Original Article

Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib

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Aim: Sorafenib is the first small molecule with significant clinical activity for advanced hepatocellular carcinoma (HCC). However, intolerable adverse events are sometimes observed. On the other hand, it has been reported that some toxicities of molecular targeted drugs, such as skin toxicities and arterial hypertension, are correlated with good clinical outcomes in other cancers.

Methods: We identified the correlations between adverse events and prognosis for sorafenib therapy in all patients with HCC treated at the institutions of the Saga Liver Cancer Study Group. The toxicities were assessed using the Common Terminology Criteria for Adverse Events version 4.0.

Results: Ninety-four patients received sorafenib until August 2010. The overall incidence of treatment-related adverse events was 98% of patients. Skin toxicities, including palmar-plantar erythrodysesthesia syndrome, rash, pruritus and alopecia, were the most common adverse events and were observed in 58 patients (62%). Hypertension was observed in 23 patients (24%). The median survival time was 12.5 months among the total patients. The patients with skin toxicities showed significantly longer survival than the patients without these toxicities (hazard ratio, 0.449; 95% confidence interval, 0.256–0.786; $P = 0.005$). Hypertension had no correlation with survival. Skin toxicities were also significant prognostic factors in a multivariate analysis (hazard ratio, 0.522; 95% confidence interval, 0.274–0.997; $P = 0.049$), along with Child–Pugh class and α-fetoprotein level. The median development time for skin toxicities was 21 days.

Conclusion: Skin toxicities occur commonly at the early phase in patients treated with sorafenib, and could be a promising surrogate marker for the treatment outcome.

Key words: adverse event, chemotherapy, liver carcinoma

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide.[1,2] It occurs frequently in the Asia–Pacific and Africa regions, and ranges 50–150/100 000 people per year. The prognosis of HCC depends on the stage of HCC at the time of diagnosis. One of the most widely used staging systems is the Barcelona Clinic Liver Cancer (BCLC) classification,[3] which comprises four categories: stage A, early HCC; stage B, intermediate HCC; stage C, advanced HCC; and stage D, end-stage HCC. Although patients with early HCC who can receive radical therapy have a good prognosis, patients with advanced HCC have a poor clinical outcome.

Sorafenib is the first targeted agent with significant clinical activity for advanced HCC. It is a small molecule that inhibits the activities of the serine-threonine kinases Raf-1 (c-Raf) and B-Raf; the receptor tyrosine kinases vascular endothelial growth factor receptor (VEGFR)-1, -2 and -3; and platelet-derived growth factor receptor (PDGFR)-α and -β.[4] Previous multicenter,

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double-blind, randomized phase III studies, the SHARP study[5] and the Asia–Pacific study,[6] showed statistically significant survival benefits compared with placebo in patients with advanced HCC, with hazard ratios of approximately 0.7. In these trials, sorafenib was characterized by a good tolerability profile, although intolerable adverse events were sometimes observed.[7] On the other hand, it has been reported that some toxicities of targeted agents, such as skin toxicities[8–15] and arterial hypertension,[16–19] are correlated with good clinical outcomes in other cancers. The aim of this study was to identify the correlations between these adverse events and prognosis for sorafenib treatment in patients with HCC.

METHODS

Patients and treatment

A RETROSPECTIVE ANALYSIS for all patients with HCC treated with sorafenib in Saga Prefecture was performed using the unified database system of the Saga Liver Cancer Study Group, which is composed of tertiary-care hospitals with specialists in liver cancer treatment in Saga Prefecture. All patients had histologically or radiologically confirmed HCC that was diagnosed as advanced, ineligible for resection or locoregional treatment, or refractory to chemoembolization. The inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status scores of 0–2, Child–Pugh scores of up to 8, and adequate hematologic and liver functions. Adequate hematologic functions were defined as a hemoglobin level of 8.5 g/dL or more, neutrophil count of more than 1500/μL and platelet count of more than 75 000/μL. Adequate liver functions were defined as alanine aminotransferase and aspartate aminotransferase levels of less than fivefold the normal upper limit and total bilirubin level of less than 2.0 mg/dL. Patients requiring hemodialysis were excluded. Patients were also considered ineligible if they received concomitant systemic therapy, including any targeted agents. The institutional review board or ethics committee of each institution approved this study protocol. All patients provided written informed consent before the treatment.

The patients received 400 mg of sorafenib twice daily. Initial dose reductions with consideration of each patient's condition were allowed. The treatment was continued until disease progression or intolerable drug-related toxicities occurred. Dose reductions (first to 400 mg once daily, and then to 400 mg every 2 days)

and interruptions were permitted for drug-related toxicities.

Assessment

Toxicities were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The patients were divided into groups according to the presence or absence of skin toxicities and hypertension related to sorafenib. Skin toxicities were considered to be palmar-plantar erythrodysesthesia syndrome, rash, pruritus and alopecia of at least grade 1 according to the CTCAE version 4.0. Patients with sorafenib-related hypertension were also chosen as at least grade 1. The treatment effects were then evaluated and compared between the groups. Radiological evaluations were carried out every 4–8 weeks using enhanced computed tomography or magnetic resonance imaging according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1.[20]

Statistical analysis

The proportions and antitumor effects between the two groups were compared using the Mann–Whitney U-test for continuous data and Cochran–Mantel–Haenszel χ^2-test for categorical data. The time to progression was calculated from the date of administration of sorafenib to the date of radiological progression or was censored at either the last follow up or at the time of death without evidence of radiological progression. The overall survival time was calculated from the date of administration of sorafenib to the date of death from any cause or was censored at the last follow up. The time to progression and survival time were estimated using the Kaplan–Meier method, and the survival curves were compared using the log–rank test. The treatment effects were adjusted using the Cox proportional hazards model. Differences with values of $P < 0.05$ were considered statistically significant. Data analyses were performed using R version 2.12.2 (The R Foundation for Statistical Computing).

RESULTS

Patient population and outcomes

S ORAFENIB MONOTHERAPY WAS initiated in 94 patients from July 2008 to August 2011, and their characteristics are shown in Table 1. Eighty-seven patients (93%) had a history of HCC treatment before sorafenib therapy. There were seven treatment-naïve patients with a BCLC stage of C. The Child–Pugh class of

Table 1 Baseline demographics

	Overall n = 94	Skin toxicities			Hypertension		
		Presence n = 58	Absence n = 36	P	Presence n = 23	Absence n = 71	P
Age, years, median (range)	75 (50–87)	75 (50–87)	76 (51–84)	0.674	75 (54–87)	75 (50–86)	0.546
Sex, n (%)							
Male	77 (82)	46 (79)	31 (86)	0.408	18 (78)	59 (83)	0.602
Female	17 (18)	12 (21)	5 (14)		5 (22)	12 (17)	
ECOG PS, n (%)							
0–1	91 (97)	57 (98)	34 (94)	0.307	23 (100)	68 (96)	0.319
2–	3 (3)	1 (2)	2 (6)		0	3 (4)	
Cause of disease, n (%)							
HCV	62 (66)	33 (57)	29 (81)	0.019	17 (74)	45 (63)	0.357
HBV	10 (11)	9 (15)	1 (2)		1 (4)	9 (13)	
Others	22 (23)	16 (28)	6 (17)		5 (22)	17 (24)	
Child–Pugh class, n (%)							
A	78 (83)	53 (91)	25 (69)	0.006	18 (78)	60 (85)	0.491
B	16 (17)	5 (9)	11 (31)		5 (22)	11 (15)	
BCLC stage, n (%)							
A	2 (2)	2 (3)	0	0.117	1 (5)	1 (2)	0.229
B	33 (35)	16 (28)	17 (47)		10 (43)	23 (32)	
C	59 (63)	40 (69)	19 (53)		12 (52)	47 (66)	
Macroscopic vascular invasion, n (%)	29 (31)	19 (33)	10 (28)	0.613	7 (30)	22 (31)	0.961
Extrahepatic spread, n (%)	40 (43)	26 (45)	14 (39)	0.573	7 (30)	33 (46)	0.179
AFP, ng/mL, median (range)	$131 (0–2.4 \times 10^5)$	$40 (0–2.4 \times 10^5)$	$753 (0–9.0 \times 10^4)$	0.050	$618 (1.8–2.4 \times 10^5)$	$91 (0–1.1 \times 10^5)$	0.667
DCP, mAU/mL, median (range)	$566 (0–1.0 \times 10^6)$	$378 (0–1.6 \times 10^5)$	$1932 (0–1.0 \times 10^6)$	0.068	$450 (12–4.9 \times 10^4)$	$639 (0–1.0 \times 10^6)$	0.510
Previous therapy, n (%)	87 (93)	54 (93)	33 (92)	0.798	21 (91)	66 (93)	0.794
Surgical resection, n (%)	38 (40)	26 (45)	12 (33)	0.272	6 (26)	32 (45)	0.109
TACE, n (%)	74 (79)	47 (81)	27 (75)	0.489	18 (78)	56 (79)	0.951
Ablation therapy, n (%)	51 (54)	33 (57)	18 (50)	0.516	15 (65)	36 (51)	0.227
Hepatic arterial infusion chemotherapy, n (%)	38 (40)	22 (38)	16 (44)	0.534	7 (30)	31 (44)	0.264
Systemic chemotherapy, n (%)	17 (18)	13 (22)	4 (11)	0.169	3 (13)	14 (20)	0.472
Radiotherapy, n (%)	9 (10)	3 (5)	6 (17)	0.067	0	9 (13)	0.074

AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; DCP, des-γ-carboxy-prothrombin; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBV, hepatitis B virus; HCV, hepatitis C virus; TACE, transarterial chemoembolization.

Table 2 Summary of treatment efficacy

	Overall n = 94	Skin toxicities		P	Hypertension		P
		Presence n = 58	Absence n = 36		Presence n = 23	Absence n = 71	
Response rate, %	6	4	12	0.170	6	6	0.902
Disease-control rate, %	49	49	50	0.940	44	51	0.637
Time to progression, months, median	2.9	3.6	2.0	0.718	5.1	2.9	0.587
Overall survival, months, median	12.5	16.8	5.9	0.004	17.0	11.1	0.332

16 patients (17%) was B. The Child–Pugh score was 7 points in nine patients and 8 points in all others. All Child–Pugh class B patients were administrated sorafenib according to the patients' wishes.

Sixty-six patients (70%) began sorafenib monotherapy at 800 mg daily. The median total dose and relative dose intensity of sorafenib in overall patients was 39 200 mg and 72%, respectively. Eighty-two patients stopped treatment because of disease progression (61%), toxicity (37%) or refusal (2%).

Fifty-one patients died, and the surviving patients had a median follow up of 9.4 months. Table 2 shows a summary of sorafenib treatment efficacies. The median time to progression and survival time in overall patients were 2.9 and 12.5 months, respectively. Eighty-one patients were evaluable for the objective tumor response. The objective response rate and disease-control rate were 6% and 49%, respectively.

Adverse events and treatment effects

The overall incidence of treatment-related adverse events was 98% of patients. Table 3 shows the incidences of sorafenib-related adverse events that occurred in at least 5% of patients. Skin toxicities were observed

Table 3 Adverse events that occurred in at least 5% of the patients as defined by the Common Terminology Criteria for Adverse Events version 4.0

Adverse events	Any n (%)	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)
Overall	92 (98)				
Skin toxicities	58 (62)				
Palmar-plantar erythrodysesthesia syndrome	52 (55)	17 (18)	25 (27)	10 (11)	–
Rash	9 (10)	6 (6)	2 (2)	1 (1)	0
Alopecia	7 (7)	5 (5)	2 (2)	–	–
Pruritus	2 (2)	1 (1)	1 (1)	0	–
Hypertension	23 (24)	1 (1)	17 (18)	5 (5)	0
Gastrointestinal disorders	44 (47)				
Diarrhea	31 (33)	13 (14)	14 (15)	4 (4)	0
Anorexia	19 (20)	11 (12)	5 (5)	3 (3)	0
Vomiting	4 (4)	2 (2)	2 (2)	0	0
Mucositis	3 (3)	3 (3)	0	0	0
Liver dysfunction	39 (41)				
AST or ALT increased	31 (33)	10 (11)	7 (7)	13 (14)	1 (1)
Bilirubin increased	10 (11)	2 (2)	2 (2)	5 (5)	1 (1)
Liver failure	6 (6)	–	–	3 (3)	3 (3)
Bleeding	9 (10)	2 (2)	3 (3)	4 (4)	0
Fever	7 (7)	5 (5)	2 (2)	0	0
Fatigue	7 (7)	3 (3)	2 (2)	2 (2)	–
Hoarseness	6 (6)	5 (5)	1 (1)	0	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

in 58 patients (62%). Among the skin toxicities, palmar-plantar erythrodysesthesia syndrome was the most common adverse event and was observed in 52 patients (55%). Hypertension was observed in 23 patients (24%). The patient baseline demographics with or without skin toxicities and hypertension are also shown in Table 1. There were significant differences in the cause of disease and the Child–Pugh class between the presence and absence of skin toxicities, while no differences were observed between the presence and absence of hypertension. The median total dose of sorafenib was 48 300 mg in the patients with skin toxicities, 23 800 mg in the patients without skin toxicities, 35 000 mg in the patients with hypertension and 39 200 mg in the patients without hypertension. There was a significant difference between the patients with and without skin toxicities ($P < 0.001$). On the other hand, the relative dose intensity in the patients with skin toxicities was lower than that in the patients without skin toxicities (median 69% and 90%, respectively; $P = 0.031$). The relative dose intensity in the patients with or without hypertension was comparable (median 73% and 71%, respectively; $P = 0.498$).

The patients with skin toxicities had a significantly longer survival than the patients without these toxicities (hazard ratio, 0.449; 95% confidence interval, 0.256–0.786; $P = 0.005$) (Fig. 1). In the patients with skin toxicities, the overall survival rate at 6 months was 79%. In the patients without skin toxicities, the overall survival rate at 6 months was 48%. The median survival time of patients with skin toxicities was 16.8 months and that of patients without skin toxicities was 5.9 months (Table 2). On the other hand, no statistically significant difference was noted among the patients stratified by sorafenib-related hypertension for survival (Table 2). According to the Cox proportional hazards model analysis, skin toxicities, Child–Pugh class A and lower serum α-fetoprotein level were significant, independent, good prognostic factors (Table 4). The median time to the first onset of skin toxicities was 21 days. There were no significant differences in antitumor responses or time to progression among the patients stratified by skin toxicities or hypertension related to sorafenib (Table 2).

DISCUSSION

THE PRESENT STUDY shows that patients with sorafenib-related skin toxicities might be associated with a good survival prognosis in HCC. There have been some reports regarding the relationships between skin toxicities caused by molecular targeted agents and their
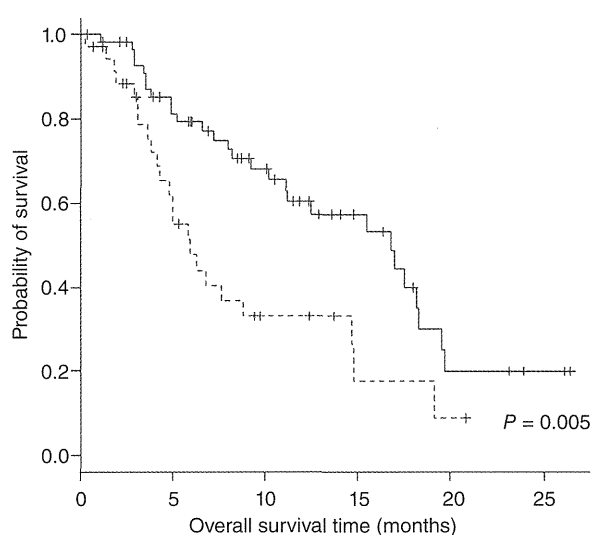

Figure 1 Kaplan–Meier analysis of overall survival stratified by skin toxicities. The patients with skin toxicities have significantly longer survival times than the patients without these toxicities ($P = 0.005$; log–rank test). (—), With skin toxicities; (---), without skin toxicities.

antitumor effects. Among patients treated with epidermal growth factor receptor inhibitors, those with drug-induced rash had a better correlation with response and/or survival in colorectal cancer,[8,9] non-small cell lung cancer,[10] head and neck squamous cell cancer,[11,12] ovarian cancer[13] and pancreatic cancer.[14,15] Regarding treatment with sorafenib, there is also a report that HCC patients who developed early skin toxicities showed a significantly longer time to disease progression.[21] However, there were no correlations between skin toxicities related to sorafenib and the antitumor response or time to progression in our study. Although targeted agents have been shown to have significant survival advantages, it is sometimes difficult to assess the antitumor response because of modest tumor shrinkage. In the SHARP study[5] and the Asia–Pacific study,[6] the objective response rates according to RECIST[22] were 2% and 3.3%, respectively. However, 71% and 54% of patients had stable disease, respectively. The RECIST criteria were originally developed to assess responses to cytotoxic agents and may not be appropriate indicators of activity for targeted agents that are associated with prolonged stable disease.[23] Modified RECIST criteria that measure viable (enhancement in the arterial phase) lesions were proposed for targeted therapies or locoregional thera-

Table 4 Univariate and multivariate Cox proportional hazard models for overall survival

Variables	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
HCV	1.012	0.577–1.778	0.966			
Child–Pugh A	0.256	0.124–0.526	<0.001	0.399	0.176–0.906	0.028
BCLC stage A-B	0.784	0.443–1.388	0.404			
AFP <100 ng/mL	0.372	0.211–0.656	<0.001	0.504	0.260–0.977	0.042
DCP <500 mAU/mL	0.766	0.442–1.329	0.343			
Relative dose intensity >50%	0.808	0.426–1.530	0.512			
Skin toxicities	0.449	0.256–0.786	0.005	0.522	0.274–0.997	0.049
Hypertension	0.713	0.362–1.406	0.329			

95% CI, 95% confidence interval; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; DCP, des-γ-carboxy-prothrombin; HCV, hepatitis C virus; HR, hazard ratio.

pies in HCC.[24] We also evaluated the antitumor response by the modified RECIST criteria, but found no links between sorafenib-related skin toxicities or hypertension and the antitumor response (data not shown). The development of an evaluation method for the antitumor response of sorafenib with regard to survival is necessary in the future.

In our cohort, the patients without skin toxicities contained more Child–Pugh class B patients than did patients with skin toxicities. The median survival time was 15.5 months in Child–Pugh A patients and 4.8 months in Child–Pugh B patients. This poor prognosis of Child–Pugh B patients might have influenced the result of our study. However, it is thought that skin toxicities associated with sorafenib therapy have a good correlation with survival because skin toxicities were a statistically significant factor for survival along with Child–Pugh class according to the multivariate analysis (Table 4). The total dose of sorafenib was higher, but the relative dose intensity was lower in the patients with skin toxicities than in the patients without skin toxicities. This paradoxical result is attributed to a longer treatment duration (median, 114 and 37 days, respectively; $P < 0.001$) and higher frequency of dose reduction or interruption of sorafenib (79% and 47%, respectively; $P = 0.001$) in the patients with and without skin toxicities.

Sorafenib is known to cause frequently occurring skin toxicities that include palmar-plantar erythrodysesthesia syndrome, facial erythema (rash or desquamation), pruritus, dry skin and alopecia.[5,6,25] Palmar-plantar erythrodysesthesia syndrome is the most common dermatological toxicity, and histological analyses of this symptom show a thickened epidermis with hyperkeratosis, non-specific inflammatory

dermal cell infiltrates and dilated dermal vessels.[25–27] Modifications in cytokeratin expression were observed after immunostaining with anti-cytokeratin antibodies, suggesting that sorafenib may affect keratinocyte differentiation. However, the pathogenesis of this syndrome caused by sorafenib has not been established because neither VEGF nor FLT-3 receptors are expressed in normal keratinocytes,[25] and immunostaining for c-kit and PDGFR showed no difference between areas of normal skin and the lesions of this syndrome.[28]

Hypertension is a common adverse event for inhibitors of angiogenesis, especially inhibitors of VEGFR signaling.[29,30] Arterial hypertension related to VEGFR inhibitors has good correlations with clinical outcomes in colorectal cancer,[16,17] pancreatic cancer[18] and renal cell cancer.[19] Although the mechanisms of the hypertension during antiangiogenic therapy have not been clarified, microvascular rarefaction may play an important role in the development of hypertension.[30] Regarding sorafenib therapy, there is a report that drug-induced hypertension can predict the clinical benefit in metastatic renal cell cancer.[31] Our study showed that there was no correlation between hypertension and clinical outcomes in patients with HCC. Further studies are needed to elucidate whether the alteration in blood pressure during sorafenib therapy is associated with an antitumor effect.

In conclusion, skin toxicities occur commonly at the early phase in HCC patients treated with sorafenib. Skin toxicities, mainly palmar-plantar erythrodysesthesia syndrome, can significantly affect the patient's quality of life, even though these toxicities are not usually life-threatening. Skin reactions could be a promising surrogate marker for the prognosis, and therefore early

identification and control of these reactions are critical for continuing sorafenib therapy.

REFERENCES

1 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557–76.

2 McGlynn KA, Tsao L, Hsing AW, Devesa SS, Fraumeni JF Jr. International trends and patterns of primary liver cancer. *Int J Cancer* 2001; **94**: 290–96.

3 Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329–38.

4 Wilhelm SM, Carter C, Tang L *et al.* BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004; **64**: 7099–109.

5 Llovet JM, Ricci S, Mazzaferro V *et al.* Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378–90.

6 Cheng AL, Kang YK, Chen Z *et al.* Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25–34.

7 Morimoto M, Numata K, Kondo M *et al.* Higher discontinuation and lower survival rates are likely in elderly Japanese patients with advanced hepatocellular carcinoma receiving sorafenib. *Hepatol Res* 2011; **41**: 296–302.

8 Cunningham D, Humblet Y, Siena S *et al.* Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337–45.

9 Van Cutsem E, Köhne CH, Hitre E *et al.* Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408–17.

10 Pérez-Soler R, Chachoua A, Hammond LA *et al.* Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *J Clin Oncol* 2004; **22**: 3238–47.

11 Soulieres D, Senzer NN, Vokes EE, Hidalgo M, Agarwala SS, Siu LL. Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. *J Clin Oncol* 2004; **22**: 77–85.

12 Herbst RS, Arquette M, Shin DM *et al.* Phase II multicenter study of the epidermal growth factor receptor antibody cetuximab and cisplatin for recurrent and refractory squamous cell carcinoma of the head and neck. *J Clin Oncol* 2005; **23**: 5578–87.

13 Gordon AN, Finkler N, Edwards RP *et al.* Efficacy and safety of erlotinib HCl, an epidermal growth factor receptor (HER1/EGFR) tyrosine kinase inhibitor, in patients with advanced ovarian carcinoma: results from a phase II multicenter study. *Int J Gynecol Cancer* 2005; **15**: 785–92.

14 Xiong HQ, Rosenberg A, LoBuglio A *et al.* Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II Trial. *J Clin Oncol* 2004; **22**: 2610–6.

15 Moore MJ, Goldstein D, Hamm J *et al.* Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960–6.

16 Scartozzi M, Galizia E, Chiorrini S *et al.* Arterial hypertension correlates with clinical outcome in colorectal cancer patients treated with first-line bevacizumab. *Ann Oncol* 2009; **20**: 227–30.

17 Österlund P, Soveri LM, Isoniemi H, Poussa T, Alanko T, Bono P. Hypertension and overall survival in metastatic colorectal cancer patients treated with bevacizumab-containing chemotherapy. *Br J Cancer* 2011; **104**: 599–604.

18 Spano JP, Chodkiewicz C, Maurel J *et al.* Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. *Lancet* 2008; **371**: 2101–8.

19 Bono P, Elfving H, Utriainen T *et al.* Hypertension and clinical benefit of bevacizumab in the treatment of advanced renal cell carcinoma. *Ann Oncol* 2009; **20**: 393–4.

20 Eisenhauer EA, Therasse P, Bogaerts J *et al.* New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228–47.

21 Vincenzi B, Santini D, Russo A *et al.* Early skin toxicity as a predictive factor for tumor control in hepatocellular carcinoma patients treated with sorafenib. *Oncologist* 2010; **15**: 85–92.

22 Therasse P, Arbuck SG, Eisenhauer EA *et al.* New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000; **92**: 205–16.

23 Ratain MJ, Eckhardt SG. Phase II studies of modern drugs directed against new targets: if you are fazed, too, then resist RECIST. *J Clin Oncol* 2004; **22**: 14S. abstract.

24 Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52–60.

25 Yang CH, Lin WC, Chuang CK *et al.* Hand-foot skin reaction in patients treated with sorafenib: a clinicopathological study of cutaneous manifestations due to multitargeted kinase inhibitor therapy. *Br J Dermatol* 2008; **158**: 592–6.

26 Autier J, Escudier B, Wechsler J, Spatz A, Robert C. Prospective study of the cutaneous adverse effects of sorafenib, a novel multikinase inhibitor. *Arch Dermatol* 2008; **144**: 886–92.

27 Robert C, Mateus C, Spatz A, Wechsler J, Escudier B. Dermatologic symptoms associated with the multikinase

inhibitor sorafenib. *J Am Acad Dermatol* 2009; **60**: 299–305.

28 Faivre S, Delbaldo C, Vera K *et al.* Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006; **24**: 25–35.

29 Sica DA. Angiogenesis inhibitors and hypertension: an emerging issue. *J Clin Oncol* 2006; **24**: 1329–31.

30 Lévy BI. Blood pressure as a potential biomarker of the efficacy angiogenesis inhibitor. *Ann Oncol* 2009; **20**: 200–3.

31 Ravaud A, Sire M. Arterial hypertension and clinical benefit of sunitinib, sorafenib and bevacizumab in first and second-line treatment of metastatic renal cell cancer. *Ann Oncol* 2009; **20**: 966–7.

Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C

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Abstract

Background Several epidemiological studies have reported that diabetes mellitus is a risk factor for hepatocellular carcinoma (HCC) in hepatitis C virus (HCV)-positive patients. However, it is unclear whether or not post-challenge hyperglycemia is a risk factor. The purpose of this study was to determine the association between post-challenge hyperglycemia and hepatocarcinogenesis in HCV-positive patients.

Methods A total of 203 HCV-RNA-positive subjects (108 males, mean age 54.3 ± 10.8 years; 95 females, mean age 56.6 ± 10.3 years; genotype 1b/2a/2b/3a: 152/38/12/1) who underwent liver biopsy and a 75-g oral glucose tolerance test, and who were treated with interferon (IFN) were enrolled in this study. None of the subjects had been treated with antidiabetic drugs. The subjects underwent ultrasonography and/or computed tomography every 6 months after the end of the IFN therapy.

Results Thirteen patients, including one patient who achieved a sustained viral response (SVR) with IFN, developed HCC. On multivariate analysis, male sex, age >65 years, excessive alcohol consumption, non-SVR, liver steatosis area >5% in liver specimens, and 120-min post-challenge hyperglycemia were risk factors for the development of HCC. After matching subjects for sex, age, alcohol intake, and response to the IFN therapy, advanced fibrosis stages [hazard ratio (HR) 2.8], liver steatosis (HR 5.4), and 120-min post-challenge hyperglycemia (HR 4.9)

were significant risk factors for the development of HCC. Furthermore, after matching for the fibrosis stage, liver steatosis (HR 5.7) and 120-min post-challenge hyperglycemia (HR 6.9) remained as significant factors for HCC development.

Conclusion Post-challenge hyperglycemia is an independent risk factor for HCC in HCV-positive patients.

Keywords Hyperglycemia · Oral glucose tolerance test · Hepatocellular carcinoma · Hepatitis C

Abbreviations

HCV	Hepatitis C virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
DM	Diabetes mellitus
HR	Hazard ratio
BMI	Body mass index
ALT	Alanine aminotransferase
HbA1c	Hemoglobin A1c
IFN	Interferon
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
HOMA-IR	Homeostasis model assessment for insulin resistance
SVR	Sustained viral response

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Introduction

Chronic hepatitis C virus (HCV) infection is a disease that can progress to cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Several factors associated with HCC

development in chronic HCV have been reported, including male sex, older age at infection, excessive alcohol consumption, coinfection with hepatitis B virus (HBV), and variations in HCV itself [3–6]. Recent epidemiological studies have shown that diabetes mellitus (DM) is also a risk factor for HCC in patients with chronic hepatitis C [7, 8], although studies in Taiwan revealed no association between DM and HCC [9, 10]. Therefore, it is still unclear whether or not DM is a significant risk factor for HCC.

Furthermore, despite the findings of these epidemiological studies, several issues remain unresolved. First, not all of the subjects in these studies underwent glucose tolerance tests, and DM was defined based on inconsistent criteria, with some studies defining diabetes based on the use of antidiabetic drugs such as insulin, the presence of fasting hyperglycemia, and/or abnormal levels of hemoglobin A1c (HbA1c). Accordingly, it is not clear whether specific components of DM, particularly post-prandial hyperglycemia, are risk factors for HCC. Second, no study has evaluated whether insulin resistance or hyperinsulinemia, which might develop in advance of hyperglycemia, is associated with the development of HCC. Third, it is unclear whether DM remains a risk factor for HCC after accounting for pathological liver findings such as fibrosis, inflammation, and steatosis, which are acknowledged risk factors for HCC [11–14]. Fourth, because many HCV-positive patients receive interferon (IFN) therapy, it is essential to consider the response to IFN therapies in such studies.

Therefore, considering these limitations of earlier studies, and the unanswered questions, we conducted a prospective cohort study of subjects with chronic hepatitis C, who underwent a 75-g oral glucose tolerance test (OGTT), liver biopsy, and IFN therapy.

Patients and methods

Patients

Overall, 203 HCV-positive subjects who underwent liver biopsy and a 75-g OGTT between 2002 and 2007 and who were treated with IFN were enrolled in this study (Table 1). All of the subjects were positive for serum HCV-RNA detected by polymerase chain reaction (PCR). Criteria for inclusion in the study were: hemoglobin ≥ 12 g/dl, leukocyte count $\geq 3,000/mm^3$, platelet count $\geq 90,000/\mu l$, and serum creatinine levels within the normal range. Patients were excluded if they had decompensated liver disease; were hepatitis B surface antigen-positive; or had a history of liver transplantation, neoplastic disease (including HCC), severe cardiac or chronic pulmonary disease, autoimmune disease, a psychiatric disorder, or severe retinopathy; or were planning on becoming pregnant. In this study, subjects who met the criteria of both fasting glucose level ≥ 126 mg/dl and HbA1c $\geq 6.5\%$, were diagnosed as having overt DM and excluded because they should be treated for DM prior to IFN therapy. Subjects who were treated with antidiabetic drugs or subcutaneous insulin infusion were excluded because it was difficult to perform the 75-g OGTT and analyze its results, and because it is unclear whether antidiabetic drugs affect HCC occurrence.

Because the duration of IFN therapy differed among the subjects, the end of the IFN regimen was defined as the start of the study observation period. The endpoint of this study was HCC occurrence. The protocol was approved by the Local Review Board in accordance with the ethical guidelines of the Declaration of Helsinki (1975, as revised in 1983). Written informed consent was obtained from all patients.

Physical examination, serum biochemistry, and OGTT

Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m²). Venous blood samples were taken from all patients at around 0800 hours after a 12-h overnight fast, to determine blood cell count and blood chemistry. Serum HCV-RNA levels were analyzed by reverse-transcriptase PCR (nested PCR or Amplicor; Roche Diagnostic Systems, CA, USA) and HCV genotypes were determined by reverse-transcriptase PCR (Roche Diagnostic Systems, CA, USA).

Insulin resistance was evaluated by the homeostasis model assessment for insulin resistance (HOMA-IR), using the following equation [15]: HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mg/dl)/405. The reference values for fasting glucose level and fasting insulin level in our clinical laboratory are 70–110 mg/dl and 4–24 μU/ml, respectively. However, we considered subjects with HOMA-IR of >2.5 as showing insulin resistance, according to a previous report [16].

All subjects underwent a 75-g OGTT. Samples were collected at baseline and every 30 min after glucose ingestion for 120 min to measure glucose and insulin levels. All examinations were performed up to 3 months before starting IFN therapy.

Liver histology

Liver needle biopsies were performed percutaneously with a 16-G needle (Super-Core™ semi-automatic biopsy instrument; InterV Clinical Products, Dartmouth, MA, USA) up to 3 months before starting IFN therapy. All subjects enrolled this study underwent liver biopsy. The

Table 1 Clinical characteristics of the patients

	Total number of patients ($n = 203$)	Patients with HCC ($n = 13$)
Age (years)[a]	55.4 ± 10.6	63.1 ± 6.5
Female % (M/F, n)	46.8 (108/95)	7.7 (11/1)
Alcohol consumption		
Excessive/daily/social or none, n	21/30/152	4/0/9
BMI[a]	23.5 ± 3.0	23.7 ± 2.7
IFN therapy history (naïve/>2), n	132/71	6/7
ALT (IU/l)[a]	71.3 ± 55.0	76.3 ± 44.8
Platelets ($\times 10^4/\mu l$)[a]	16.3 ± 6.2	12.1 ± 3.2
AFP (ng/ml)[a]	15.0 ± 37.8	14.3 ± 9.6
Viral load ($\times 10^6$ IU/ml)[a]	1.8 ± 1.5	1.5 ± 1.1
Genotype (1b/2a/2b/3a)[a]	152/38/12/1	12/0/1/0
Fasting glucose (mg/dl)[a]	86.7 ± 9.3	89.9 ± 14.4
Fasting insulin (μU/ml)[a]	9.4 ± 5.5	10.9 ± 7.8
HOMA-IR[a]	2.0 ± 1.3	2.7 ± 1.9
Liver histology		
A0/A1/A2/A3, n	1/71/106/25	0/3/8/2
F0/F1/F2/F3/F4, n	2/91/63/37/10	0/1/6/5/1
Steatosis <5/5–9/>10%, n	175/15/13	7/3/3
Response to IFN therapy; SVR, n (%)	89 (44.3)	1 (7.7)

HCC hepatocellular carcinoma, *BMI* body mass index, *IFN* interferon, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance, *SVR* sustained viral response

[a] Data are expressed as means ± SD

liver biopsy specimen was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin–eosin and Azan for histological evaluation. Pathological liver fibrosis and inflammation activity were evaluated according to the METAVIR scoring system (stages 0–4 for fibrosis and grades 0–4 for inflammatory activity) [17]. The area of steatosis in the liver specimen was calculated using Image J 1.42 (http://rsb.info.nih.gov/ij/). All liver biopsy specimens were evaluated by three experienced pathologists who were unaware of the clinical conditions of the patients.

Therapy and follow-up protocol

Between 2002 and 2003, all of the treatment-naïve (hereafter, 'naïve') patients with genotype 1b/high viral load (>100 KIU/ml) and patients refractory to prior IFN therapy were treated with either IFNα2a or IFNβ plus oral ribavirin at body weight-dependent doses (total dose: 600 mg for patients <60 kg; 800 mg for patients weighing 60–80 kg; 1,000 mg for patients weighing ≥80 kg). Between 2004 and 2007, all of the naïve patients with genotype 1b/high viral load and patients refractory to prior IFN therapy were treated with either pegylated (Peg)-IFNα2a (180 μg/week subcutaneously) or Peg-IFNα2b (1.5 μg/kg/week subcutaneously) plus oral ribavirin in body weight-dependent doses. Patients with genotype 1b were treated for 48 weeks, while all other patients were treated for 24 weeks. Between 2002 and 2003, non-genotype 1b and low viral load

(<100 KIU/ml) naïve patients were treated with IFNα2a or IFNβ for 24 weeks, and between 2004 and 2007, such patients were treated with Peg-IFNα2a (180 μg/week subcutaneously) monotherapy for 24 weeks. Patients were not randomized to therapy and the selection of the therapeutic protocol was at the study physicians' discretion.

Ultrasonography and/or computed tomography were performed every 6 months in all patients. At 6 months after the end of treatment, patients with a negative qualitative HCV-RNA test were considered to have a sustained viral response (SVR). Patients with a negative qualitative HCV-RNA test at the end of therapy and a positive HCV RNA text after therapy were considered to show relapse. Patients who never achieved viral clearance during therapy were considered non-responders.

Statistical analysis

Comparisons between groups were made using the Mann–Whitney U test for continuous variables and the χ^2 test for categorical data. Changes in biological parameters in each group were assessed using paired t tests. Continuous variables are summarized as means ± SD. Differences were considered significant at $P < 0.05$. The Cox proportional hazard regression model was used for univariate and multivariate analyses to determine the risk of HCC occurrence. Significant variables on univariate analyses were included in the multivariate analyses. In the multivariate analyses, up to three subjects without HCC

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occurrence were randomly selected for each patient with HCC, and were matched by sex, age, alcohol intake, response to IFN therapy, and fibrosis stage. Statistical analyses were performed with SPSS II (SPSS Japan, Tokyo, Japan).

Results

Subject characteristics

The clinical characteristics of the 203 patients (108 males, mean age 54.3 ± 10.8 years; 95 females, mean age 56.6 ± 10.3 years) enrolled in this study are summarized in Table 1. The average observation time was 52.0 ± 19.5 months. Twenty-one (10.3%) patients were classified as having excessive alcohol intake (>50 g ethanol per day). Using the METAVIR scoring system, fibrosis was staged as F0 in two patients (1%), F1 in 91 (44.8%), F2 in 63 (31%), F3 in 37 (18.2%), and F4 in 10 (4.9%). All liver biopsy specimens taken before therapy showed typical features of chronic HCV infection, including infiltration of lymphocytes in Glisson's capsule, piecemeal necrosis, and periportal fibrosis. The average area of steatosis in the liver specimens was 2.6 ± 3.1%. During the observation period, 13 patients, including one patient who achieved SVR with IFN therapy, developed HCC (12 males and 1 female, mean age 62.8 ± 6.7 years).

OGTT results

The serum glucose and insulin levels during the 75-g OGTT are shown in Fig. 1a, b. In patients who developed HCC (HCC group), the glucose levels at 30 ($P = 0.002$), 90 ($P = 0.033$), and 120 ($P = 0.001$) min, and the insulin levels at 30 min ($P = 0.017$) were significantly higher than those in patients without HCC (non-HCC group). There

were no significant differences in fasting glucose or insulin levels between the HCC group and the non-HCC group.

Univariate and multivariate analyses of risk factors for HCC

On univariate analyses, male sex [hazard ratio (HR) 10.5], age >65 years (HR 5.3), excessive alcohol consumption (HR 4.6), non-SVR after IFN therapy (HR 9.5), advanced liver fibrosis (HR 2.9), α-fetoprotein >10 ng/ml (HR 4.6), liver steatosis area >5% (HR 5.7), and 120-min post-challenge hyperglycemia (>200 mg/dl; HR 6.3) were significant risk factors for the development of HCC (Table 2). BMI, fasting glucose, fasting insulin, insulin levels during the 75-g OGTT, HOMA-IR, cholesterol, and triglyceride were not associated with the development of HCC. Furthermore, viral load and genotype, and IFN therapy protocols were not associated with the development of HCC.

On multivariate analyses, male sex, age >65 years, excessive alcohol consumption, non-SVR, liver steatosis area >5%, and 120-min post-challenge hyperglycemia were risk factors for the development of HCC (Table 3). When we limited the analyses to the HCC group ($n = 13$) and non-HCC patients ($n = 30$) matched for sex (male; $n = 27$), age (>65 years; $n = 8$), alcohol intake (excessive alcohol intake; $n = 8$), and response to IFN therapy (SVR; $n = 3$), advanced fibrosis stage (HR 2.8), liver steatosis area >5% (HR 5.4), and 120-min post-challenge hyperglycemia (HR 4.9) were significant risk factors for the development of HCC. When we matched patients for fibrosis stage (advanced fibrosis stage; $n = 10$) as well as the above factors (male; $n = 27$, age >65 years; $n = 9$, excessive alcohol intake; $n = 8$, SVR; $n = 3$), liver steatosis area >5% (HR 5.7), and 120-min post-challenge hyperglycemia (HR 6.9) remained as significant factors associated with the development of HCC.

Fig. 1 a Serum glucose levels and b insulin levels on 75-g oral glucose tolerance test (OGTT). *Open triangles* patients who developed hepatocellular carcinoma (*HCC*). *Open circles* patients without HCC. *P < 0.05 by Mann–Whitney *U* test. *Error bar* ± standard deviation
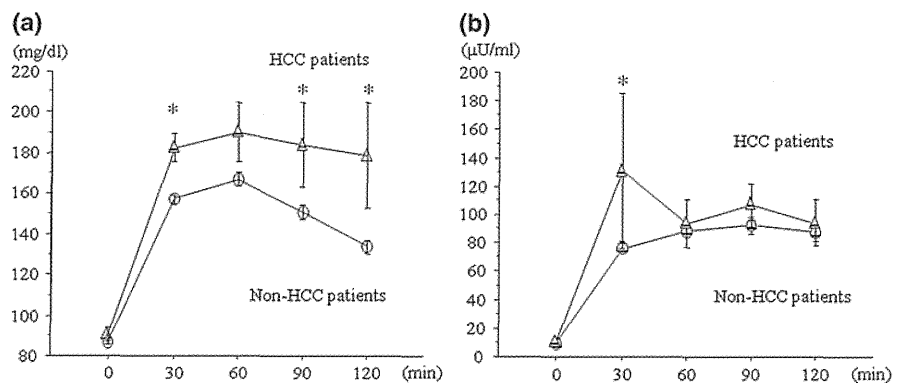
Table 2 Univariate analyses: comparison of the risk factors for HCC between 13 patients with HCC and non-HCC patients

Variable	HCC ($n = 13$)	Non-HCC ($n = 190$)	HR (95% CI)
Male	12 (92.3)	96 (50.5)	10.5 (1.4–81.0)*
Age >65 years	6 (46.2)	29 (15.3)	5.3 (1.8–16.0)*
Excessive alcohol consumption[a]	4 (30.8)	17 (8.9)	4.6 (1.4–15.0)*
Response to IFN therapy; non-SVR	12 (92.3)	101 (53.2)	9.5 (1.2–73.2)*
Fibrosis stage; F3 and F4	6 (46.2)	41 (21.6)	2.9 (1.1–8.7)*
BMI >25	5 (38.5)	53 (27.9)	1.5 (0.5–4.7)
AFP >10 ng/ml	8 (61.5)	50 (26.3)	4.6 (1.4–15.2)*
Steatosis >5%	6 (46.2)	22 (11.6)	5.7 (1.9–17.1)*
Fasting glucose ≥126 mg/dl	1 (7.7)	2 (1.1)	6.4 (0.8–50.0)
Fasting insulin ≥15 μU/ml	2 (15.4)	29 (15.3)	0.9 (0.2–4.0)
HOMA-IR ≥3	2 (15.4)	33 (17.4)	0.7 (0.2–3.4)
120-min post-challenge hyperglycemia[b]	5 (38.5)	15 (7.9)	6.3 (2.0–19.1)*

Data are expressed as numbers (%)

HR hazard ratio, *CI* confidence interval, *BMI* body mass index, *IFN* interferon, *SVR* sustained viral response, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance

* $P < 0.05$

[a] More than 50 g ethanol/day

[b] Serum glucose level was more than 200 mg/dl at 120 min on 75-g oral glucose tolerance test (OGTT)

Table 3 Multivariate analyses: comparison of the risk factors for HCC between 13 patients with HCC and non-HCC patients

Variable	HCC	Non-matched		Sex, age, alcohol intake, response to IFN matched		Sex, age, alcohol intake, response to IFN, fibrosis stage matched	
	$n = 13$	$n = 190$	HR (95% CI)	$n = 30$	HR (95% CI)	$n = 30$	HR (95% CI)
Male	12 (92.3)	96 (50.5)	18.8 (2.2–161.4)*	Matched	–	matched	–
Age >65 years	6 (46.2)	29 (15.3)	9.9 (2.5–39.9)*	Matched	–	Matched	–
Excessive alcohol consumption[a]	4 (30.8)	17 (8.9)	7.2 (1.4–37.5)*	Matched	–	Matched	–
Response to IFN therapy; non-SVR	12 (92.3)	101 (53.2)	20.4 (2.1–200.9)*	Matched	–	Matched	–
Fibrosis stage; F3 and F4	6 (46.2)	41 (21.6)	6.3 (1.2–33.3)*	8 (26.7)	2.8 (1.0–11.3)*	Matched	–
AFP >10 ng/ml	8 (61.5)	50 (26.3)	1.3 (0.4–1.8)	15 (50)	0.5 (0.7–3.1)	15 (50)	0.4 (0.1–2.2)
Steatosis >5%	6 (46.2)	22 (11.6)	5.6 (1.4–22.6)*	3 (10)	5.4 (1.1–27.3)*	2 (3.3)	5.7 (1.2–27.1)*
120-min post-challenge hyperglycemia[b]	5 (38.5)	15 (7.9)	19.5 (3.7–104.1)**	4 (13.3)	4.9 (1.3–18.9)*	2 (6.7)	6.9 (1.7–28.4)*

Data are expressed as numbers (%)

HR hazard ratio, *CI* confidence interval, *IFN* interferon, *SVR* sustained viral response, *AFP* alpha-fetoprotein

* $P < 0.05$, ** $P < 0.001$

[a] More than 50 g ethanol/day

[b] Serum glucose level was more than 200 mg/dl at 120 min on 75-g OGTT

Clinical characteristics of patients with post-challenge hyperglycemia

The clinical characteristics of 20 patients with 120-min post-challenge hyperglycemia on the 75-g OGTT and the remaining 183 patients are summarized and compared in Table 4. Fasting glucose levels and the HCC occurrence rate were significantly higher in patients with post-challenge hyperglycemia. On the other hand, the SVR rates were not significantly different, being 40% in patients with post-challenge hyperglycemia and 44.8% in patients without post-challenge hyperglycemia. The rate of patients with advanced liver fibrosis was higher in patients with post-challenge hyperglycemia than in the other patients, although the difference was not statistically significant.

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Table 4 Clinical characteristics of the patients with/without 120-min post-challenge hyperglycemia

	Post-challenge hyperglycemia		P value
	With ($n = 20$)	Without ($n = 183$)	
Age (years)[a]	58.2 ± 8.3	55.1 ± 10.8	0.223
Female % (M/F, n)	35 (13/7)	48 (95/88)	0.265
Alcohol consumption			
Excessive or habitual/social or none, n	2/18	28/155	0.523
BMI[a]	23.9 ± 2.5	23.4 ± 3.0	0.477
ALT (IU/l)[a]	68.1 ± 35.1	71.7 ± 56.6	0.781
Platelets ($\times 10^4/\mu l$)[a]	16.0 ± 6.0	16.3 ± 6.2	0.830
AFP (ng/ml)[a]	21.0 ± 44.4	14.4 ± 37.0	0.458
Viral load ($\times 10^6$ IU/ml)[a]	1.4 ± 1.3	1.8 ± 1.6	0.205
Genotype (1b/non-1b), n	15/5	137/46	0.989
Fasting glucose (mg/dl)[a]	96.5 ± 15.9	85.8 ± 8.5	<0.001
Fasting insulin (μU/ml)[a]	9.4 ± 4.5	9.3 ± 5.6	0.978
HOMA-IR[a]	2.4 ± 1.4	2.0 ± 1.2	0.228
Liver histology			
A0–1/A2–3, n	4/16	68/115	0.128
F0–2/F3–4, n	12/8	144/39	0.060
Steatosis <5/5–9/>10%, n	17/0/3	158/15/10	0.122
Response to IFN therapy; SVR, n (%)	8 (40)	82 (44.8)	0.681
HCC occurrence, n (%)	5 (25)	8 (4.4)	<0.001

BMI body mass index, *IFN* interferon, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance, *SVR* sustained viral response

[a] Data are expressed as means ± SD

Cumulative HCC occurrence rate

The cumulative HCC occurrence rates in the patients with 120-min post-challenge glucose levels of ≥200 and those with levels of <200 mg/dl are shown in Fig. 2a. While the HCC occurrence rates at 3 and 5 years were 3.3 and 4.3% in patients with 120-min glucose <200 mg/dl, the corresponding rates were 15.0 and 28.1% in patients with 120-min glucose ≥200 mg/dl. There was a significant difference in the HCC occurrence rate between patients with 120-min glucose <200 versus those with ≥200 mg/dl ($P < 0.001$).

Figure 2b shows the cumulative HCC occurrence rates in patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5%. The rates at 3 and 5 years were 14.3 and 20.4% in patients with a liver steatosis area of >5% versus 2.9% and 4.7%, respectively, in patients with a liver steatosis area of ≤5%. There was a significant difference in the HCC occurrence rate between patients with a liver steatosis area of ≤5% versus those with a liver steatosis area of >5% ($P < 0.001$).

Comparison of 75-g OGTT results between patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5%

The serum glucose and insulin levels during the 75-g OGTT in patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5% are shown in Fig. 3a, b. There were no differences in glucose levels between the two groups. In contrast, fasting and 30-min insulin levels were significantly higher in patients with a liver steatosis area of >5% versus those with a liver steatosis area of ≤5% (fasting insulin: 11.4 ± 6.0 vs. 9.0 ± 5.3 μU/ml, $P = 0.035$; 30-min insulin: 118.9 ± 147.6 vs. 73.4 ± 51.8 μU/ml, $P = 0.003$).

Discussion

This study has revealed that post-glucose challenge hyperglycemia is an independent risk factor for the development of HCC in chronic hepatitis C patients without overt DM or those who are not being treated with antidiabetic drugs.

Although it is unclear why post-challenge hyperglycemia influences hepatic carcinogenesis, we assumed that the mechanism might involve oxidative stress associated with an acute increase in glucose levels. Four of the five patients with HCC and glucose levels of >200 mg/dl at 120-min after the glucose load had normal fasting glucose levels. A previous study showed that acute glucose fluctuations caused greater oxidative stress than sustained chronic hyperglycemia in patients with type 2 DM [18]. Moreover, the activation of oxidative stress as a result of hyperglycemia plays an important role in the pathogenesis of
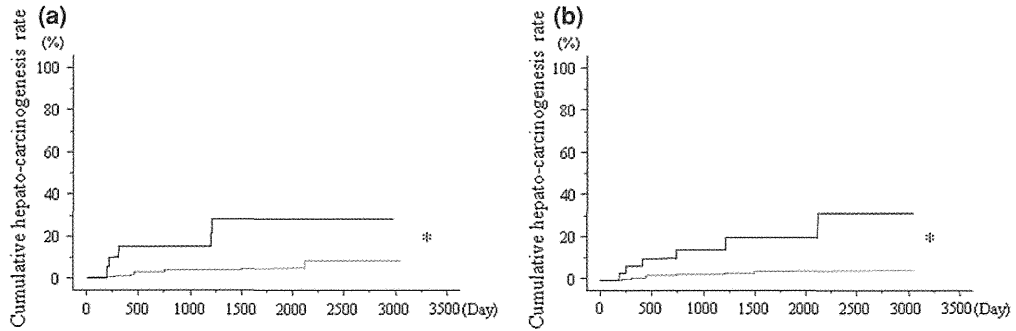
Fig. 2 a The cumulative HCC occurrence rates in patients with 120 min post-challenge hyperglycemia (serum glucose level more than 200 mg/dl at 120 min on 75-g GTT; *thick line*) and patients without hyperglycemia (serum glucose level less than 200 mg/dl at 120 min on 75-g GTT; *thin line*). **b** The cumulative HCC occurrence rates in patients with liver steatosis of more than 5% (*thick line*) and patients with liver steatosis of 5% or less (*thin line*). *$P < 0.001$ by log-rank test

Fig. 3 a Serum glucose levels and **b** insulin levels on 75-g OGTT. *Open triangles* patients with liver steatosis of more than 5%. *Open circles* patients with liver steatosis of 5% or less. *$P < 0.05$ by Mann–Whitney *U* test. *Error bar* ± standard deviation
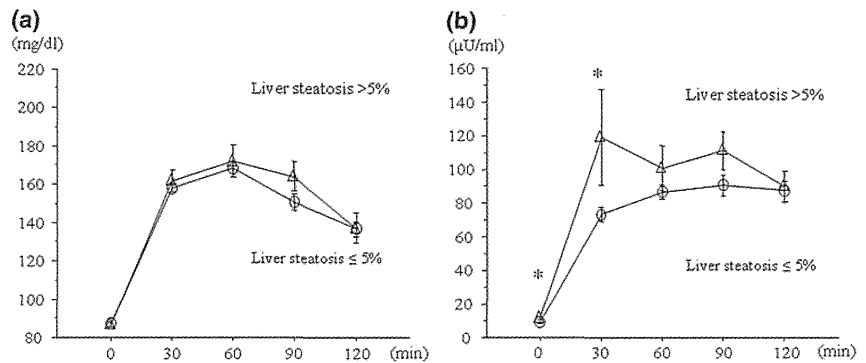
diabetic complications and carcinogenesis [19]. It was also reported that DNA damage caused by oxidative stress could be associated with hepatocarcinogenesis [20, 21]. Because it was previously demonstrated that the post-challenge glucose level was correlated with post-prandial glucose and HbA1c levels [22, 23], the patients with post-challenge hyperglycemia were assumed to have been exposed to daily fluctuations in glucose levels and oxidative stress. These findings might explain why the post-challenge glucose level, but not fasting glucose, was associated with HCC occurrence in the present study. However, further studies that include the assessment of oxidative stress are needed to elucidate the association between acute glucose fluctuations and hepatocarcinogenesis.

Hyperinsulinemia caused by insulin resistance is a well-known carcinogenic factor in several organs, including the liver [24, 25]. It has been shown that HCV itself, including its core protein, induces insulin resistance by impairing the insulin signaling pathway [26, 27]. It was also reported that insulin resistance was more severe in chronic HCV-infected patients than in patients with chronic hepatitis caused by another etiology [28, 29]. However, our study failed to show any associations between HOMA-IR or fasting

insulin and the development of HCC. Instead, we found that hepatic steatosis was an independent risk factor for HCC. Moreover, we found significant differences in the fasting and 30-min insulin levels after a glucose load, but not in glucose levels at any time, between patients with and without steatosis, so that HOMA-IR and the area under the curve of insulin concentrations during the 75-g OGTT were higher in patients with liver steatosis (data was not shown). These findings suggest that hyperinsulinemia or insulin resistance might influence hepatic carcinogenesis via hepatic steatosis.

Konishi et al. [30] reported that post-challenge hyperglycemia, but not insulin resistance, was a risk factor for HCC occurrence in chronic hepatitis C patients. Although their results are similar to our own, there is a difference in terms of whether or not hepatic steatosis is a risk factor for HCC occurrence. The discrepancy between these two studies might be due to the methods used to measure liver steatosis. In our study, an image analyzer was used to precisely measure the fat-occupied area, thus allowing us to include the actual area of steatosis in the analyses.

Liver fibrosis can not only cause hepatic cancer, but it can also cause insulin resistance and glucose intolerance.

To overcome any potential bias due to liver fibrosis, we performed case-matched multivariate analyses. These analyses showed that post-challenge hyperglycemia and hepatic steatosis were associated with the development of HCC, independent of hepatic fibrosis.

DM is generally diagnosed based on pre- or post-prandial blood glucose, HbA1c, or glycoalbumin levels. However, it was previously reported that fasting glucose, HbA1c, and glycoalbumin were inadequate tests for the diagnosis of impaired glucose tolerance in patients with advanced liver fibrosis [31, 32]. Although the measurement of post-prandial blood glucose might be an easy method to determine post-challenge hyperglycemia, these values are likely to fluctuate according to the meal content or the length of time after the meal. Therefore, we believe that OGTTs are an indispensable and useful method to detect post-challenge hyperglycemia and to predict the risk of HCC in chronic HCV-infected patients.

It is still unclear what stage in the progression of glucose intolerance carries the greatest risk for HCC. This is because the earlier cohort studies that investigated possible associations between DM and HCC occurrence did not use consistent diagnostic criteria for "overt DM" [7–10]. Because the glucose levels at 120 min during an OGTT are more precise and sensitive parameters for the diagnosis of glucose intolerance than the evaluation of pre- and/or post-prandial hyperglycemia [33, 34], our data suggest that the stages of DM/glucose intolerance preceding "overt DM" may also be associated with HCC occurrence.

Our study revealed significant differences in glucose levels not only at 120 min, but also at 30 and 90 min during OGTTs, between the HCC and non-HCC patients. Furthermore, the 30- and 90-min glucose levels were significant risk factors for HCC on univariate analyses (30 min > 175 mg/dl: HR 4.3, 95% CI 1.4–13.1; 90 min > 175 mg/dl: HR 3.6, 95% CI 1.2–11.1). However, these HRs were smaller than the HR for 120-min and they were not significant on multivariate analysis. Interestingly, according to previous studies, such as DECODE and DECODA, 120-min post-challenge glucose levels were associated with increased risks for macrovascular events and heart disease-related death [35, 36]. Although the mechanisms underlying these associations are not yet fully understood, it seems that 120-min post-challenge hyperglycemia is an important factor involved in several events.

There is no doubt that the eradication of HCV with IFN is an effective approach to reduce the risk of HCC in chronic HCV-infected patients [37]. Our data indicate that the SVR achieved by IFN treatment is a significant factor that inhibits the development of HCC. Recently, it was reported that HCV infection per se downregulated the cell surface expression of the glucose transporter [38]. We have previously reported that the eradication of HCV contributes to improvements in insulin resistance and post-challenge hyperglycemia [39]. These findings suggest that the eradication of HCV by IFN therapy contributes to improvements in glucose intolerance. According to our present results, however, post-challenge hyperglycemia was independent of the IFN response, which means that patients with both glucose intolerance and sustained HCV infection are at increased risk for HCC. These results indicate that improvement of glucose intolerance should be considered as one of the strategies to prevent HCC in patients with chronic hepatitis C, particularly those in whom HCV cannot be eradicated.

A limitation of our study is that the severity of glucose intolerance might change following IFN therapy, because of HCV eradication or because of adverse effects of IFN such as anorexia and body weight loss. To confirm whether or not glucose intolerance is a true risk factor for HCC, future studies should include continued assessment of glucose tolerance following IFN therapy.

In conclusion, the assessment of post-challenge hyperglycemia using a 75-g OGTT is useful for estimating the risk of HCC in HCV-positive patients. Future studies are needed to elucidate the underlying mechanism and identify possible treatments to further reduce the risk of HCC.

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41–52.
2. Poynard T, Bedossa P, Oplon P, for the OBSTIRC, METAVIR, CLINIVIR and DOSVIRC groups. Nature history of liver fibrous progression in patients with chronic hepatitis C. Lancet. 1997;349:825–32.
3. Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med. 1999;340:1228–33.
4. Wiley TE, McCarthy M, Breidi L, McCarthy M, Layden TJ. Impact of alcohol on the histological and clinical progression of hepatitis C infection. Hepatology. 1998;28:805–9.
5. Liaw YF, Chen YC, Sheen IS, Chien RN, Yeh CT, Chu CM. Impact of acute hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. Gastroenterology. 2004;126: 1024–9.
6. Hung CH, Chen CH, Lee CM, Wu CM, Hu TH, Wang JH, et al. Association of amino acid variations in the NS5A and E2-PePHD region of hepatitis C virus 1b with hepatocellular carcinoma. J Viral Hepat. 2008;15:58–65.
7. Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatology. 2008;47:1856–62.
8. Chen CL, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, et al. Metabolic factors and risk of hepatocellular carcinoma by chronic

hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology. 2008;135:111–21.

9. Tung HD, Wang JH, Tseng PL, Hung CH, Kee KM, Chen CH, et al. Neither diabetes mellitus nor overweight is a risk factor for hepatocellular carcinoma in a dual HBV and HCV endemic area: community cross-sectional and case–control studies. Am J Gastroenterol. 2010;105:624–31.

10. Lai MS, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: a cohort study in high prevalence area of hepatitis virus infection. Hepatology. 2006;43:1295–302.

11. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Inhibition of hepatocarcinogenesis by interferon therapy. Ann Intern Med. 1999;131:174–81.

12. Fattovich G, Giustina G, Degos F, Diodati G, Tremolada F, Nevens F, et al. Effectiveness of interferon alfa on incidence of hepatocellular carcinoma, decompensation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol. 1997;27:201–5.

13. Tarao K, Rino Y, Ohkawa S, Shimizu A, Tamai S, Miyakawa K, et al. Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus associated cirrhosis. Cancer. 1999;86:589–95.

14. Pekow JR, Bhan AK, Zheng H, Chung RT. Hepatic steatosis is associated with increased frequency of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. Cancer. 2007;109: 2490–946.

15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.

16. Ikeda Y, Suehiro T, Nakamura T, Kumon Y, Hashimoto K. Clinical significance of the insulin resistance index as assessed by homeostasis model assessment. Endocr J. 2001;48:81–6.

17. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996; 24: 289-293.

18. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA. 2006;295:1681–7.

19. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615–25.

20. Tanaka H, Fujita N, Sugimoto R, Urawa N, Horiike S, Kobayashi Y, et al. Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. Br J Cancer. 2008;98:580–6.

21. Jüngst C, Cheng B, Gehrke R, Schmitz V, Nischalke HD, Ramakers J, et al. Oxidative damage is increased in human liver tissue adjacent to hepatocellular carcinoma. Hepatology. 2004; 39:1663–72.

22. Oka R, Hifumi S, Kobayashi J, Mabuchi H, Asano A, Yagi K, et al. The relationship between post-prandial plasma glucose and post-challenge plasma glucose in Japanese population. Diabetes Res Clin Pract. 2007;78:282–8.

23. Ito C, Maeda R, Ishida S, Sasaki H, Harada H. Correlation among fasting plasma glucose, two-hour plasma glucose levels in OGTT and HbA1c. Diabetes Res Clin Pract. 2000;50:225–30.

24. Tsugane S, Inoue M. Insulin resistance and cancer: epidemiological evidence. Cancer Sci. 2010;101:1073–9.

25. El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol. 2006;4:369–80.

26. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology. 2004;126:840–8.

27. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol. 2004;165:1499–508.

28. Persico M, Masarone M, La Mura V, Persico E, Moschella F, Svelto M, et al. Clinical expression of insulin resistance in hepatitis C and B virus-related chronic hepatitis: differences and similarities. World J Gastroenterol. 2009;15:462–6.

29. Eguchi Y, Mizuta T, Ishibashi E, Kitajima Y, Oza N, Nakashita S, et al. Hepatitis C virus infection enhances insulin resistance induced by visceral fat accumulation. Liver Int. 2009;29:213–20.

30. Konishi I, Hiasa Y, Shigematsu S, Hirooka M, Furukawa S, Abe M, et al. Diabetes pattern on the 75 g oral glucose tolerance test is a risk factor for hepatocellular carcinoma in patients with hepatitis C virus. Liver Int. 2009;29:1194–201.

31. Cacciatore L, Cozzolino G, Giardina MG, De Marco F, Sacca L, Esposito P, et al. Abnormalities of glucose metabolism induced by liver cirrhosis and glycosylated hemoglobin levels in chronic liver disease. Diabetes Res. 1988;7:185–8.

32. Bando Y, Kanehara H, Toya D, Tanaka N, Kasayama S, Koga M. Association of serum glycated albumin to haemoglobin A1C ratio with hepatic function tests in patients with chronic liver disease. Ann Clin Biochem. 2009;46:368–72.

33. Qiao Q, Lindström J, Valle TT, Tuomilehto J. Progression to clinically diagnosed and treated diabetes from impaired glucose tolerance and impaired fasting glycaemia. Diabet Med. 2003; 20:1027–33.

34. Nakagami T, Tajima N, Oizumi T, Karasawa S, Wada K, Kameda W, et al. Hemoglobin A1c in predicting progression to diabetes. Diabetes Res Clin Pract. 2010;87:126–31.

35. DECODE Study Group, European Diabetes Epidemiology Group. Is the current definition for diabetes relevant to mortality risk from all causes and cardiovascular and noncardiovascular diseases? Diabetes Care. 2003;26:688–96.

36. Nakagami T, DECODA Study Group. Hyperglycaemia and mortality from all causes and from cardiovascular disease in five populations of Asian origin. Diabetologia. 2004;47:385–94.

37. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet. 1995;346:1051–5.

38. Kasai D, Adachi T, Deng L, Nagano-Fujii M, Sada K, Ikeda M, et al. HCV replication suppresses cellular glucose uptake through down-regulation of cell surface expression of glucose transporters. J Hepatol. 2009;50:883–94.

39. Kawaguchi T, Mizuta T, Oza N, Takahashi H, Ario K, Akiyama T, et al. Eradication of hepatitis C virus by interferon improves whole-body insulin resistance and hyperinsulinaemia in patients with chronic hepatitis C. Liver Int. 2009;29:871–7.

Editorial

Can exercise be a new approach for chronic hepatitis C?

Hepatitis C virus (HCV) is a major cause of chronic liver disease with an estimated 170 million carriers worldwide, and leads to cirrhosis and hepatocellular carcinoma (HCC).[1,2] Although therapies for chronic hepatitis C (CHC) have improved substantially during the last two decades, the sustained virological response rate is still around 50% in patients infected with HCV genotype 1 and around 80% in patients infected with HCV genotype 2 or 3. Therefore, we need to take measures to restrain the disease progression, together with the development of stronger antiviral treatments.

Recently, several host and virus-related factors have become well-established predictors of response to antiviral therapy and clinical outcome in CHC patients.[3–5] Among these, obesity and its associated metabolic complications are increasingly recognized as independent risk factors for diminished response to therapy and more severe liver disease.[6–8]

Concerning the response to antiviral therapy, it has been reported that obesity or metabolic syndrome is a risk factor for non-response to pegylated interferon (PEG-IFN) monotherapy or PEG-IFN plus ribavirin (RBV) independent of genotype and presence of cirrhosis in CHC patients.[9,10] Regarding the insulin resistance strongly associated with metabolic syndrome, Romero-Gómez *et al.*[11] showed that increased homeostasis model assessment as an index of insulin resistance is an independent predictor of decreased viral response to PEG-IFN plus RBV therapy for CHC. The author and colleagues also previously reported that the whole-body insulin sensitivity index, which is calculated by a 75-g oral glucose tolerance test and correlated with whole-body (primarily muscle) insulin sensitivity, is a highly specific marker for predicting the antiviral effect of PEG-IFN plus RBV therapy.[12] These findings suggest that excessive fat accumulation and accompanying insulin resistance in the liver and muscle definitely interfere with the antiviral effect of IFN. Two recent studies evaluated the effects of adding insulin sensitizers, pioglitazone[13] or metformin,[14] to the standard antiviral therapy. However, neither drug provided sufficient additive effects. There are no studies to date assessing whether lifestyle interventions impact on the response to antiviral treatment.

Many previous reports indicated that metabolic abnormalities, including liver steatosis, obesity and diabetes, can worsen the clinical course of CHC.[15–20] These findings suggest that obesity, especially visceral adiposity, and accompanying insulin resistance and glucose intolerance can vigorously cause progression of fibrosis and hepatocarcinogenesis in CHC patients, although it is well known that HCV itself can induce insulin resistance and oxidative stress in infected hepatocytes.[21]

Taking these observations together, lifestyle modifications should take priority and are likely to be very important for management for CHC patients. There is a report that weight reduction is associated with decreases in serum liver enzymes, hepatic steatosis and fibrosis scores in obese patients with CHC.[22] However, there is little information to date concerning the effects of physical exercise for CHC patients.

In this issue of *Hepatolology Research*, Konishi *et al.*[23] show that aerobic exercise can improve insulin sensitivity and decrease serum leptin secreted from adipose tissue. Since a previous study indicated that high serum leptin is a negative predictive factor for response to IFN,[24] this new study suggests the possibility that aerobic exercise may improve the response to antiviral treatment in CHC patients. However, they could not show significant changes in serum adiponectin, interleukin-6 and tumor necrosis factor-α, which are well-known to be more important cytokines than leptin for insulin resistance in metabolic disorders, non-alcoholic fatty liver disease (NAFLD) and CHC. The authors should more carefully address these non-comprehensive results.

Despite many lines of evidence indicating the effectiveness of physical exercise for improvement of NAFLD, the precise mechanisms by which it reduces hepatic steatosis remain unknown. Generally, it is thought that physical exercise can reduce visceral adiposity, decrease fatty acid delivery to the liver and improve insulin sensitivity at the skeletal muscle level, resulting in decreased hepatic steatosis. Recent data from animal studies using Otsuka Long-Evans Tokushima Fatty rats indicate that daily physical activity can directly stimulate lipid oxidation and inhibit lipid synthesis in the liver through activation of the

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adenosine monophosphate (AMP)-activated protein kinase pathway and upregulation of hepatic mitochondrial function.[25,26]

In HCV infection, HCV itself is known to modulate lipid homeostasis by increasing lipogenesis via sterol regulatory element-binding protein activation and reducing oxidation and lipid export, leading to steatosis.[27] If physical exercise can also modulate HCV-induced lipid metabolism abnormalities in the liver, it will provide new strategies for managing or treating CHC patients. Future studies will need to address the exact role of physical activity with or without weight loss and its beneficial effects on the histological features of CHC, or focus on how the intensity or duration of exercise is appropriate for CHC patients.

Finally, clinical physicians have experienced difficulties in clinical practice in making patients adhere to structured programs of physical activity for metabolic syndrome or NAFLD.[28] Therefore, if lifestyle modifications are applied to clinical management of CHC, a multidisciplinary team approach, including not only physicians but also dieticians and physical activity specialists, is needed to maximize adherence to physical exercise intervention.

The time has come that we, as hepatologists, must seriously commit to lifestyle-related disorders, similar to diabetologists and endocrinologists.

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REFERENCES

1 Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41–52.

2 Liang TJ, Rehermann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med* 2000; **132**: 296–305.

3 Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002; **36**: S47–S56.

4 Namiki I, Nishiguchi S, Hino K *et al.* Management of hepatitis C: Report of the Consensus Meeting at the 45th Annual Meeting of the Japan Society of Hepatology (2009). *Hepatol Res* 2010; **40**: 347–68.

5 Romero-Gomez M, Eslam M, Ruiz A, Maraver M. Genes and hepatitis C: susceptibility, fibrosis progression and response to treatment. *Liver Int* 2011; **3**: 443–60.

6 Hourigan LF, Macdonald GA, Purdie D *et al.* Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999; **29**: 1215–19.

7 Ortiz V, Berenguer M, Rayón JM, Carrasco D, Berenguer J. Contribution of obesity to hepatitis C-related fibrosis progression. *Am J Gastroenterol* 2002; **97**: 2408–14.

8 Hickman IJ, Powell EE, Prins JB *et al.* In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. *J Hepatol* 2003; **39**: 1042–8.

9 Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**: 639–44.

10 Hanouneh IA, Feldstein AE, Lopez R *et al.* Clinical significance of metabolic syndrome in the setting of chronic hepatitis C infection. *Clin Gastroenterol Hepatol* 2008; **6**: 584–9.

11 Romero-Gómez M, Del Mar Viloria M, Andrade RJ *et al.* Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**: 636–41.

12 Mizuta T, Kawaguchi Y, Eguchi Y *et al.* Whole-body insulin sensitivity index is a highly specific predictive marker for virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients with genotype 1b and high viral load. *Dig Dis Sci* 2010; **55**: 183–9.

13 Overbeck K, Genné D, Golay A, Negro F, Swiss Association for the Study of the Liver (SASL). Pioglitazone in chronic hepatitis C not responding to pegylated interferon-alpha and ribavirin. *J Hepatol* 2008; **49**: 295–8.

14 Romero-Gómez M, Diago M, Andrade RJ *et al.* Treatment of insulin resistance with metformin in naïve genotype 1 chronic hepatitis C patients receiving peginterferon alfa-2a plus ribavirin. *Hepatology* 2009; **50**: 1702–8.

15 Sanyal AJ. Role of insulin resistance and hepatic steatosis in the progression of fibrosis and response to treatment in hepatitis C. *Liver Int* 2011; **31** (Suppl 1): 23–8.

16 Negro F, Alaei M. Hepatitis C virus and type 2 diabetes. *World J Gastroenterol* 2009; **15**: 1537–47.

17 Eguchi Y, Mizuta T, Ishibashi E *et al.* Hepatitis C virus infection enhances insulin resistance induced by visceral fat accumulation. *Liver Int* 2009; **29**: 213–20.

18 Kobayashi Y, Kawaguchi Y, Mizuta T *et al.* Metabolic factors are associated with serum alanine aminotransferase levels in patients with chronic hepatitis C. *J Gastroenterol* 2011; **46**: 529–35.

19 Takahashi H, Mizuta T, Eguchi Y *et al.* Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. *J Gastroenterol* 2011; **46**: 790–8.

20 Akiyama T, Mizuta T, Kawazoe S *et al.* Body mass index is associated with age-at-onset of HCV-infected hepatocellular carcinoma patients. *World J Gastroenterol* 2011; **17**: 914–21.

21 Koike K, Moriya K, Matsuura Y. Animal models for hepatitis C and related liver disease. *Hepatol Res* 2010; **40**: 69–82.

22 Hickman IJ, Clouston AD, Macdonald GA *et al.* Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. *Gut* 2002; **51**: 89–94.

23 Konishi I, Hiasa Y, Tokumoto Y *et al.* Aerobic exercise improves insulin resistance and decreases body fat and serum levels of leptin in patients with hepatitis C virus. *Hepatol Res* 2011; **41**: 928–35 (this issue).

24 Eguchi Y, Mizuta T, Yasutake T *et al.* High serum leptin is an independent risk factor for non-response patients with low viremia to antiviral treatment in chronic hepatitis C. *World J Gastroenterol* 2006; **12**: 556–60.

25 Lavoie JM, Gauthier MS. Regulation of fat metabolism in the liver: link to non-alcoholic hepatic steatosis and impact of physical exercise. *Cell Mol Life Sci* 2006; **63**: 1393–409.

26 Rector RS, Uptergrove GM, Morris EM *et al.* Daily exercise vs. caloric restriction for prevention of nonalcoholic fatty liver disease in the OLETF rat model. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G874–83.

27 Syed GH, Amako Y, Siddiqui A. Hepatitis C virus hijacks host lipid metabolism. *Trends Endocrinol Metab* 2010; **21**: 33–40.

28 Oza N, Eguchi Y, Mizuta T *et al.* A pilot trial of body weight reduction for nonalcoholic fatty liver disease with a home-based lifestyle modification intervention delivered in collaboration with interdisciplinary medical staff. *J Gastroenterol* 2009; **44**: 1203–8.