Table 1 Profiles of the patients

	Hx ($n = 29$)	PD ($n = 32$)	HPD ($n = 14$)	p value*
Age (range)	65 ± 2 (43–81)	67 ± 2 (48–81)	62 ± 2 (50–78)	.18
Sex, M/F	18/11	23/9	10/4	.68
Predominant location of tumor				
Perihilar	29	0	9	
Distal	0	32	5	
Operative procedure				
RH	13 (2)		6	
LH	13 (2)		7	
CBS	2			
Seg	1		1	
PPPD		25	11	
PD		7	3	
PVR	13 (4)	4 (1)	5 (2)	
HAR	6		2	
Operative time, min (range)	429 ± 21 (247–655)	275 ± 22 (131–585)	550 ± 30 (370–1,025)	<.0001
Blood loss, ml (range)	1,096 ± 107 (390–2,497)	863 ± 115 (230–2,470)	1,354 ± 153 (754–2,900)	.041

The figures in parentheses indicate the number of graft reconstructions

RH right hepatectomy (two right trisectionecomy), *LH* left hepatectomy (two left trisectionectomy), *CBS* central bisegmentectomy, *Seg* segmentecomy, *PPPD* pylorus-preserving pancreatoduodenectomy, *PD* pancreatoduodenectomy, *PVR* (segmental) portal vein reconstruction, *HAR* hepatic artery reconstruction

* p values compare variables among the three surgical methods

PVR or HAR was performed, HAR method, operative time, total blood loss, histopathologic features of the resected tumor, morbidity, follow-up time, site of recurrence, and survival time.

Statistics

Continuous variables are expressed as mean ± standard error of the mean. Differences between groups were analyzed by chi-square or Mann–Whitney test, as appropriate. Survival was plotted by the Kaplan–Meier method and analyzed by log-rank test (univariate analysis) to identify potential prognostic factors. Multivariate analysis was carried out by means of a Cox proportional hazards model to identify independent risk factors influencing survival. Statistical significance was accepted at $p < .05$. Data analysis was performed with SPSS, version 17.0 (SPSS Japan Inc., Tokyo, Japan).

Results

Clinical variables

Mean age of the 75 study patients was 65 ± 1 years (range: 43–81 years); the male/female ratio was 51/24 (Table 1). Both perihilar ($n = 9$) and widespread distal ($n = 5$) cholangiocarcinomas were treated by HPD. The numbers of right and left hepatectomies were evenly distributed in the Hx and HPD groups, whereas for pancreatic resection, pylorus-preserving pancreaticoduodenectomy (PPPD) was most prevalent procedure, according to our institutional policy. Portal vein reconstruction was performed for macroscopic cancer invasion in 13 patients during Hx, in 4 patients during PD, and in 5 patients during HPD, for an overall PVR rate of 29%. HAR was required for 6 patients who underwent Hx and 2 patients who underwent HPD, for a total HAR rate of 11%. Six patients underwent both PVR and HAR. Regarding arterial reconstruction, simple end-to-end arterial reconstruction was performed for 1 patient during Hx, whereas others required mobilization of another artery (either the gastroduodenal or middle colic artery) for anastomosis to the future remnant hepatic artery.

As expected, operative time and blood loss differed between groups, with PD resulting in the shortest operative time and least blood loss.

Follow-up time

Mean follow-up for all patients was 46 ± 4 months. There were 4 in-hospital deaths (in-hospital mortality, 5.3%). Thirty-nine patients died from cancer relapse, and 3 died from other diseases. The remaining 29 patients were alive as of December 31, 2009. The minimum follow-up period for any patient was 31 months.

Postoperative complications

Morbidity and mortality did not differ statistically between groups (Table 2). The overall incidence of morbidity was lower in the Hx group than in the other two groups, but

Table 2 Postoperative complications

	Hx ($n = 29$)	PD ($n = 32$)	HPD ($n = 14$)	p value*
Morbidity	10 (34)	14 (44)	8 (57)	.37
Liver failure	4 (14)		3 (21)	
Pancreatic fistula		8 (25)	3 (21)	
Bile leak	3 (10)		3 (21)	
Abscess	2 (7)	1 (3)	1 (7)	
Pleural effusion/ascites	2 (7)		2 (14)	
Sepsis	1 (3)	1 (3)		
DGE		2 (6)	1 (7)	
Cerebral infarction		2 (6)		
Mortality (in-hospital)	3 (10)	1 (3)	0	.28
Hospital stay, days (median)	32	35	44	.40

Numbers in parentheses indicate the percentages

DGE delayed gastric empting

* p values compare variables among the three surgical methods

Table 3 Histopathologic factors

	Hx ($n = 29$)	PD ($n = 32$)	HPD ($n = 14$)	p value*
Tumor grade				
wel[a]	9 (31)	13 (41)	5 (36)	.12
mod[b]	19 (66)	15 (47)	5 (36)	
por[c]	1 (3)	4 (13)	4 (29)	
Perineural invasion	23 (79)	16 (50)	11 (79)	.031
Arterial invasion	11 (38)	3 (9)	3 (21)	.029
Portal vein invasion	8 (28)	4 (12)	2 (14)	.29
Node involvement	16 (55)	12 (38)	6 (43)	.38
Positive ductal margin	8 (28)[d]	2 (6)	2 (14)	.075
Positive radial margin	3 (10)	4 (13)	2 (14)	.93
R-classification				
R0	17 (59)	27 (84)	9 (64)	.074
R1/2	12 (41)	5 (16)	5 (36)	
T-classification				
≤2	5 (17)	9 (28)	7 (50)	.081
3, 4	24 (83)	23 (72)	7 (50)	
Stage				
0, I	5	8	5	.58
II	12	16	5	
III	5	4	1	
IV	7	4	3	

Numbers in parentheses indicate the percentages

* p values compare variables among three surgical methods

[a] Well differentiated, including papillary adenocarcinoma

[b] Moderately differentiated

[c] Poorly differentiated

[d] Six hepatic side bile ducts, 2 duodenal side bile ducts

severe liver failure developed in 4 patients undergoing Hx, contributing to the relatively high mortality rate in this group. Liver failure and bile leakage rates were higher in the HPD group than the Hx group. The pancreatic fistula rate and DGE rate did not differ between the HPD group and the PD group. Reoperation was required in 1 patient after PD for refractory bleeding due to rupture of the gastroduodenal artery secondary to pancreatic fistula and in a second patient following HLPD who underwent re-anastomosis of the pancreaticojejunostomy due to a sustained pancreatic fistula requiring readmission (5 months after HLPD). The causes of mortality were liver failure in 2 patients (on POD 32 and POD 116) and sepsis due to cholangitis in 1 patient (on POD 44) in the Hx group, and cerebral infarction in 1 patient (POD 42) in the PD group who suffered from prolonged DGE. Hospital stay was approximately 10 days longer after HPD than after the other procedures, but the differences in hospital stay between groups were not significant.

Patency of the vascular reconstruction was questionable in 2 patients who underwent HAR; blood flow was not seen postoperatively at the anastomotic site. However, there were no associated complications because collateral flow was well established. There were no complications in patients with PVR in this study.

Histopathologic factors

Histopathologic features of the tumors were compared between groups. Tumor grade, portal vein invasion, nodal involvement, surgical margin status, R-classification, UICC T-classification, and UICC stage did not differ significantly between groups (Table 3). Perineural invasion and associated arterial invasion occurred more frequently in the Hx and HPD groups than in the PD group ($p = .031$ and $p = .029$, respectively). Cancer invasion into the portal vein wall was histologically proven in 14 of 22 patients (64%) who underwent PVR. Although curability did not differ statistically between groups, R0 (histologically curative) resection was achieved in 84% of patients who underwent PD, but in only 59% of patients who underwent Hx and 64% of those who underwent HPD. Histologically positive bile duct margins (R1 resection) were present in 8 patients who underwent Hx, but in only 2 patients each who underwent HPD and PD. Radial margins were comparable between groups. Histologic para-aortic lymph nodes metastases accounted for R2 resections (macroscopically residual cancer) in 3 patients who underwent Hx, 3 patients who underwent PD, and 2 patients who underwent HPD, with no statistical differences between groups. Other factors contributing to R2 resection were liver metastases (Hx group, $n = 3$; HPD group, $n = 1$) and localized peritoneal dissemination around the PTBD catheter (Hx group, $n = 2$).

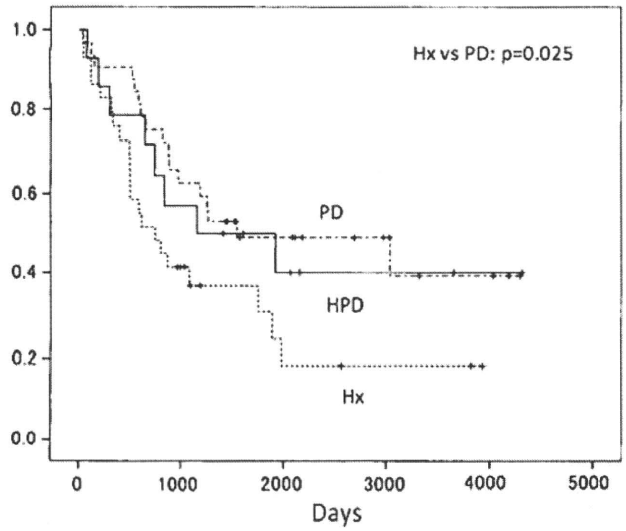

Fig. 3 Cumulative survival of patients according to operative procedure. *Hx* hepatectomy, *PD* pancreatoduodenectomy, *HPD* hepatopancreatoduodenectomy

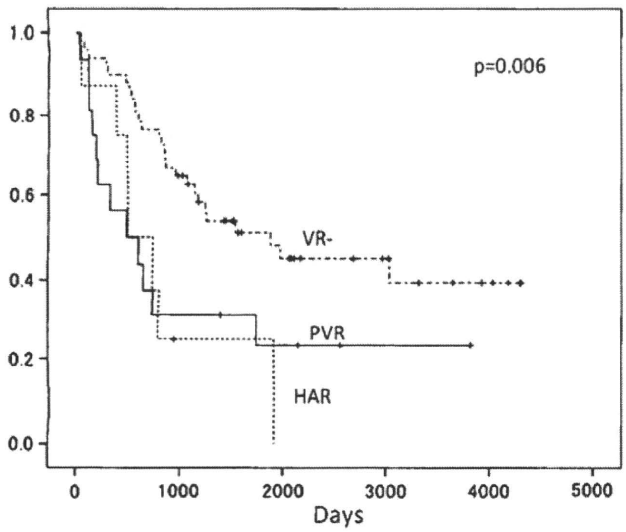

Fig. 4 Cumulative survival of patients according to vascular reconstruction. *VR–* no vascular reconstruction, *PVR* portal vein reconstruction, *HAR* hepatic artery reconstruction

Survival

Overall median survival time was 39 months, and the 5-year survival rate was 42%. Median survival time and 5-year survival per group were as follows: 24 months and 31% in the Hx group, 51 months and 49% in the PD group, and 63 months and 50% in the HPD group (Fig. 3). The difference in survival rate between the Hx group and the PD group was significant ($p = .025$), but that between the Hx group and the HPD group was not ($p = .21$).

Five-year survival rates were comparable between patients who underwent PVR (23%) and those who underwent HAR (25%), but the 5-year survival rate was substantially increased (51%) for patients who did not undergo vascular reconstruction (Fig. 4).

According to the types of invasion in HPD, survival time associated with each of 3 types of cancer spread did not differ significantly ($p = .78$), and 5-year survival rates were the same (50% each).

Sites of recurrence were identified in 17 patients after Hx, 15 patients after PD, and 7 patients after HPD. Liver metastases occurred in 3 patients (18%) after Hx, 5 patients (33%) after PD, and 2 patients (29%) after HPD. Local recurrence (with or without peritoneal dissemination) was the most prevalent pattern of recurrence, seen in 12 patients (71%) after Hx, 6 patients (40%) after PD, and 4 patients (57%) after HPD. Other types of recurrence were lymph

Table 4 Univariate analysis of risk factors for all patients

	No. (months)	MST (%)	5-year survival	*p* value
Tumor grade				
wel[a]	27	100	57	.079
mod[b]	39	28	33	
por[c]	9	21	33	
Perineural invasion				
No	25	–	75	<.0001
Yes	50	24	25	
Arterial invasion				
No	58	58	50	<.0001
Yes	17	19	18	
Portal vein invasion				
No	61	51	47	.003
Yes	14	24	14	
Node involvement				
No	41	100	55	.0012
Yes	34	24	23	
Positive ductal margin				
No	63	51	47	.011
Yes	12	20	13	
Positive radial margin				
No	66	51	48	<.0001
Yes	9	19	0	
R-classification				
R0	53	63	56	<.0001
R1/2	22	19	12	
T-classification				
≤2	21	–	71	.009
3, 4	54	28	30	

MST median survival time

[a] Well differentiated including papillary adenocarcinoma

[b] Moderately differentiated

[c] Poorly differentiated

node metastasis ($n = 11$), lung metastasis ($n = 2$), and bone metastasis ($n = 1$) (including the overlapping data).

Risk factors

Univariate analysis showed perineural invasion ($p < .0001$), arterial invasion ($p < .0001$), R classification (R1/2 versus R0, $p < .0001$), a positive radial margin ($p < .0001$), nodal involvement ($p = .0012$), portal vein invasion ($p = .003$), UICC T classification (T3/4 versus T0/1/2, $p = .009$), and a positive ductal margin ($p = .011$) to significantly influence survival (Table 4). Absence of perineural invasion was associated with a high 5-year survival rate (75%). Patients with lymph node metastases had relatively good survival, with a median survival time of 24 months and a 5-year survival rate of 23%. When positive nodes were present in the *hepatoduodenal ligament* ($n = 13$) and *peripancreatic space* ($n = 13$) mean survival times were 35 and 31 months, respectively, but when positive *para-aortic* ($n = 8$) nodes (leading to R2 resection) were present, mean survival was only 16 months, with no patient living more than 2 years.

When factors shown by univariate analysis to be statistically significant were entered into the Cox regression model, only perineural invasion (relative risk [RR], 3.39; 95% confidence interval [CI] 1.40–8.19; $p = .007$) and R classification (RR, 2.30; 95%CI, 1.16–4.53; $p = .017$) were shown to be independent predictors of outcome.

Discussion

Many institutions are now starting to treat perihilar and distal cholangiocarcinomas by hepatectomy with either bile duct resection or PD as an attempt at curative resection [1–6]. Bile duct resection alone should be restricted to early-stage papillary carcinoma present in a limited area [4]. With the idea that radical surgery could contribute to better survival, we have performed radical surgeries including hepatecomy with bile duct resection, PD, and HPD for biliary malignancies over the last several years [17, 18]. Regarding HPD, in the early 1990 s, morbidity and mortality associated with this surgery were approximately 90–100% and 25–29%, respectively [8, 9]. These numbers have decreased over time to 30–43% and 0–14%, respectively [19, 20]. The Japanese Society of Biliary Surgery recently reported that HPD was performed for 29 of 255 hilar cholangiocarcinomas (11.4%) in selected institutions in Japan during the period 1998–2002 [21]. These data indicate that HPD is going to be the standard surgery for biliary malignancies. Appropriate preoperative biliary drainage following portal vein embolization to optimize residual liver function as standard preoperative treatment before extended surgery, such as HPD, has improved outcomes [22–25]. Portal vein embolization introduced by Makuuchi [26], in particular, has become key to improving the safety of major hepatectomy, and approximate 10% increase in residual liver volume was reported [27, 28].

Only a few articles have addressed the clinical significance of HPD in comparison to hepatectomy or PD for cholangiocarcinoma [29]. According to our single institutional study, morbidity associated with HPD was higher than that associated with the other procedures, but the difference did not reach statistical significance. Additional complications with HPD were related to the number of required reconstructions in the alimentary tract. Morbidity associated with Hx was lower than anticipated (34%), but the associated mortality was much higher (10%). We speculate that liver function after Hx is typically most significantly affected by bilateral or segmental obstruction of the intrahepatic bile ducts, which might cause postoperative septicemia, and also some patients with Hx had far advanced stage of disease, which might decrease the patient's immunologic defenses against surgical stress. In contrast, HPD is typically performed for cases in which liver function is relatively good and the UICC T-classification is relatively low, indicating limited cancer cell invasion around the tumor, with a concomitant decrease in the need for vascular reconstruction. In addition, our criteria for HPD, in which the proximal extent of the residual hepatic duct by the tumor had to be limited within the first order branch, might decrease the operative risk, which was seen in the Hx group. Hence, no in-hospital mortality occurred among our study patients who underwent HPD. Incidences of pancreatic fistula and also DGE were not different between patients with PD and HPD; therefore, we believe a two-stage operation [30], in which reconstruction of the pancreatic duct is the second step, is not necessary for HPD.

Histopathologic analysis demonstrated perineural invasion, and the associated arterial invasion was significantly different between the groups, with the Hx and HPD group revealing greater prevalence of these factors than the PD group. This difference in incidence may be related to anatomic differences of the perihilar and distal portion of the bile duct conferring an inherent difference in distance from adjacent vessels. Although the incidences of portal vein invasion and nodal involvement did not differ significantly between groups, both were more prevalent in the Hx group. Positive ductal margin was also more frequently identified in the Hx group than in the others. Although R-classification and the stage of disease were not different statistically between the groups, these data strongly suggested that the Hx group showed more extensive disease and received less curable surgery than the other groups.

The 5-year survival rate of 31% in our Hx group, in comparison to the 49% in our PD group and 50% in our

HPD group, was the worst among our patients. The 31% was similar to other reported rates for Hx [25], despite the high incidence of very advanced tumors in our patient series. Distant metastases apparently prevented many R0 resections in the Hx group; however, aggressive resection was possible in many cases in this group because of the limited extent of metastatic disease. Unexpectedly high survival rates were achieved with PD and HPD and may be due to the comparatively early stage at which our patients' tumors were treated and the aggressive surgical tactics we used. Bile duct resection was not performed in isolation, and concomitant vascular resection was vigorously applied [31–33]. However, the rate at which R0 resection was achieved with HPD was not as high as expected. In general, it might be difficult to secure negative surgical margins in cases of biliary malignancy; the recently reported rate of positive surgical margins is about 60%, regardless of the type of surgery [34]. When survival was examined in relation to vascular reconstruction, it was clear that survival associated with PVR and HAR was low in comparison to that associated with absence of vascular reconstruction. Between PVR and HAR, survival was equivalent, contrary to some reports indicating no survival benefit with HAR [35]. We suppose our surgical tactics, i.e., en bloc resection of the tumor with the adherent vessels, might confer favorable results. In this study, HAR was almost done using the mobilized artery to keep a safe distance from the tumor. Historically, HPD has been performed for superficially spreading perihilar cholangiocarcinoma or retropancreatic lymph *node metastases* [36]. In our patient series, HPD was performed for any of 3 indications: widespread intramural invasion, superficial spread, and hepatoduodenal ligament invasion. Survival did not differ statistically between these three groups. The number of patients in our study was too low to determine the pertinent indications for HPD among the types of cholangiocarcinoma, but our results suggest preliminarily that any type of cholangiocarcinoma in which curative resection can be obtained is a possible candidate for surgery. Miwa et al. [37] reported results similar to ours: *in their* report 14 patients with diffuse bile duct cancer underwent HPD with no postoperative mortality and 51.9% 5-year survival. Concomitant vascular resection including HLPD was applied to decrease the incidence of positive radial margins in our patient series. It is possible that HLPD for highly advanced intrahepatic cholangiocarcinoma may prove beneficial, providing survival beyond 5 years and a positive palliative effect [38].

Risk factors were analyzed to identify the biologic behaviors of extrahepatic cholangiocarcinomas following the three aggressive surgical approaches. We determined that R classification, a positive surgical stump, perineural *invasion*, UICC T-classification, arterial invasion, lymph node metastasis, and portal vein invasion significantly influenced survival. As Sakamoto et al. [34] pointed out, the radial margin rather than the duct margin significantly influenced survival, and there was no 5-year survival with the positive radial margin in the present study. Unlike other researchers [24, 25, 39, 40], we did not find tumor grade and lymph node metastasis to be statistically significant by multivariate analyses, and only perineural invasion and R classification (R1/2 versus R0) were shown to be independent risk factors. Most studies performed outside Japan did not include perineural invasion as a prognostic factor; thus, a consensus definition for positive perineural invasion has not yet been established worldwide. In the present study, positive perineural invasion was defined as an intermediate or greater degree of invasion as set forth in the *Japanese General Rules for Surgical and Pathological Studies on Cancer of the Biliary Tract* [16]. As previously reported [41, 42], we found perineural invasion to be one of the major obstacles to obtaining histologically curative resection for biliary malignancies. In addition, our results indicate that regional (periductal and peripancreatic) lymph node metastasis does not have to be a contraindication for surgery; when positive nodes were present in the regional nodes mean survival times was over 31 months. However, patients with para-aortic lymph node metastases did not survive beyond 2 years. Randomized controlled trials examining the survival benefits of adjuvant chemotherapies in patients with biliary cancer are underway. Perhaps a standard adjuvant chemotherapy regimen for *biliary* cancer will be established in the near future.

In conclusion, our aggressive surgical strategy provides a long-term survival benefit to patients with perihilar or distal cholangiocarcinoma. The zero in-hospital mortality and favorable long-term survival among our 14 consecutive patients who underwent HPD suggest that HPD is worthy of consideration as a standard operation for cholangiocarcinoma with limited tumor extent, regardless of the type of tumor invasion. Because of the small number of patients in this study, further investigation in larger study populations is needed to confirm our policy.

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Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy

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SUMMARY. Mutations in two regions of hepatitis C virus (HCV) have been implicated in influencing response to interferon (IFN) therapy. Substitutions in the NS5A region of HCV have been associated with response to IFN therapy, and this region has been known as the IFN sensitivity-determining region (ISDR). The mutations in the core region of HCV have also been reported to predict IFN response. The aim of this study was to investigate whether amino acid substitutions in the core region and ISDR among patients with HCV genotype 1b affect the response to IFN therapy. A total of 213 patients who completed IFN treatment were randomly selected. All patients received pegylated-IFN-alpha 2b once each week, plus oral ribavirin daily for 48 weeks. Of the 213 patients, 117 (54.9%) showed early virologic response (EVR), with HCV-negativity, at 12 weeks. Factors related to EVR on multivariate analysis were non-Gln70 and Leu91 in the core region, and ISDR mutant-type. One hundred and two (47.9%) showed a sustained virologic response (SVR). SVR occurred more frequently in patients without Gln70 (55.4%) than in those with Gln70 (21.3%) ($P < 0.0001$). SVR was achieved in 43.6% of patients with wild-type ISDR and 62.5% of patients with mutant-type ($P = 0.0227$). Of the 34 patients who simultaneously had non-Gln70 and mutant-type ISDR, 26 (76.5%) achieved SVR. Factors related to SVR on multivariate analysis were non-Gln70 and ISDR mutant-type. In conclusion, amino acid substitutions in the core region and ISDR were useful for predicting the response to IFN in patients with HCV genotype 1b.

Keywords: core region, genotype 1b, hepatitis C virus, interferon sensitivity-determining region, interferon therapy, NS5A.

INTRODUCTION

Hepatitis C virus (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into potentially fatal cirrhosis and hepatocellular carcinoma [1]. It has been estimated that 170 million people are infected with HCV worldwide. Therefore, HCV infection is a major global health problem. HCV consists of four structural proteins (core, envelope 1, envelope 2 and p7) and six nonstructural proteins (NS2–NS5) [2]. HCV core protein was thought to inhibit the antiviral action of interferon (IFN) through down-regulation of transcription of IFN-induced antiviral genes [3,4]. The NS5A region includes the PKR-binding domain, which is associated with viral replication that is affected by IFN [5]. Thus, the core and NS5A regions of HCV appear to be important factors that may affect the response to IFN therapy, and mutations in the core and NS5A regions of HCV have been reported to affect response to IFN therapy [6–10]. The core region of HCV is well conserved, but substitutions of amino acid (aa) 70 and aa 91 are frequently found. Several studies reported a relation between these substitutions in the core region and IFN responsiveness [8,10]. The substitutions in the NS5A region of HCV have been closely associated with response to IFN therapy, and this region is known as the IFN sensitivity-determining region (ISDR) [6]. However, these

Abbreviations: Aa, amino acid; ALT, alanine aminotransferase; EVR, early virologic response; HCV, hepatitis C virus; IFN, interferon; ISDR, interferon sensitivity-determining region; SVR, sustained virologic response.

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relationships are little known and still controversial [10]. The aim of this study was to investigate whether amino acid substitutions in the core region and ISDR among patients with HCV genotype 1b affect the response to pegylated-IFN-alpha 2b and ribavirin combination therapy.

MATERIAL AND METHODS

A total of 891 patients with chronic hepatitis C genotype 1b and high viral load who were treated at Nagoya University Hospital and Affiliated Hospitals were enrolled; 213 patients who completed IFN treatment were randomly selected for this study. The patients' clinical characteristics are summarized in Table 1. Patients whose HCV-RNA levels were <100 KIU/mL were excluded. The core region (aa 30–110) and ISDR (aa 2209–2248) were examined by direct sequencing. All patients received subcutaneous injections of pegylated-IFN-alpha 2b (1.5 μg/kg) once each week plus oral ribavirin daily for 48 weeks. HCV-RNA in serum samples was examined at 12 weeks, at the end of IFN therapy and at 6 months after the end of treatment. Serum was stored at −80 °C for virologic examination. Early virologic response (EVR) was defined as HCV-negative at 12 weeks. Patients who were persistently negative for serum HCV-RNA and who had a normal serum alanine aminotransferase (ALT) level at 24 weeks after withdrawal of IFN treatment were considered to have sustained virologic response (SVR). Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virologic analysis

HCV-RNA quantitative viremia load was determined by polymerase chain reaction (PCR). HCV was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions as described previously [11,12]. Genotypes were classified according to the nomenclature proposed by Simmonds *et al.* [13]. Direct sequencing of the core and NS5A-ISDR region was carried out as reported previously, but with modifications [7,14]. In brief, RNA was extracted from 140 μL serum with a commercial kit (QIAamp Viral RNA Kit; Qiagen, Valencia, CA, USA) and dissolved in 50 μL diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligo and random hexamer primers with a commercial kit (iScript cDNA Synthesis Kit; Bio-Rad, Hercules, CA, USA). HCV core region and NS5A-ISDR were amplified by nested PCR. In brief, each 50-μL PCR reaction contained 100 nM of each primer, 1 ng template cDNA, 5 μL GeneAmp 10 × PCR buffer, 2 μL dNTPs and 1.25 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). Primers for core region were sense 5′-GGGAGGTCTCGTAGACCGTGCACCATG-3′ and antisense 5′-GAGMGGKATRTACCCCATGAGRTCGGC-3′ and primers for the NS5A-ISDR were sense 5′-TGGATGGAGTGCGGTTGCACAGGTA-3′ and antisense 5′-TCTTTCTCCGTGGAGGTGGTATTG-3′. Amplification conditions consisted of 10 min at 94 °C, followed by 40 cycles of 94 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The second PCR was performed in the same reaction buffer with the first-round PCR product as template, and the following sets of primers: for the core region, sense primer 5′-AGACCGTGCACCATGAGCAC-3′ and antisense 5′-TACGCCGGGGGTCAKTRGGGCCCCA-3′; and for the NS5A-ISDR, sense 5′-CAGGTACGCTCCGGCGTGCA-3′ and antisense 5′-GGGGCCTTGGTAGGTGGCAA-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems). A mutation mixture was defined as viral mutants that constituted 50% or more of the total viral population.

Table 1 Clinical characteristics

Clinical characteristics	*N* =2 13
Age (years)	55.2 ± 10.6
Sex: male/female	120/93
AST(IU/L)	58.5 ± 37.7
ALT(IU/L)	66.0 ± 53.9
Platelet count (10^4/uL)	17.1 ± 5.1
HCV RNA level (KIU/mL)	1720 (100–7200)
Treatment: naive/retreatment	117/96
Body weight (kg)	55.3 ± 19.9

Data are expressed as mean ± standard deviation HCV RNA level was shown by median (range). AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

Statistical analysis

Data are expressed as means ± standard deviation (SD). The paired *t*-test, the chi-square and the Fisher's exact tests were used to analyze differences in variables. A *P*-value of <0.05 was considered statistically significant. Multiple logistic regression models were used to identify factors predictive of EVR and SVR. Statview 5.0 software (SAS Institute, Inc., Cary, NC, USA) was used for all analyses.

RESULTS

Genetic heterogeneity in NS5A-ISDR and core regions of the HCV genome

The mutations in the HCV core region were measured by direct sequencing. The core region of HCV is well conserved,

Table 2 Prevalence of amino acid substitutions at 70, 75, and 91

Core 70	
Histidine	$n = 6$
Glutamine	$n = 46$
Glutamine/Histidine	$n = 1$
Arginine	$n = 160$
Core 75	
Alanine	$n = 112$
Alanine/Serine	$n = 1$
Alanine/Threonine	$n = 2$
Glutamine	$n = 1$
Serine	$n = 5$
Threonine	$n = 91$
Valine	$n = 1$
Core 91	
Leucine	$n = 162$
Methionine	$n = 51$

but substitutions of aa 70, aa 75 and aa 91 were frequently found, as previously reported. The distribution of mutations in the HCV core region at aa 70, aa 75 and aa 91 is shown in Table 2. The sequence of the HCVJ strain was defined as the consensus sequence, and the approach of counting the number of mutations to the chosen consensus sequence in ISDR was used to analyze the ISDR system. The number of NS5A-ISDR mutations was as follows: none ($n = 102$), 1 ($n = 63$), 2 ($n = 14$), 3 ($n = 8$), 4 ($n = 8$), 5 ($n = 7$), 6 ($n = 2$), 7 ($n = 4$) and 8 ($n = 5$). The relationships between substitutions of amino acids in the HCV core region and NS5A-ISDR are shown in Fig. 1. There were no significant relationships between the two regions. Thus, the HCV core region and the NS5A-ISDR were independent factors.

Virological response

Of 213 patients, 117 (54.9%) showed EVR, with HCV-negativity, at 12 weeks, and 76 became HCV-negative after 12 weeks; overall, 187 patients became HCV-negative at the end of treatment (87.8%). However, 85 patients continued to be HCV-positive after withdrawal of IFN treatment, and 102 of 213 (47.9%) patients were defined as achieving a SVR. Of 117 patients with EVR, 87 (74.4%) achieved SVR. Of 96 patients without EVR, 81 became non-SVR (84.4%). Thus, EVR was strongly associated with SVR.

Factors associated with early virologic response

The results of univariate analysis for factors predictive of EVR are shown in Table 3. The EVR rate according to amino acid substitutions of ISDR are shown in Table 4. The EVR rate of patients with more than two mutations in the ISDR (mutant-type) was 68.9%. Of 166 patients without glutamine (Gln) at aa 70 in the core region, 100 achieved EVR. The EVR rate of patients with Leu91 in the core region was 61.1%. The results of multivariate analysis for factors predictive of EVR are shown in Table 5. Factors related to EVR on multivariate analysis were non-Gln70, Leu91 and ISDR mutant-type.

Factors associated with sustained virologic response

The results of univariate analysis for factors predictive of SVR are shown in Table 6. The SVR rate according to amino acid substitutions of ISDR are shown in Table 4. SVR occurred more frequently in patients without Gln70 (55.4%) than in those with Gln70 (21.3%) (odds ratio, 0.217; 95% confidence interval (CI), 0.101–0.466; $P < 0.0001$). SVR was achieved in 43.6% of patients with wild-type ISDR and 62.5% with mutant-type ISDR (odds ratio, 0.465; 95% CI, 0.240–0.899; $P = 0.0227$). Factors related to SVR on multivariate analysis were non-Gln70 and ISDR mutant-type, as shown in Table 7.

The virological response according to amino acid substitutions in the 70 core region and ISDR

The SVR and EVR rates according to amino acid substitutions in the 70 core region and ISDR are shown in Table 8. The best response for both SVR and EVR was achieved in patients with non-Gln70 and mutant-type ISDR, and the

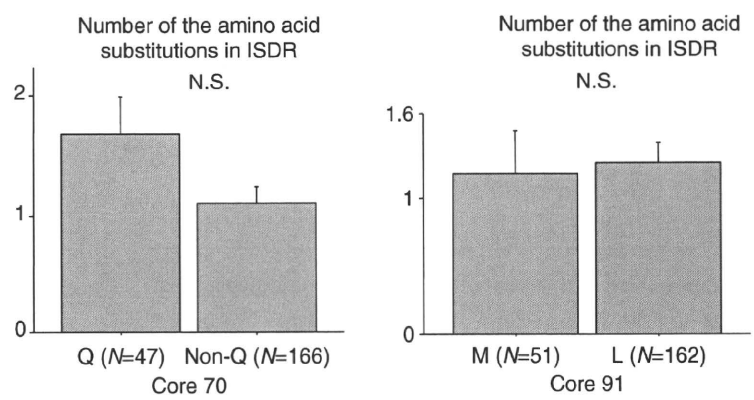

Fig. 1 The association between amino acid substitutions in core region and ISDR. ISDR, interferon sensitivity-determining region; Q, glutamine; L, leucine; M, methionine; NS, not significant.

Table 3 Univariate analysis: Factors predictive of EVR

Factors	EVR (n = 117)	Non-EVR (n = 96)	P-value
Age (years)	54.7 ± 11.3	55.9 ± 9.7	0.4511
Gender: male/female	63/54	57/39	0.7830
ALT (IU/L)	69.6 ± 64.8	61.5 ± 36.2	0.3002
AST (IU/L)	59.4 ± 40.9	57.3 ± 33.5	0.7026
PLT ($\times 10^4/mm^3$)	17.4 ± 5.1	16.9 ± 5.18	0.4955
HCV RNA level (KIU/mL)	2051.3 ± 1373.4	2006.1 ± 1462.7	0.8216
Core 70:non-Q/Q	100/17	66/30	0.0046
Core 75: A/non-A	58/59	54/42	0.3387
Core 91: L/M	99/18	63/33	0.0020
ISDR: wild/mutant	84/33	81/15	0.0327

EVR, early virologic response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; Q, glutamine; A, alanine; L, leucine; M, methionine; ISDR, interferon sensitivity-determining region

Table 4 Amino acid substitutions of ISDR and virologic response

ISDR; number of the amino acid substitutions	0 N = 102	1 N = 63	2 N = 14	3 N = 8	4 N = 8	5 N = 7	6 N = 2	7 N = 4	8 N = 5
EVR rate (%)	51 (50.0)	33 (52.4)	10 (71.4)	4 (50.0)	7 (87.5)	4 (80.0)	0 (0)	3 (75.0)	5 (100)
SVR rate (%)	41 (40.2)	31 (49.2)	10 (71.4)	4 (50.0)	4 (50.0)	5 (71.4)	0 (0)	3 (75.0)	4 (80.0)

EVR, early virologic response; SVR, sustained virologic response.

Table 5 Multivariate analysis: Factors predictive of EVR

Factors	P-value	Risk ratio	95% CI	
Gender: male	0.3760	0.754	0.403	1.410
Age: <60 years	0.8247	0.915	0.416	2.012
AST: <60 IU/L	0.3301	1.525	0.652	3.569
ALT: <60 IU/L	0.2484	0.613	0.267	1.407
PLT: $<17 \times 10^4/mm^3$	0.0666	0.530	0.269	1.044
Core 70: nonQ	0.0242	2.406	1.121	5.165
Core 91: A	0.0022	3.409	1.557	7.463
Core 75: M	0.0683	1.863	0.954	3.635
ISDR: mutant	0.0085	0.338	0.151	0.759

EVR, early virologic response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, Interferon sensitivity-determining region; Q, glutamine; A, alanine; L, leucine; M; methionine.

worst response was achieved in patients with Gln70 and wild type ISDR. The SVR rates according to amino acid substitutions in the 70 core region and ISDR and EVR are shown in Table 9. The positive predictive values for SVR and non-SVR improved to 88.9% and 90.9%, respectively, when EVR was considered with the 70 core region and ISDR.

DISCUSSION

Peginterferon and ribavirin combination therapy has been standard treatment for patients with chronic hepatitis C. However, the SVR rate was almost 50% for HCV genotype 1b, which is a refractory strain. The standard doses and duration of peginterferon plus ribavirin may be suboptimal for half of the patients; patients need a new approach for eradicating HCV. Peginterferon and ribavirin therapy has been a useful treatment, but cost and adverse events have been problems. To select patients who could attain cure from HCV by current standard treatment, it is necessary to predict the response before therapy. Current guidelines for HCV treatment recommend that the selection of IFN treatment regimen depends on HCV genotypes and viral loads. Several studies have focused on sequence variation of the HCV genome and response to IFN therapy, but prediction of IFN responsiveness has been less well characterized. NS5A-ISDR heterogeneity is an important factor that may affect response to IFN, especially in Asia [6,7,9]. The ISDR interacts with PKR and regulates replication of HCV *in vitro* [5]. Mutations in the ISDR affect the interaction with PKR and may inhibit viral replication. Therefore, ISDR of not only HCV genotype 1b but also 2a and 2b could also play an important role as a predictor of IFN responsiveness in clinical research of standard IFN or Peg-IFN monotherapy [15,16]. The differences in HCV 1b subtype and race affect the utility of ISDR

Table 6 Univariate analysis: factors predictive of SVR

Factors	SVR (n = 102)	Non-SVR (n = 111)	P-value
Age (years)	53.6 ± 10.8	56.7 ± 10.2	0.0319
Gender: male/female	57/45	63/48	0.7830
ALT (IU/L)	69.6 ± 66.7	62.6 ± 38.5	0.3606
AST (IU/L)	58.8 ± 40.9	58.3 ± 34.8	0.9469
PLT ($\times 10^4/mm^3$)	17.7 ± 5.1	16.7 ± 5.0	0.1563
HCV RNA level (KIU/mL)	2111.1 ± 1504.9	1956.4 ± 1319.8	0.4386
Core 70:non-Q/Q	92/10	74/37	0.0001
Core 75: A/non-A	50/52	62/49	0.3388
Core 91: L/M	82/20	80/31	0.1984
ISDR: wild/mutant	72/30	93/18	0.0227

SVR, sustained virologic response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; Q, glutamine; A, alanine; L, leucine; M, methionine, ISDR, Interferon sensitivity-determining region.

Table 7 Multivariate analysis: factors predictive of SVR

Factors	P-value	Risk ratio	95% CI	
Age: <60 years	0.5219	0.770	0.346	1.714
Gender: male	0.6775	1.140	0.614	2.116
AST: <60 IU/L	0.1017	0.487	0.206	1.153
ALT: <60 IU/L	0.1690	1.799	0.779	4.157
PLT: $<17 \times 10^4/mm^3$	0.4067	1.324	0.682	2.573
HCV RNA levels: <106 IU/mL	0.6409	0.841	0.405	1.743
Core70: nonQ	0.0004	0.220	0.094	0.512
Core91: M	0.5643	0.799	0.373	1.711
Core75: A	0.3993	0.757	0.396	1.446
ISDR: mutant	0.0096	2.879	1.294	6.407

SVR, sustained virologic response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count, HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; Q, glutamine; A, alanine; L, leucine; M, methionine.

Table 8 The SVR and EVR rate according to amino acid substitutions in 70 core region and ISDR

Core70/ISDR	SVR (n = 102)	EVR (n = 117)
Q/wild (n = 33)	6 (18.2%)	11 (33.3%)
Q/mutant (n = 14)	4 (28.6%)	6(42.9%)
Non-Q/wild (n = 132)	66 (50.0%)	73 (55.3%)
Non-Q/mutant (n = 34)	26 (76.5%)	27 (79.4%)

SVR, sustained virologic response; EVR, early virologic response; SDR, interferon sensitivity-determining region; Q, Glutamine; ISDR, interferon sensitivity-determining region.

sequences for predicting IFN responsiveness [7,17,18]. Thus, ISDR was found to be good for predicting IFN outcome of patients in Asian countries rather than of patients in Western countries. The approach of counting the number of mutations to the HCV-J strain in the ISDR was used in the original report by Enomoto *et al.*, [6] and they classified the mutations into three groups: wild type (no mutation), intermediate (1–3 mutations) and mutant-type (more than four mutations). SVR did not occur in any of the 30 patients with wild type ISDR in the original report using standard IFN monotherapy. In the present study, 41 of 102 patients (40.2%) with the wild type ISDR (no mutation) achieved SVR because of improvement of Peg-IFN plus RBV combination therapy. We examined the association between the number of mutations and SVR with adjustment for current standard treatment. We were unable to identify a significant relation between no mutation and one mutation in ISDR and SVR. Thus, sequences of the HCV-J strain and HCV-J strain with single substitutions were defined as the wild-type, and ISDR sequences with more than two mutations were defined as the mutant-type. SVR was achieved in 43.6% of patients with wild-type ISDR and 62.5% of patients with mutant-type ISDR in this study. ISDR alone was insufficient to predict IFN responsiveness in patients who received peginterferon plus ribavirin combination therapy. We speculated that the other region would explain differences in IFN sensitivity in patients infected with wild type ISDR. HCV core, E2-PePHD and NS5A-V3 regions were reported to be associated with IFN response [8,10,19,20]. The HCV core interacts with several cell factors and modulates numerous gene expressions, including down-regulating transcription of IFN-induced antiviral genes, and it affects the inhibition of the antiviral action of IFN. Several studies indicated that the HCV core region could predict IFN responsiveness [8,10]. Therefore, the utility of substitutions of amino acids in the HCV core region combined with NS5A-ISDR sequences for predicting

Table 9 The SVR rate according to EVR amino acid substitutions in 70 core region and ISDR

Core70/ISDR	SVR of patients with EVR (n = 87)	Non SVR of patients with EVR (n = 30)	SVR of patients without EVR (n = 15)	Non SVR of patients without EVR (n = 81)
Q/wild (n = 33)	4 (40%*)	7	2	20 (90.9%**)
Q/mutant (n = 14)	3 (50%*)	3	1	7 (87.5%**)
Non-Q/wild (n = 132)	56 (76.7%*)	17	10	49 (83.1%**)
Non-Q/mutant (n = 34)	24 (88.9%*)	3	2	5 (71.4%**)

*Positive predictive value for SVR. **Positive predictive value for non-SVR. SVR, sustained virologic response; EVR, early virologic response; ISDR, interferon sensitivity-determining region; Q, glutamine.

IFN responsiveness was investigated. The non-Gln70 amino acid substitution in the HCV core region was related to SVR on univariate and multivariate analysis. SVR occurred more frequently in patients without Gln70 (50.6%) than with Gln70 (14.3%). SVR was not associated with aa 75 and aa 91 in the core region. When core 70 was considered in the analysis of ISDR, the SVR rates varied widely according to amino acid substitutions in core region 70 and ISDR. For instance, only 18.1% of patients with Gln70 and wild type ISDR achieved SVR compared with 76.4% in those with non-Gln70 and mutant-type ISDR. Despite having genotype 1b, patients with non-Gln70 and mutant-type ISDR responded to IFN as well as those with genotypes 2 and 3. Pegylated-IFN-alpha 2b and ribavirin combination therapy was suitable for treatment of Japanese patients with HCV genotype 1b, particularly those with non-Gln70 and mutant-type ISDR. Optimal duration of IFN therapy in some patients with non-Gln70 and mutant-type ISDR could be shorter than 48 weeks; and in these patients, costs and side effects could be reduced without reducing the efficacy of IFN therapy by using a shorter regimen. On the other hand, patients with Gln70 and wild type ISDR resistant to pegylated-IFN-alpha 2b and ribavirin combination therapy should receive much more powerful treatment, such as triple therapy including the new protease inhibitor, peginterferon alfa and ribavirin as their first regimen [21,22]. This is an important consideration to achieve optimal therapy and avoid unnecessary treatment. The effects of amino acid substitutions in core 70 on gene expression and core protein function were unclear, and further studies are needed to determine their mechanism. Although the effects of amino acid substitutions of the core region and ISDR were unclear, the mutation at core 70 and the ISDR system could be clinically used as a simple diagnostic tool to predict SVR in patients infected with genotype 1b. It is not easier to routinely measure the HCV sequence to determine the core 70 and ISDR sequence. Virologic response, as rapid virologic response and EVR, could be easy to measure by commercial kits in clinical practice and would be useful for prediction of achieving SVR for chronic hepatitis C patients. The present study also confirmed that EVR has been associated with SVR, but virologic response cannot be assessed before treatment. HCV sequencing analysis will become a convenient method because of progression of sequencing technology and cost reduction. In this respect, the core region and ISDR were useful predictors of virologic response. Analysis of EVR in combination with the core region and ISDR revealed that 24 of 34 patients with non-Gln70 and mutant-type ISDR and EVR achieved SVR. EVR, core region and ISDR are considered strong indicators of SVR for patients with HCV genotype 1b. Although validation of these observations in larger cohorts is required, amino acid substitutions in the core region of HCV and ISDR were useful for predicting the response to pegylated-IFN-alpha 2b and ribavirin combination therapy in patients with chronic hepatitis C genotype 1b. Combining amino acid substitutions in the core region and ISDR could improve the predictive value of SVR in patients with genotype 1b, but the efficacy is still not satisfactory. The explanation for the lack of SVR in patients with non-Gln70 and mutant-type ISDR remains unclear. The other regions of HCV or host factors are candidates for a third factor for improving the prediction of SVR [23,24].

CONCLUSION

Amino acid substitutions in the 70 core region of HCV and ISDR were useful for predicting the response to pegylated-IFN-alpha 2b and ribavirin combination therapy in patients with chronic hepatitis C genotype 1b.

Data of this study were presented in part at the 59th annual meeting of the American association for the study of liver diseases (AASLD), October 31-November 4, 2008, San Francisco, CA, USA.

DISCLOSURE

All people have nothing to disclose.

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Phase I/II Study of Hepatic Arterial Infusion Chemotherapy With Gemcitabine in Patients With Unresectable Intrahepatic Cholangiocarcinoma (JIVROSG-0301)

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Objectives: No established therapy exists for unresectable intrahepatic cholangiocarcinoma (ICC). We conducted a phase I/II study to ascertain the recommended dose (RD) of hepatic arterial infusion using gemcitabine (GEM) for ICC and to assess the efficacy and safety.

Methods: For patients with unresectable ICC, GEM was administered through the hepatic artery via the port system as a 30-minute infusion on days 1, 8, and 15 every 4 weeks for 5 cycles. In phase I, dosage for levels 1, 2, and 3 was set at 600, 800, and 1000 mg/m^2, respectively, and was increased in 3 to 6 patients at a time. Maximum tolerated dose was defined as a dosage resulting in dose-limiting toxicity in 2 of 3 patients or 3 of 6 patients, and RD was estimated during the first cycle. In the phase II, more RD patients were added to assess tumor response and toxicity.

Results: During the phase I, 16 patients were enrolled. Maximum tolerated dose was not reached. Assuming RD at 1000 mg/m^2, the phase II enrolled a total of 13 patients. The following Grade 3 toxicities were observed: neutropenia 20%, increased gamma-glutamyl transpeptidase 8%, increased aspartate aminotransferase 4%, increased alanine aminotransferase 4%, increased bilirubin 4%, nausea 4%, and fatigue 4%. The tumor response rate was 7.7% (complete response 0, partial response 1, stable disease 8, and progressive disease 4).

Conclusion: Whereas the toxicity of hepatic arterial infusion with 1000 mg/m^2 GEM for ICC was tolerable, expected efficacy could not be obtained, thus suggesting only minimal activity.

Key Words: intrahepatic cholangiocarcinoma, hepatic arterial infusion, gemcitabine, phase I/II study, clinical trial

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Intrahepatic cholangiocarcinoma (ICC) constitutes 5% to 15% of cases of the primary hepatic cancer in Japan. It is a cancer with a relatively low incidence, but is characterized by spread from the biliary epithelium to Glisson capsule. ICC has a high incidence of lymph node metastasis and vascular invasion and also tends to invade adjacent organs, so that in a fair number of cases it is already advanced and unresectable at the time of detection.[1–3] Chemotherapy is the treatment option for unresectable ICC, but no standard therapy has been established.[4,5] Typically, drug regimens centered on 5-fluorouracil (5-FU) have been used, but recently, gemcitabine hydrochloride (GEM) has appeared promising.[6]

Hepatic arterial infusion (HAI) chemotherapy is one local therapy for unresectable malignant hepatic tumors and its anticancer effect is obtained by raising the local concentration of the anticancer agent. Local therapy also reduces systemic adverse response and can increase the effect on the hepatic lesions by infusing the active medicinal agent into a hepatic artery.[7] In Japan, HAI with percutaneous placement of a catheter-port system is highly feasible,[8–10] and HAI of GEM can be continued systematically. If a local effect for ICC supplying from the hepatic artery can be obtained with HAI of GEM, this treatment may contribute to prolonging patient survival.

With this as background, we designed a phase I and II clinical trial to evaluate HAI chemotherapy with GEM for unresectable ICC, and a multicenter study was carried out by the Japan Interventional Radiology in Oncology Study Group.

MATERIALS AND METHODS

Study Design and Patient Eligibility

A phase I and II clinical trial at multiple institutions was designed to determine the dose-limiting toxicity (DLT) and recommended dose (RD) for HAI chemotherapy with GEM to treat unresectable ICC, as well as to evaluate its safety and tumor response effect. Dose-limiting toxicity and recommended dose of hepatic arterial infusion of GEM were determined as the primary end point, and the frequency and severity of adverse events, tumor response effect in the liver only, and tumor response effect in the whole body were the secondary end points. In phase I portion, DLT was assessed and RD was estimated, and in phase II portion, cases were added at the estimated RD, and the tumor response effect was evaluated. Toxicity assessment was conducted in all patients with HAI chemotherapy.

The inclusion criteria were the following conditions for cases of unresectable ICC:

1. Cases of histologically confirmed ICC (initial tumor or recurrence after resection), which was determined to be unresectable by a hepatic surgeon at each institution, or it was judged to be the prognosis-determining factor, even when metastasis was found as extrahepatic lesions.
2. Cases that were previously untreated with GEM or that were previously treated with agents other than GEM in the past, but had received no chemotherapy for at least 4 weeks from the last session, and were not responded by the chemotherapy.
3. Cases in which measurable lesions that corresponded to the target lesions on response evaluation criteria in solid tumors were located in the liver and had maximum tumor diameters of 20 mm or more

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on computed tomography (CT) images with 10-mm slices or 10 mm or more on CT images with slices of 5 mm or less.
4. Cases in which a port-catheter system for HAI was placed percutaneously, and arterially infused contrast medium was distributed through the entire liver or at least the entire hepatic lesions and in whom it was confirmed that there was no distribution of the arterially infused contrast medium in the surrounding extrahepatic organs based on CT angiography or MR angiography from the implanted port.
5. Cases aged 20 years or more with an Eastern Cooperative Oncology Group performance status classification of 2 or less.
6. Cases in which major organ function was maintained (white blood cell count $\geq 3000/mm^3$ and $\leq 12{,}000/mm^3$, platelets $\geq 100{,}000/mm^3$, transaminase ≤ 5 times the institution's upper limit of normal, serum total bilirubin ≤ 3.0 mg/dL, serum creatinine ≤ 1.5 mg/dL, electrocardiogram not indicating the need for treatment) and in whom hepatic function was Grade 2 or less on National Cancer Institute-Common Toxicity Criteria (NCI-CTC) (version 2.0) with consideration of the influence of the hepatic lesion.
7. Cases of life expectancy of more than 8 weeks.
8. Cases in which written informed consent was obtained.

Patients excluded from the trial were the patients who scheduled for radiation therapy for the hepatic portal region because of hepatic portal region invasion or lymph node metastasis, or who had previously undergone radiation therapy; patients with concurrent infection excluding viral hepatitis, fever of 38°C or above, or who required antibiotics; patients with serious complications (intestinal paralysis, intestinal obstruction, interstitial pneumonia, pulmonary fibrosis, intractable diabetes mellitus, cardiac failure, renal failure, hepatic failure, etc); patients with other concurrent cancer; patients who could not undergo angiography because of allergy to iodinated contrast material; patients with serious mental disabilities; patients who were pregnant or may have been pregnant, and nursing mothers; and patients whose catheters for HAI chemotherapy were placed via laparotomy.

This study protocol was approved by the ethics committee of the Japanese Society of Interventional Radiology and the institutional review boards of the participating hospitals.

Treatment Protocol and Evaluation Methods

Using a percutaneously placed HAI catheter-port system, 1 course was defined as HAI of GEM on days 1, 8, and 15; a course was performed every 4 weeks for a total of 5 courses.

In phase I portion, the GEM dosage was set at Level −1, 400 mg/m^2; Level 1, 600 mg/m^2; Level 2, 800 mg/m^2; and Level 3, 1000 mg/m^2. Because the approval dosage of GEM is 1000 mg/m^2 in Japan, we defined it as the upper limit in this study. The design called for increase at each level in 3 to 6 patients from Level 1. Three patients were enrolled at each level. The study on the next dose level was not conducted until all 3 patients had completed the first cycle without any problems regarding safety and tolerance. If a DLT of any type was detected in 1 of 3 patients during the first cycle, an additional 3 patients were enrolled. If DLT was detected in more than 2 patients, the dose was defined as the maximum tolerated dose (MTD). RD was estimated to be one level below that judged to be MTD. DLT was defined as follows and judged during the first course: Grade 4 leukopenia or neutropenia; Grade 4 thrombocytopenia; nonhematologic toxicities of Grade 3 or more (excluding that from PD, nausea/vomiting, and alopecia; for patients whose pre-enrollment level of transaminase or serum total bilirubin was Grade 2, DLT was taken to be more than twice the pre-enrollment level); not meeting the criteria to start administration (same as the enrollment criteria) for the next course on day 29 because of toxicity.

In phase II portion, up to 13 patients were added at the dose found to be RD in phase I portion and the tumor response effect was judged using response evaluation criteria in solid tumors. Because HAI was being used, the target lesion was limited to hepatic lesions. Tumor size was measured on intravenous contrast-enhanced CT within 2 weeks before enrollment, and the tumor response effect was judged after the completion of courses 1, 3, and 5, and as needed.

Toxicity assessment was done in all cases using NCI-CTC (version 2.0) and the frequency of the worst grade was obtained during all courses. Physical examination and blood tests were done immediately before the start of each treatment and recorded.

Statistical Analysis

In phase I portion, the number of enrolled patients per level from Level −1 to Level 1 was minimum 6. The maximum number of patients up to Level 3, in case that MTD was reached, was 18 patients in the dose finding stage. In phase II portion, when the threshold tumor response rate was taken to be 20% and the expected efficacy rate was set at 50%, 13 patients would be needed to judge the tumor response effect under conditions of $\alpha = 0.1$ and $\beta = 0.2$, and 7 to 10 cases would need to be added at the estimated RD. For the entire study, a maximum of 25 patients was needed.

RESULTS

Patient Backgrounds

A total of 16 patients were enrolled in the phase I portion (May 2004–November 2005), and 9 patients were added for the phase II portion (February 2006–November 2006). All patients met the eligibility requirements. A summary of all 25 patients is shown in Table 1.

Phase I Portion

In phase I portion, 6 patients were registered at Level 1, 6 at Level 2, and 4 at Level 3. DLT appeared in 2 of the 6 patients at Level 1, and 2 of the 6 patients at Level 2, but DLT did not appear at Level 3. The third and fourth patients at Level 3 were registered at almost the same time. Four patients did not meet the criteria to start administration for the second course on day 29. In these 4 patients, the administration of drugs had been delayed because of Grade 1 and 2 leukopenia (n = 3) or thrombocytopenia (n = 4) in the first course. No Grade 4 hematologic toxicity or nonhematologic toxicity of Grade 3 or more was seen in the first course (Tables 2, 3). MTD was not reached up to Level 3. Accordingly, the RD was assumed to be the Level 3 dose of 1000 mg/m^2.

Phase II Portion

Nine patients were added at GEM 1000 mg/m^2. In these patients, together with the patients at Level 3 in phase I portion (total: 13 patients), the tumor response effect was complete response 0/partial response 1/stable disease 8/progressive disease 3/not evaluated 0 in the liver only, and complete response 0/partial response 1/stable disease 8/progressive disease 4/not evaluated 0 in the whole body. The response rate was 7.7% (95% confidence interval [CI], 0.2%–36.0%). Although disease control was not one of the assessment items, the disease control rate with SD added was 69% (95% CI, 38.6%–90.9%). The tumor response effect and survival in all 25 treated patients are shown in Table 4 and Figure 1.

Toxicity

The incidence of adverse events (NCI-CTC version 2.0) of Grade 3 or more in all treated cases was 20% neutropenia, 8% elevated gamma-glutamyl transpeptidase (GGT), 4% elevated aspartate aminotransferase (AST), 4% elevated alanine aminotransferase (ALT), 4% elevated bilirubin, 4% nausea, and 4% fatigue. The only

TABLE 1. Patients' Characteristics

Phase	Phase I			Phase II	
Level of GEM Dose	Level 1	Level 2	Level 3	Estimated RD	All Patients
GEM dose	600 mg/m^2	800 mg/m^2	1000 mg/m^2	1000 mg/m^2	600, 800, 1000 mg/m^2
No. patients	6	6	4	9	25
Age (yr)					
Median (range)		64 (34–76)		56 (46–74)	58 (34–76)
Gender					
Male	3	5	3	7	18
Female	3	1	1	2	7
ECOG PS					
0	4	5	3	7	19
1	1	1	1	2	5
2	1	0	0	0	1
Previous therapy					
None	4	2	3	4	13
Resection	1	3	1	5	10
Chemotherapy	1	0	1	2	4
Embolization or ablation	0	2	0	1	3
Extrahepatic lesions					
None	3	3	2	8	16
Lymph node	3	3	2	0	8
Peritoneum	1	0	0	0	1
Lung	0	1	2	1	4
Median no. courses administered	5	4.5	4		5
Median no. administrations	15	14	12		15
Relative dose intensity	81.9%	87.3%	84.8%		84.7%

ECOG indicates Eastern Cooperative Oncology Group performance status.

TABLE 2. No. Patients With Hematologic Toxicities (Cycle 1, Phase I Portion, n = 16)

	Level 1 600 mg/m^2 6				Levele 2 800 mg/m^2 6				Level 3 1000 mg/m^2 4			
Level Dose n Grade	1	2	3	4	1	2	3	4	1	2	3	4
Leucocytes	1	2	0	0	1	3	0	0	2	1	0	0
Neutrophils	0	2	1	0	1	1	2	0	1	1	0	0
Hemoglobin	0	1	0	0	0	0	0	0	0	0	0	0
Platelets	2	2	0	0	2	1	0	0	1	1	0	0

Grade 4 event was elevated bilirubin in 1 patient in the second course, but this was accompanied by portal vein tumor thrombosis (Tables 5, 6).

Events related to the HAI procedure included difficulties with the placed catheter-port system in 5 patients (catheter obstruction in 3 patients, port damage in 2 patients), and hepatic artery occlusion in 1 patient. In 2 of the patients with catheter obstruction and the 2 patients with port damage the catheter or port was exchanged and the treatment continued. The remaining patient with catheter obstruction showed an antitumor effect of PD, so the catheter was not replaced and the treatment was stopped. In the patient with hepatic artery occlusion, a left hepatic artery occlusion occurred in the second course, which meant that the drug was not reaching the left lobe of the liver, and the treatment was discontinued.

TABLE 3. No. Patients With Adverse Events (Cycle 1, Phase I Portion, n = 16)

	Level 1 600 mg/m^2 6				Level 2 800 mg/m^2 6				Level 3 1000 mg/m^2 6			
Level Dose n Grade	1	2	3	4	1	2	3	4	1	2	3	4
Nausea	0	2	0	0	2	0	0	0	3	0	0	0
Vomiting	0	1	0	0	0	0	0	0	2	0	0	0
Fatigue	1	1	0	0	3	0	0	0	0	0	0	0
Stomatitis	0	0	0	0	1	0	0	0	0	0	0	0
Headache	0	0	0	0	1	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	0	0	0	0	0
Fever without neutropenia	0	0	0	0	0	0	0	0	1	0	0	0
Anorexia	0	0	0	0	0	0	0	0	0	0	0	0
Alopecia	0	0	0	0	1	0	0	0	0	0	0	0
Alkaline phosphatase	2	0	0	0	1	0	0	0	1	0	0	0
Bilirubin	1	0	0	0	0	0	0	0	0	0	0	0
GGT	1	0	0	0	0	1	0	0	0	0	0	0
Hypoalbuminemia	0	0	0	0	0	0	0	0	1	0	0	0
SGOT (AST)	1	0	0	0	0	0	0	0	1	0	0	0
SGPT (ALT)	0	0	0	0	0	1	0	0	1	0	0	0
Hyperkalemia	0	0	0	0	1	0	0	0	0	0	0	0
Hyponatremia	0	0	0	0	0	0	0	0	1	0	0	0

TABLE 4. Objective Response and Clinical Outcome

GEM Dose	600 mg/m²		800 mg/m²		1000 mg/m² (Phase II)		All Patients	
No. Patients	6		6		13		25	
Evaluation Site	Liver	Whole Body	Liver	Whole Body	Liver	Whole Body	Liver	Whole Body
Best response								
CR	0	0	0	0	0	0	0	0
PR	0	0	2	2	1	1	3	3
SD	4	4	3	3	9	8	16	15
PD	2	2	0	0	3	4	5	6
NE	0	0	1	1	0	0	1	1
Response rate	0%	0%	33.3%	33.3%	7.7%	7.7%	12.0%	12.0%
95% CI	0%–45.9%	0%–45.9%	4.3%–77.7%	4.3%–77.7%	0.2%–36.0%	0.2%–36.0%	2.5%–31.2%	2.5%–31.2%
Disease control rate	66.7%	66.7%	83.3%	83.3%	76.9%	69.2%	76.0%	72.0%
95% CI	22.3%–95.7%	22.3%–95.7%	35.9%–99.6%	35.9%–99.6%	46.2%–95.0%	38.6%–90.9%	54.9%–90.6%	50.6%–87.9%
Median survival time	297 d		298 d		389 d		340 d	
95% CI	140–454 d		0–747 d		158–620 d		198–482 d	

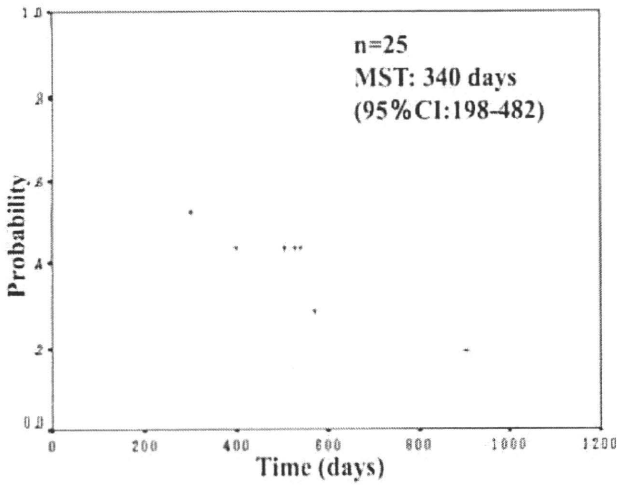

FIGURE 1. Survival time in all 25 patients received hepatic arterial infusion with gemcitabine.

TABLE 5. No. Patients With Hematologic Toxicities (Cycle 1–5, Phase I–II Portion, n = 25)

	600 mg/m²				800 mg/m²				1000 mg/m²			
	6				6				13			
Dose n												
Grade	1	2	3	4	1	2	3	4	1	2	3	4
Leucocytes	1	3	0	0	0	4	0	0	4	6	0	0
Neutrophils	0	2	1	0	1	1	2	0	1	7	2	0
Hemoglobin	0	1	0	0	0	1	0	0	2	1	0	0
Platelets	2	2	0	0	2	1	0	0	6	3	0	0

DISCUSSION

ICC originates in the biliary epithelium and is almost always adenocarcinoma. In Japan, it has been reported to account for 5% to 15% of primary hepatic cancers. The only curative treatment is surgical resection. However, at the time of detection, the cancer is often judged to be unresectable because of liver metastasis, vascular invasion, lymph node metastasis, or other distant metastasis.[1–3]

TABLE 6. No. Patients With Adverse Events (Cycle 1–5, Phase I–II Portion, n = 25)

	600 mg/m²				800 mg/m²				1000 mg/m²			
	6				6				13			
Dose n Grade	1	2	3	4	1	2	3	4	1	2	3	4
Nausea	0	2	0	0	3	0	1	0	7	1	0	0
Vomiting	0	0	0	0	1	1	0	0	3	0	0	0
Fatigue	1	1	0	0	3	0	1	0	3	2	0	0
Stomatitis	0	0	0	0	1	0	0	0	0	0	0	0
Headache	0	0	0	0	1	0	0	0	1	0	0	0
Diarrhea	1	0	0	0	0	0	0	0	0	0	0	0
Fever without neutropenia	0	0	0	0	1	0	0	0	4	1	0	0
Anorexia	0	0	0	0	0	0	0	0	4	1	0	0
Alopecia	0	0	0	0	1	0	0	0	1	0	0	0
Alkaline phosphatase	3	0	0	0	1	0	0	0	2	4	0	0
Bilirubin	1	0	0	0	3	0	0	0	1	1	0	1
GGT	1	0	0	0	0	1	0	0	1	0	2	0
Hypoalbuminemia	0	0	0	0	0	0	0	0	3	2	0	0
SGOT (AST)	1	0	0	0	1	0	0	0	4	2	1	0
SGPT (ALT)	0	0	0	0	1	1	0	0	3	2	1	0
Hyperkalemia	0	0	0	0	1	0	0	0	1	0	0	0
Hyponatremia	0	0	0	0	1	0	0	0	1	0	0	0

Chemotherapy is the treatment option for unresectable ICC but no standard therapy has been established.[4,5] Multiagent treatment has been reported with drugs such as 5-FU, mitomycin C (MMC), adriamycin, and epirubicin hydrochloride similar to biliary tract cancer (extrahepatic bile duct cancer, gallbladder cancer). Combined use of cisplatin and 5-FU is reportedly effective but all of these reports are from case studies only.[11,12] HAI chemotherapy has also been attempted for unresectable intrahepatic bile duct cancer and regimens such as FAM (5-FU + adriamycin + MMC), FEM (5-FU + epirubicin hydrochloride + MMC), high-dose 5-FU, and low-dose FP (5-FU + cisplatin) have been reported to be effective.[13] Again, however, all of these reports are from case studies only.

A new anticancer agent of GEM has been introduced for pancreatic cancer and biliary tract cancer, which has no standard therapy like ICC.[6] For pancreatic cancer chemotherapy, it is the drug of choice.[14,15] In treating ICC with GEM, good results were reported in 2001 from a phase II trial in Germany in which the tumor response effect was reported to be 30% and the median survival time (MST) was 9.3 months.[16] Because ICC is classified as a primary hepatic cancer in Japan, HAI of GEM has also been attempted. Tsujino et al performed HAI of GEM at the recommended dose of 1000 mg/m^2 with intravenous infusion, and they observed tumor size and tumor marker reductions.[17]

Whereas no consensus has been reached with regard to the contribution of HAI to extending survival in cases of hepatic metastasis of colorectal cancer, the local tumor response effect is considered to be superior to that with systemic chemotherapy.[18–20] Moreover, in hepatocellular carcinoma which is a primary hepatic cancer like ICC, the intra-arterial local therapy for hepatic arterial chemoembolization is thought to significantly prolong survival in unresectable cases compared with the results of symptomatic treatment.[21,22] It is possible that local therapy can also prolong survival in cases of ICC.

This study was designed with consideration of the above to establish the DLT for HAI of GEM and estimate the RD; the tumor response effect with the estimated RD was then determined and safety was evaluated. In phase I portion, GEM was increased from 600 mg/m^2 to 800 mg/m^2 and 1000 mg/m^2. A delay in the start of the second course because of Grade 1 and 2 leukopenia or thrombocytopenia as DLT was seen in 4 cases (25%). MTD was not reached up to dosage Level 3. Thus, RD was estimated to be 1000 mg/m^2, and more patients were added in phase II portion.

The incidence of adverse events of Grade 3 or more in all courses was 20% neutropenia, 8% elevated GGT, 4% elevated AST, 4% elevated ALT, 4% elevated bilirubin, 4% nausea, and 4% fatigue. The only Grade 4 event was elevated bilirubin in 1 case during the second course. However, this was a case of portal vein tumor thrombosis, which was thought to have caused the elevated bilirubin. Toxicity with HAI of GEM was generally tolerable throughout all courses and it was milder than in reports of systemic administration.[23]

Events related to the HAI itself or the implanted catheter-port system occurred in 6 cases (24%). Most were dealt with by replacing the port in order that HAI could be continued. Hepatic artery occlusion occurred in only 1 case. Compared with other reports,[8–10] more of the present cases were within the tolerable range. No catheter or port infection or induced thrombosis was observed.

The response rate of HAI of GEM at the estimated RD of 1000 mg/m^2 in 13 cases of unresectable ICC was 7.7% (CR, n = 0; PR, n = 1), which was below the established threshold efficacy rate of 20%. Although disease control was not one of the items investigated in this study, the disease control rate including SD (n = 8) was 69% and MST in all 25 patients was 340 days (95% CI: 198–482 days).

In conclusion, DLT was the delay in the start of the second course because of Grade 1 and 2 leukopenia or thrombocytopenia and RD was estimated to be 1000 mg/m^2 in HAI of GEM for unresectable ICC. Toxicity was within the tolerable range. However, the tumor response effect of HAI of GEM at 1000 mg/m^2 was low, and it was judged that no improvement in treatment results can be expected with HAI. The disease control rate and MST were acceptable, but, considering that the subjects in this study were patients whose hepatic lesions were predominant and that the implanted catheter-port system was required for HAI as a painful procedure, it cannot be claimed that this protocol has an advantage over systemic treatment.

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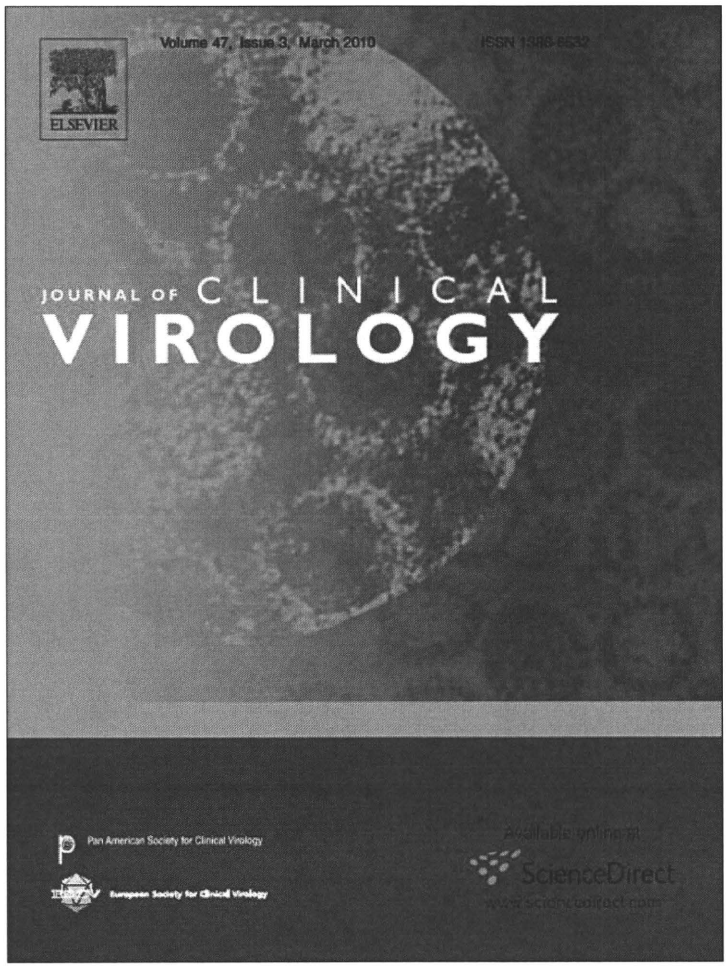

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Transient reappearance of serum hepatitis C virus RNA observed by real-time PCR during antiviral therapy with peginterferon and ribavirin in patients with HCV genotype 1b

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ABSTRACT

Background: The "response-guided therapy" based on response of hepatitis C virus (HCV) during antiviral combination therapy with peginterferon and ribavirin is important for patients with HCV genotype 1. However, the sensitivity of previous assays for serum HCV RNA is limited.
Objectives: We evaluated the changes in serum HCV RNA during the combination therapy using a novel method for measurement based on real-time PCR.
Study design: Changes in serum HCV RNA during the combination therapy were reanalyzed using TaqMan PCR assay in 144 patients with chronic HCV genotype 1b infection who underwent the therapy under HCV RNA monitoring with the Amplicor Monitor assay. Treatment duration was elongated from 48 weeks to 72 weeks in 17 patients based on the time when serum HCV RNA became negative.
Results: In 9 of 144 (6.3%) patients, serum HCV RNA transiently appeared again on the TaqMan PCR assay after having previously become negative. At the point of reappearance, the Amplicor Monitor assay gave a negative result in all patients, and no flare of alanine aminotransferase activity was observed. Each of the 9 patients achieved an end-of-treatment response but relapsed after the end of treatment, including 3 patients in whom the treatment duration was elongated to 72 weeks.
Conclusions: Attention should be paid to this phenomenon in the antiviral treatment for patients with HCV infection. The transient reappearance of HCV RNA in the serum indicates a high likelihood of relapse, and is likely to be missed without frequent measurements by a sensitive detection method.

1. Background

The current standard antiviral therapy for patients with chronic hepatitis C is combination therapy with peginterferon (PEG-IFN) and ribavirin.[1] Although the rate of sustained virologic response (SVR), which indicates the eradication of hepatitis C virus (HCV), has increased with the use of the current regimen, it is still only around 50% in patients infected with HCV genotype 1.[2–8] The response of HCV during combination therapy, i.e., the changes in serum HCV RNA after the start of therapy have been reported to be predictors of the therapeutic outcome[9–12]; therefore "response-guided therapy" based on this response is now favored,[12,13] especially for patients with HCV genotype 1.

To improve outcome prediction and the selection of treatment duration for response-guided therapy, more precise and sensitive evaluation of serum HCV RNA is necessary. Serum HCV RNA concentration has previously been measured by the branched-DNA probe assay and, more recently, by the Amplicor Monitor assay.[14,15] However, the sensitivity of these assays is limited. Very recently, a novel method for measurement of serum HCV RNA, based on real-time PCR, has been established, and is reported to have high sensitivity for the detection of serum HCV RNA.[16–18]

2. Objectives

In the present study, we used the real-time PCR-based TaqMan assay to reanalyze the changes in serum HCV RNA from stored serum samples of patients with chronic HCV genotype 1 infection. These patients had undergone antiviral combination therapy with PEG-IFN and ribavirin under monitoring of serum HCV RNA using the Amplicor Monitor assay. In some patients, we observed a reappearance of serum HCV RNA during the treatment after hav-

Abbreviations: HCV, hepatitis C virus; PEG-IFN, peginterferon; SVR, sustained virologic response; RVR, rapid virologic response; cEVR, complete early virologic response; ETR, end-of-treatment response.

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