group, RFS was significantly improved in the DAA (P=0.014) and IFN groups (P=0.009). However, there was no significant difference in RFS between the DAA and IFN groups (P=0.564).

Possible risk factors for HCC recurrence. Table II shows the possible risk factors for HCC recurrence by Cox proportional hazards model, and IFN therapy (HR 0.327, 95% CI 0.145-0.742, P=0.07) and DAA therapy (HR 0.222, 95% CI 0.069-0.758, P=0.011) were independent factors, which could reduce the risk of HCC recurrence.

Discussion

The results of the present study revealed that the patients in whom HCV infection was successfully eradicated by DAAs after curative treatment of initial HCC had a similar recurrence rate as those who underwent IFN-based therapy. Furthermore, compared with the non-treatment group, successful treatment with DAAs reduced the risk of HCC recurrence after curative treatment by about one-fifth, which was almost the same as that reported previously for IFN-based therapy (10). These results strongly encourage us to introduce DAA treatment among patients treated with curative treatment for primary HCV-related HCC.

Recently, DAAs have become the dominant therapy for HCV infection in place of conventional IFN-based therapy because of its higher tolerability and SVR rate (11). In fact, all the patients in the DAA group did not develop serious

Table I. Baseline demographic and clinical characteristics of the DAA, IFN and no treatment groups.

Variables	DAA (n=13)	IFN (n=14)	No treatment (n=64)	P-value
Sex (male/female)	9/4	12/2	44/20	0.513
Age (years)	74.1±3.8	65.5±6.8	73.2±8.0	0.002[a]
BMI (kg/m^2)	21.9±3.4	22.0±2.9	22.4±2.8	0.759
Child-Pugh score (5/6/7/8/9)	7/4/1/1/0	8/4/2/0/0	33/17/7/6/1	0.985
ALB (g/dl)	3.6±0.5	3.7±0.4	3.5±0.5	0.437
ALT (IU/l)	40.4±22.3	79.5±49.8	44.7±26.9	0.001[b]
T-Bil (mg/dl)	0.9±0.4	0.9±0.3	1.1±0.6	0.513
PLT (x10^4/μl)	13.5±6.0	12.0±6.0	11.2±4.7	0.313
PT (%)	83.3±17.0	84.5±11.2	85.7±14.9	0.856
AFP (ng/dl)	500±913	202±670	174±617	0.363
PIVKA-II (mAU/ml)	469±1256	2,170±7,952	816±2648	0.428
Tumor size (cm)	2.0±1.1	2.0±1.2	2.4±1.4	0.541
Tumor number (1/≥2)	11/2	11/3	49/15	0.947
Stage (I/II/III)	7/6/0	6/7/1	26/31/7	0.873
Initial treatment (resection/RFA)	5/8	6/8	17/47	0.590

[a]DAA vs. IFN (P=0.007), DAA vs. no treatment (P=0.694), IFN vs. no treatment (P=0.002). [b]DAA vs. IFN (P=0.003), DAA vs. no treatment (P=0.645), IFN vs. no treatment (P<0.001). Values are presented as the mean ± standard deviation. P-values were calculated using one-way analysis of variance for continuous variables or the χ^2 test for categorical variables. The two factors (age and ALT) that exhibited significant differences were then analyzed using the Holm method as a post-hoc test, and the results of this were indicated using superscripted letters. DAA, direct-acting antiviral; IFN, interferon; BMI, body mass index; ALB, albumin; ALT, alanine aminotransferase; T-Bil, total bilirubin; PLT, platelet count; PT, prothrombin time; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonists-II; RFA, radiofrequency ablation.
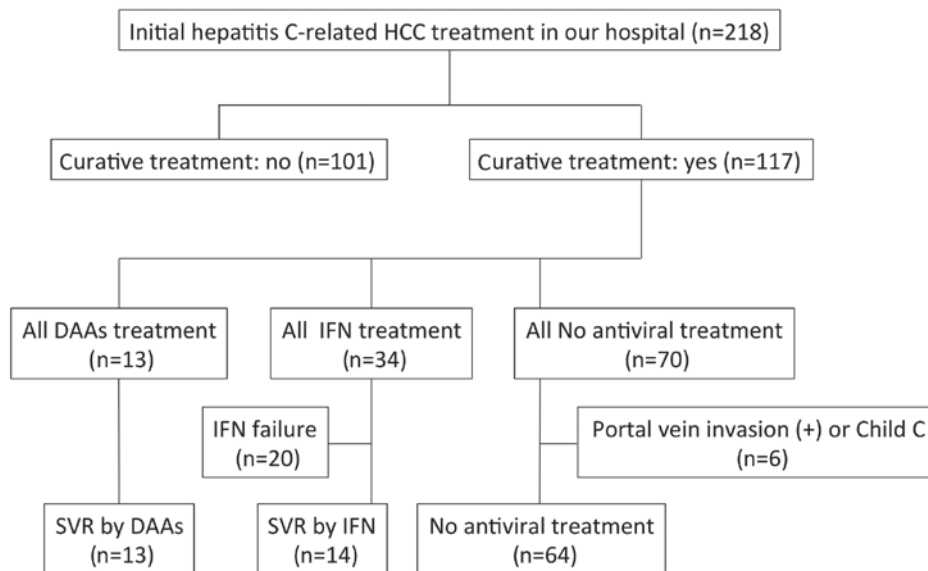
Figure 1. Patient flow in the present study. Child C refers to the worst liver functional reserve (Child-Pugh score ≥10). HCC, hepatocellular carcinoma; IFN, interferon; DAAs, direct-acting antivirals; SVR, sustained virological response.

complications during DAA treatment and achieved SVR. On the other hand, it is reported that patients with HCC had significantly higher DAA failure (19). The presence of active HCC was the primary predictor of DAA failure even after controlling for other variables (19). Therefore, we did not introduce DAA treatment to HCV positive patients with active HCC and this might lead to the achievement of extremely high

SVR rate in this study. In order to achieve SVR, it is important to confirm that there is no active HCC before introducing DAA treatment.

Importantly, it is also expected that treatment with DAAs may exert an anti-liver tumorigenesis effect, which was proven by IFN-based therapy in patients with cirrhosis (10). Several studies have revealed the association between successful

Table II. Analysis of possible risk factors for HCC recurrence using a Cox proportional hazards model.

	Univariate analysis	
Variables	HR (95% CI)	P-value
Sex (male vs. female)	1.084 (0.612-1.920)	0.782
Age (years)	0.986 (0.948-1.025)	0.475
BMI (kg/m^2)	1.002 (0.914-1.099)	0.960
Child-Pugh grade (A vs. B)	0.970 (0.488-1.927)	0.931
ALB (g/dl)	0.769 (0.436-1.357)	0.364
ALT (IU/l)	0.995 (0.987-1.004)	0.265
T-Bil (mg/dl)	1.398 (0.759-2.578)	0.283
PLT (x10^4/μl)	0.962 (0.908-1.019)	0.184
PT (%)	0.999 (0.980-1.019)	0.925
AFP (ng/dl)	0.999 (0.999-1.000)	0.424
Tumor size (cm)	1.124 (0.882-1.431)	0.345
Tumor number (1 vs. ≥2)	1.111 (0.663-1.861)	0.689
Cancer stage	1.016 (0.652-1.584)	0.944
Initial treatment (RFA vs. resection)	1.286 (0.711-2.33)	0.406
Antiviral therapy		
IFN vs. no treatment	0.327 (0.145-0.742)	0.007
DAA vs. IFN	1.470 (0.373-5.788)	0.581
DAA vs. no treatment	0.222 (0.069-0.758)	0.011

HR, hazard ratio; BMI, body mass index; ALB, albumin; ALT, alanine aminotransferase; T-Bil, total bilirubin; PLT, platelet count; PT, prothrombin time; AFP, α-fetoprotein; RFA, radiofrequency ablation; IFN, interferon; DAA, direct-acting antiviral.
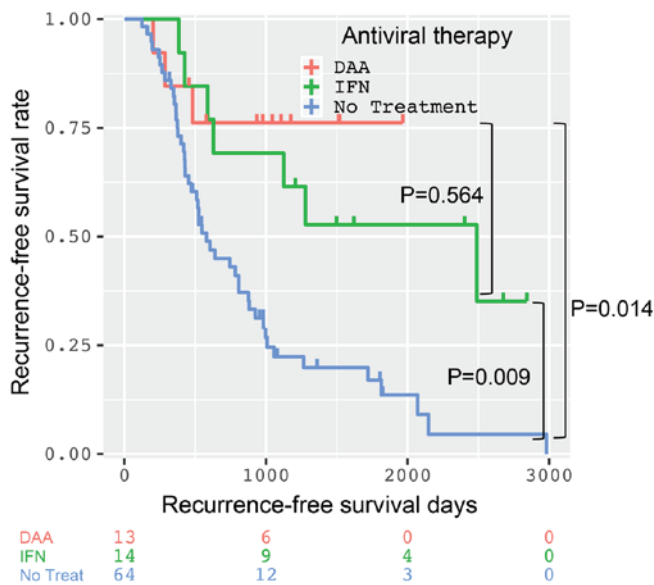

Figure 2. Kaplan-Meier curves for recurrence-free survival time of the DAA, IFN and no treatment groups. IFN, interferon; DAAs, direct-acting antivirals.

DAA treatment and the reduction of the risk of HCC (8,9,14). HCV eradication can improve hepatic inflammation and fibrosis, both of which are associated with the progression of precancerous lesions into malignant cell clones (20). Therefore, HCV eradication from the liver exerts a protective effect against HCC development, regardless of the type of antiviral treatment. Importantly, not the type of antiviral treatment (IFN or DAA) but treatment response (SVR or non-SVR) was the only independent predictive factor of HCC recurrence after curative treatment (21).

On the other hand, some studies have indicated that DAAs could instead promote the development of HCC (15-17). To explain these contrasting results, the following hypotheses can be considered: DAAs cause a deregulation of the immune system through an abrupt reduction in the HCV load, leaving immune cells less active against tumor cells already present at the beginning of antiviral treatment (22,23). Moreover, it can persist even after successful DAA treatment, while exogenous IFN used as part of HCV treatment plays a protective role in HCC by switching on inflammatory cells with pro-apoptotic and anti-tumoral activities (24,25).

However, it should be recognized that patients treated with DAAs have a significantly higher risk of HCC occurrence or recurrence than those with IFN-based therapy because of their background factors. Namely, DAA-treated patients have a significantly higher rate of known risk factors for HCC, including cirrhosis, older age, and a higher baseline alpha-feto-protein (AFP) level (14). Age and serum AFP levels were higher and liver fibrosis was more advanced in patients who achieved SVR with DAA therapy than those in patients who achieved SVR with IFN-based therapy (26). The higher prevalence of underlying HCC risks among DAA-treated individuals could be because patients treated with earlier DAA regimens had previously failed treatment with IFN-based therapy (14). Indeed, patients were significantly older in the DAA group in the present study. Furthermore, two patients had recurrent HCC

within a year in the DAA group, while none did in the IFN group.

Another emphasized point of the present study is that even though HCV could be eradicated successfully by either DAA- or IFN-based therapy, the risk of recurrence almost never changes within a year. Because DAAs might not be able to inactivate tumor cells already present at the beginning of antiviral treatment, close follow-up for surveying HCC development is required. All of the guidelines from the American Associated for the Study of Liver Diseases, the European Association for the Study of the Liver, and the Japan Society of Hepatology recommended surveillance every 3-4 months using image examination with or without tumor maker measurements after curative treatment of HCC (27-29). The appropriate surveillance interval also should be determined on the basis of risk factors of HCC recurrence such as male sex, older age, presence of cirrhosis, alcohol consumption, higher AFP levels, and advanced stage of primary HCC (1,27-33).

The main limitation of our study is the small sample size and relatively short follow-up time. Our study is also limited by its study design as a retrospective-cohort study. Further prospective multicenter studies with larger sample sizes and longer follow-up periods are warranted to further verify the anti-liver tumorigenesis effect of DAAs. However, although there are such limitations, the result of the present study indicating the anti-liver tumorigenesis effect of DAAs is extremely important because there is a concern that DAAs might promote HCC (15-17).

In conclusion, SVR by DAAs showed a suppressive effect on HCV-related HCC recurrence after curative treatment, which was almost equal to that obtained by IFN-based therapy. However, the risk of HCC recurrence almost never changes within a year and, therefore, strict surveillance should be performed at least within a year after curative treatment for primary HCC even if successful DAA treatment is achieved.

Acknowledgements

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KI, KT, TH, AS, MShir and MShim designed the study. KI analyzed the data and drafted the manuscript. KT supervised the treatment of the participants. KT, TH, AS and MShir contributed to the selection of the participants and collected the data. KT, TH, AS and MShir revised the manuscript, and MShim mainly reviewed and amended the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All study participants provided verbal informed consent, which was considered sufficient as the present study followed an observational research design that did not require new human specimens and instead relied only on preexisting samples. The authors declare that publication of clinical datasets does not compromise anonymity or confidentiality or breach local data protection laws, for the dataset to be considered for publication. The study design, including this consent procedure, was approved by the Ethics Committee of the Gifu University School of Medicine (Gifu, Japan).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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