antiviral activities. The direct antineoplastic effects include cell damage (27), induction of cyclin-dependent kinase inhibitors involved in G1/G0 arrest (22) and delayed cell cycle (28). The indirect antineoplastic effects include activation of natural killer cells, T cells and macrophages (29–31). In various cultures of malignant cells, IFN-α exhibited a biomodulatory effect that enhanced the antineoplastic activity of 5-FU partly because of the arrangement of metabolism of 5-FU to fluoro-deoxy-uridylate (32–36). Furthermore, 5-FU and IFN-α synergize the antineoplastic effects of each other. The antineoplastic effects of the combination therapy of intra-arterial 5-FU and IFN are also considered to be mediated by modulating tumour necrosis factor-related apoptosis-inducing ligand receptor-induced cytotoxic pathway (37).

Several subtypes of natural IFN-α have been described (38), while only one subtype is available for recombinant IFN-α. Patients treated with natural IFN-α barely have antibodies to IFN, whereas circulating antibodies to IFN are sometimes detected in patients treated with recombinant IFN-α (12, 13). Antibodies to IFN weaken the therapeutic effects of IFN. Therefore, antibodies to IFN may dampen the effects of the combination therapy of intra-arterial 5-FU and IFN. This hypothesis favours the combination therapy of intra-arterial 5-FU and natural IFN-α relative to 5-FU and recombinant IFN-α.

Interferon-α subtypes exhibit several variations in biological activity. With regard to the antiviral activity, IFN-α8 is reported to be the most potent while IFN-α1 the least potent (39). IFN-α8 was the most potent in the induction of antineoplastic effect on renal cell carcinoma (40). OIF®, but not Intron A®, contains IFN-α8. Considered with the deficiency of the subtypes *in vitro*, the effect of combination therapy with IFN-α may be different based on the IFN.

Our results, however, showed no significant differences between the two groups with respect to the early response, adverse reactions, TTP and survival rate. What are the reasons for the lack of differences *in vivo*? One reason may relate to the dose and antineoplastic activity of IFN (19–31, 39, 40). Several groups have studied the impact of IFN treatment on HCC. Two controlled trials reported by Lai *et al.* (41, 42) using very high doses of IFN (50×10^6 IU/m^2) showed a 30% response rate and improvement in survival compared with no treatment. In comparison, another study using a low dose of IFN (3×10^6 IU/m^2) did not show any survival advantage (43). Considered together, it appears that for IFN alone to be effective against HCC, its dose must be higher than that used for the treatment of chronic hepatitis B and C. Administration of high-dose IFN may improve the effect of the combination therapy. However, under such circumstances, many patients could potentially DO because of the adverse reactions. Thus, our protocol is safe regardless of the type of IFN used. The second reason relates to the relationship between IFN subtype and the mechanism of action of the combination therapy (32–40). Although the mechanism is not yet clear, the direct effect of inhibition of cancer cells and the anti-angiogenic effect of IFN might play minor roles in our protocol *in vivo*. The most important mechanism of action of the combination therapy of our protocol may be enhancement of the antineoplastic effect of 5-FU by IFN. Thus, the IFN subtype does not seem to strongly influence the effect of the combination therapy. The third reason may relate to the several limitations in our study (e.g. small sample size, not randomized-controlled trial).

Most of the adverse reactions were controllable in the present cohort. The adverse reactions of anaemia, leucopenia and thrombocytopenia were controllable without G-CSF or blood transfusion. Depression owing to IFN was not observed in our patients. Thus, the lack of severe pancytopenia in patients with advanced HCC treated by the current protocol reflects the safety of intra-arterial 5-FU and IFN. It is recommended, however, that careful treatment should be provided to patients who develop pancytopenia.

Our study fell somewhat short of conclusiveness owing to the small number of patients. Thus, our study should be extended to include a long-term follow-up and a large sample size. In our protocol of the combination therapy, there were no significant differences between recombinant IFN-α-2b and natural IFN-α with regard to early response to therapy, adverse effects, TTP and survival rates. Recombinant IFN-α-2b is inexpensive compared with natural IFN-α. Our analysis showed a better cost-effectiveness ratio for recombinant IFN-α-2b than natural IFN-α. Thus, assuming no difference in outcomes between the two regimens, we recommend the use of recombinant IFN-α-2b based on the cost-effectiveness.

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Biliary Complications after Duct-to-duct Biliary Reconstruction in Living-donor Liver Transplantation: Causes and Treatment

Hirotaka Tashiro · Toshiyuki Itamoto · Tamito Sasaki · Hideki Ohdan · Yasuhiro Fudaba · Hironobu Amano · Saburo Fukuda · Hideki Nakahara · Kohei Ishiyama · Akihiko Ohshita · Toshihiko Kohashi · Hiroshi Mitsuta · Kazuaki Chayama · Toshimasa Asahara

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Abstract
Background In living-donor liver transplantation (LDLT), biliary complications are recognized as a significant cause of post-transplantation morbidity.
Methods Eighty patients who underwent LDLT with duct-to-duct biliary reconstruction at Hiroshima University Hospital were enrolled in this study. The mean follow-up was 24 months (range, 3–72 months). Eighteen patients underwent the basiliximab-based immunosuppressive therapy, and 62 patients underwent non-basiliximab-based immunosuppressive therapy. The development of biliary complications after LDLT was retrospectively analyzed. Biliary complications were initially treated by endoscopic or radiological modalities.
Results Biliary leakages and strictures occurred in 12 (15%) and 20 (25%) of the 80 patients, respectively. Stepwise multivariate analysis demonstrated bile leakage to be an independent risk factor for the development of biliary stricture ($p = 0.001$) and basiliximab-based immunosuppressive therapy to be an independent protective factor for postoperative biliary leakage ($p = 0.005$). The 1-week total doses of steroids were significantly lower in the basiliximab-based immunosuppressive regimes (mean dose: 573mg) than in the non-basiliximab-based ones (mean dose: 1,121mg) ($p = 0.01$). All patients with biliary leakage were successfully treated with endoscopic or radiological modalities, except one patient who was treated by surgical treatment. Endoscopic or radiological modalities were successful as primary treatment modalities in 12 (60%) of 20 patients with biliary strictures. Lastly, six patients were treated surgically with long-term success, except for one patient with chronic cholangitis who died after 16 months.
Conclusions Steroid-sparing basiliximab-based immunosuppressive therapy reduced the incidence of biliary leakage, and biliary leakage was the independent factor for biliary stricture. The non-surgical and surgical treatments for biliary complications were satisfactory.

Various refinements in surgical techniques, postoperative management, and immunosuppressive management have reduced the incidence of complications after liver transplantation. Biliary complications, however, continue to be a significant cause of morbidity after liver transplantation [1, 2]. In living-donor liver transplantation (LDLT), the biliary system is usually reconstructed by performing a Roux-en-Y hepaticojejunostomy (RYHJ), which results in biliary complications in 12%-18% of recipients [3, 4]. In 1998, Wachs et al. first reported duct-to-duct reconstruction for LDLT [5]. Duct-to-duct direct biliary reconstruction has been performed in many institutes, and the advantages of duct-to-duct biliary reconstruction over hepaticojejunostomy have been pointed out in several reports. For example, it preserves the physiological bilioenteric and bowel continuity, thus preventing delayed bowel movements. Further, it permits easy endoscopic access to the biliary tree for diagnostic and therapeutic instrumentation and assists the prevention and management

H. Tashiro (✉) · T. Itamoto · H. Ohdan · Y. Fudaba · H. Amano · S. Fukuda · H. Nakahara · K. Ishiyama · A. Ohshita · T. Kohashi · H. Mitsuta · T. Asahara
Second Department of Surgery, Hiroshima University, 1-2-3, Kasumi, Hiroshima 734-8551, Japan
e-mail: htashiro@hiroshima-u.ac.jp

T. Sasaki · K. Chayama
First Department of Internal Medicine, Hiroshima University, 1-2-3, Kasumi, Hiroshima 734-8551, Japan

of ascending cholangitis [6–9]. As the number of patients who have undergone LDLT with duct-to-duct biliary reconstruction has increased, however, a variety of biliary complications have emerged [10–12]. Some reports have addressed the occurrence of late biliary complications, particularly biliary strictures, in many patients with a significant median follow-up duration (>12 months) [9, 13]. In the present study, to evaluate the safety of duct-to-duct biliary reconstruction in LDLT, we retrospectively analyzed the biliary complications observed, with focus on biliary leakage and stricture.

Materials and methods

Patients and surgical procedures

Between May 2000 and September 2006, 85 patients underwent LDLT at Hiroshima University. Among these patients, the 80 patients who underwent duct-to-duct biliary reconstruction along with LDLT were enrolled in this study. Patient, graft, and operative characteristics are summarized in Table 1. The series comprised 47 men and 33 women (average age: 50 years). The most common indications for LDLT were viral hepatitis and cirrhosis with or without hepatocellular carcinoma (n = 54), followed by fulminant hepatic failure (n = 10), primary biliary cirrhosis (n = 8), autoimmune hepatitis (n = 6), and others (n = 2). The most commonly used graft type was a right hemi-liver (n = 66), followed by a left hemi-liver (n = 14). The donors included 49 men and 31 women (average age: 35 years).

The mean model for end-stage liver diseases (MELD) score at the time of LDLT was 17.9 (range: 5–50). The mean graft-to-recipient weight ratio (GRWR) was 1.08 (range: 0.5–2.4); moreover, none of the grafts included a middle hepatic vein. The mean operative time was 12 h 8 min (range: 9–25 h). The mean total ischemic time was 108 min (range: 43–240 min), and the warm ischemic time was 45 min (range: 32–220 min).

The immunosuppressive regimen comprised cyclosporine with mycophenolate mofetil (MMF) and methylprednisolone and basiliximab (n = 18), or cyclosporine with MMF and methylprednisolone (n = 8), or tacrolimus with methylprednisolone (n = 56). The steroid-sparing basiliximab-based immuosuppressive therapy was indicated for viral hepatitis, because the steroid might contribute to the acceleration of hepatitis viral replication. Basiliximab 20 mg was given intravenously on both day 0 and day 4 after surgery. Tacrolimus was administered with a level of 5 ng/ml for the first 48 h postoperatively in order to maintain renal function. Then, the dose of tacrolimus was adjusted to maintain a level of 10–15 ng/ml during the first month, and afterward tapered to achieve a level of 5–10 ng/ml. Cyclosporine was also administered at a level of 50–100 ng/ml for the first 48 h postoperatively in order to maintain renal function. Then, the dose of cyclosporine was adjusted to maintain a level of 250–300 ng/ml during the first month, after which it was tapered to achieve a level of 150–250 ng/ml. Dose reductions of both tacrolimus and cyclosporine were performed primarily on the basis of renal and liver function. For patients with renal insufficiency, tacrolimus or cyclosporine was not given until renal function improved. After oral medication capsules were tolerated, MMF was given at a dose of 500–1,000 mg a day. Mycophenolate mofetil was tapered and discontinued, based on gastrointestinal toxicity and myelosuppression. Treatment with steroids was discontinued 2–3 months after LDLT. In basiliximab-based immuosuppressive therapy, patients either received no methylpredonisolone or they received 250 mg methylpredonisolone intravenously during surgery, followed by daily tapering (starting at 120 mg/day and ending at a baseline 40 mg/day, intravenously).

Table 1 Living-donor liver transplantation patient demographics

Characteristics	Number = 80
Age (range, years)	50 (20–69)
Male	47 (59%)
MELD (range)	17.9 (5–50)
Indication	
Liver cirrhosis (HCC)	54 (35)
Cholestatic disease	8
Fulminant hepatic failure	10
Autoimmune hepatitis	6
Others	2
Donor	
Age (range, years)	35 (18–64)
Male	49 (31)
Graft	
Left lobe	14
Right lobe	66
GRWR	1.08 (0.5–2.4)
Immunosuppresive therapy	
Tac + steroid	54
CyA + steroids + MMF	8
CyA + steroids + MMF + Bax	18
Operation	
Time (range)	12 h 8 min (9h–25h)
Blood loss (range)	4875ml (345–39500)
Total ischmic time (range)	108min (43–240)
Warm ischmic time (range)	45min (32–220)

MELD model for end-stage liver disease; GRWR graft: recipient weight ratio; Tac tacrolimus; CyA cyclosporin; MMF mycophenolate mofetil; Bax basiliximab

Treatment with oral methylpredonisone (32 mg/day) was initiated on postoperative day 7–10. In non-basiliximab based immunosuppression therapy, patients received 500 mg methylpredonisolone intravenously during surgery, followed by daily taper (starting at 250 mg/day and ending at a baseline 40 mg/day, intravenously). Treatment with oral methylpredonisone (32 mg/day) was initiated on day 7–10. Subsequent adjustment in maintenance methylpredonisolone was dependent on the patient's clinical course.

Donor assessment and surgery

The donors underwent several preoperative examinations, including computed tomography (CT) and drip-infusion cholangiography-CT, in order to assess the biliary and vascular system. The surgical techniques for donor hepatectomy have been described elsewhere [14]. Briefly, prior to parenchymal transection, routine intraoperative cholangiography was performed with fluoroscopy to determine the transection point of the hepatic duct. Minimal dissection was performed at the hilar plate around the hepatic duct. The liver was then transected with an ultrasonic dissector without inflow occlusion. The hepatic duct was sharply severed near the confluence, and the remnant stump was carefully closed with 6–0 polydioxanone monofilament sutures (PDS; Ethicon, Inc., Tokyo, Japan). The liver graft was perfused with University of Wisconsin (UW) solution. The diameters of the bile duct and vessels of the graft and the graft weight were directly measured. The average intraoperative blood loss was 310 ml. None of the 80 donors were given a blood transfusion.

Recipient surgery

In total hepatectomy, the hilar plate was dissected sharply at or distal to the second-order branch of the bile duct. In the dissection, careful attention was paid in order to preserve as much as possible of the surrounding tissues with an adequate blood supply to the bile duct. To maintain the blood supply to the bile duct from the right hepatic artery, dissection between the right hepatic artery and the bile duct was avoided. Bile duct anastomosis was performed after completion of all vascular anastomoses and reperfusion of the liver graft. Wherever possible, we prospectively performed duct-to-duct biliary reconstruction. An end-to-end anastomosis between the graft and recipient bile ducts was performed using an interrupted 6–0 PDS, beginning from the posterior wall and terminating at the anterior wall. In the case of more than one ductal opening in the graft, if the openings were adjacent to each other, ductoplasty was performed to suture them to form a single orifice. If two ductal openings in the graft were far apart, separate duct-to-duct anastomoses were performed without ductoplasty. A stent tube was routinely placed through the anastomosis as a splint and was pulled out through the common bile duct above the duodenum. A cholangiogram was obtained by using the inserted stent tube 1 month after LDLT, and then the stent tube was clamped. The tubes for bile duct stenting were removed 3 months after LDLT.

Diagnosis and treatment of biliary complications

Biliary leakage was diagnosed clinically and radiologically on the basis of a bile leak through abdominal drains, evacuation of extrahepatic biloma through a newly inserted drain under ultrasound guidance, or identification of a leak by endoscopic retrograde cholangiography (ERC) or cholangiography via an inserted stent tube. For biliary leakage, endoscopic retrograde nasobiliary drainage (ENBD) or percutaneous drainage under ultrasound guidance were the techniques most commonly undertaken.

Biliary stricture is primarily suspected when cholestatic enzymes that are assessed by liver function tests, including alkaline phosphatase and γ-glutamyl transpeptidase, are elevated and/or if there is sonographic evidence of a dilated biliary system. If the total bilirubin was not elevated, drip-infusion cholangiography-CT was performed. The presence of strictures was confirmed by ERC and/or percutaneous transhepatic cholangiography (PTC). Biliary stenosis was diagnosed on the basis of an abrupt luminal narrowing with an overt dilatation of the intrahepatic duct.

Primary transpapillary intervention was attempted in all patients who underwent duct-to-duct biliary reconstruction. Endoscopic retrograde balloon cholangioplasty was performed; this was followed by the placement of a plastic internal stent tube. When endoscopic treatment failed, percutaneous management of the biliary stricture was undertaken. Surgical revision was indicated when both these modalities failed.

Statistical analysis

Category variables were compared with the chi-square test. Continuous data were compared by the Mann-Whitney test. Patient survival after liver transplantation was analyzed by the Kaplan-Meier survival method. The statistical comparison of survival data was performed with the log-rank test. Stepwise logistic regression analysis was carried out in order to identify the independent predictors of biliary complications. A p value < 0.05 was considered to be significant. All statistical analyses were performed with the statistical software package SPSS version 11.0 (SPSS Inc. Chicago, IL).

Results

Type of biliary reconstruction

Table 2 shows the type of bile duct reconstruction with the corresponding incidence rate of biliary stricture. Forty-eight (60%) grafts had a single duct for anastomosis, 29 grafts (36%) had two ducts, and 3 grafts (4%) had three ducts. After ductoplasty in 14 grafts, 62 grafts had a single duct for anastomosis, 15 grafts had two ducts for anastomosis, 2 grafts had three ducts for anastomosis, and 1 graft had two ducts for anastomosis.

Overall incidence of biliary complications, risk factors, and outcomes after LDLT

Biliary leaks developed in 12 patients (15%), and 20 (25%) of the 80 patients suffered from a post-transplantation biliary stricture (Table 2). The mean follow-up was 24 months (range: 3–72 months). The onset of biliary leakage was 20 ± 8 days. No patient developed a de novo biliary stricture beyond 20 months after LDLT. Seven patients (8.8%) developed both the biliary complications. None of the five patients that underwent hepaticojejunostomy developed a biliary stricture. Further, there were no hepatic arterial complications in our series. By univariate analysis, we found two variables to be associated with an increased risk of biliary stricture: a postoperative bile leakage and non-basiliximab-based immunosuppressive therapy (Table 3). After stepwise multivariate analysis, one variable remained significant, i.e., postoperative bile leakage ($p = 0.001$) (Table 3). There were no significant differences in the incidence of biliary stricture with respect to donor age, MELD score, graft type, the number of bile ducts, and ductoplasty. There was no significant difference in the incidence of biliary stricture with respect to the number and mode of anastomotic sutures. However, in the grafts that had three ducts, we observed a high incidence of biliary stricture (2/3, 66.6%) (Table 2). We next examined the incidence of biliary stricture according to the diameter of the anastomosis. Graft duct sizes were classified into small (diameter <4 mm), medium (diameter 4–5 mm), and large (diameter >5 mm). Recipients with two or three biliary ducts were excluded in order to avoid bias from complex biliary reconstructions. We observed no association between the diameter of the bile ducts and the incidence of anastomotic suture.

Interestingly, both biliary leaks and strictures developed less frequently in patients with basiliximab-based immunosuppressive regimes. In stepwise multivariate analysis, non-basiliximab-based immunosuppressive therapy was associated with an increased risk for postoperative bile leakage ($p = 0.005$) (Table 4). Further, we found that the 1-week doses of methylpredonisolone after LDLT were significantly lower in basiliximab-based immunosuppressive regimes than in non-basiliximab-based ones ($p = 0.01$) (Fig. 1).

Freedom from biliary stricture was 73% at 1 year and 69% at 2 years (Fig. 2). The 1-year and 5-year survival rates for patients with biliary stricture were 69% and 53%, respectively, compared with 79% and 70% for those without biliary strictures ($p = 0.31$) (Fig. 3).

Table 2 Biliary complications after duct-to-duct biliary reconstruction in living donor liver transplantation

	n	Leakage (%)	stricture (%)
Number of bile ducts and anastomoses	80	12 (15)	20 (25)
1 duct / 1 anastomoses	48	5 (10.4)	11 (22.9)
2 ducts / 1 anastomoses (plasty)	14	3 (21.4)	5 (35.7)
2 ducts / 2 anastomoses	15	2 (13.3)	2 (13.3)
3 ducts / 3 anastomoses	2	1 (50)	2 (100)
3 ducts / 2 anastomoses (plasty)	1	0	0

Table 3 Univariate and multivariate analysis of risk factors for biliary strictures

Risk factors	No. of patients with biliary stricture	*p* Value (Univariate)	*p* Value (Multivariate)
Immunosuppression		0.044	0.124
Bax (*n*=18)	1 (5%)		
Non-Bax (*n*=62)	19 (30.6%)		
Biliary leakage		0.0001	0.001
Yes (*n*=12)	7 (58%)		
No (*n*=68)	13 (19%)		
No. of bile ducts		0.501	0.165
Single (*n*=48)	11 (22.9%)		
Non-single (*n*=32)	9 (28.1%)		
Ductoplasty		0.216	0.121
Yes (*n*=15)	5 (33.3%)		
No (*n*=65)	15 (23.1%)		
Donor age		0.072	0.152
> 50 year (*n*=28)	10 (35.7%)		
< 50 year (*n*=52)	10 (19.2%)		
Graft		0.647	0.917
Right (*n*=66)	17 (25.7%)		
Left (*n*=14)	3 (21.4%)		
MELD		0.837	0.806
> 25 (*n*=17)	4 (23.5%)		
< 25 (*n*=63)	16 (25.3%)		

Table 4 Univariate and multivariate analysis of risk factors for biliary leakage

Risk factors	No. of patients with biliary leakage	*p* Value (Univariate)	*p* Value (Multivariate)
Immunosuppression		0.033	0.005
Bax (*n*=18)	0 (0%)		
Non-Bax (*n*=62)	12 (19%)		
No. of bile ducts		0.569	0.901
Single (*n*=48)	6 (12.5%)		
Non-single (*n*=32)	6 (19%)		
Ductoplasty		0.639	0.702
Yes (*n*=16)	3 (18.8%)		
No (*n*=64)	9 (14%)		
Donor age		0.066	0.065
> 50 year (*n*=28)	7 (25%)		
< 50 year (*n*=52)	5 (9.6%)		
Graft		0.636	0.512
Right (*n*=66)	9 (13.6%)		
Left (*n*=14)	3 (21%)		
MELD		0.674	0.42
> 25 (*n*=17)	2 (12.5%)		
< 25 (*n*=63)	10 (15.6%)		

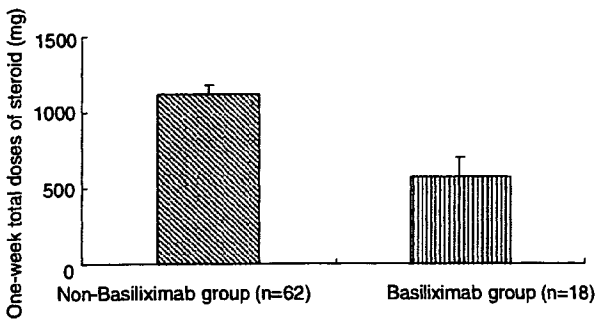

Fig. 1 Total doses of methylpredonisolone that were administered for one week after living donor liver transplant (LDLT). All values are expressed as the mean ± standard error

Management of biliary complications

For biliary leakage, ENBD was possible in five patients. Six patients with bilairy leakage underwent percutaneous drainage under ultrasound guidance. One patient underwent Roux-en-Y hepaticojejunostomy. All patients were successfully treated by these modalities (Fig. 4).

Figure 5 shows the summary of the various modalities used for the treatment of biliary strictures. Initially, the patients with biliary strictures were referred for ERC. In 10 of the 20 patients, a guidewire could pass through the stricture, and these patients were treated by endoscopic internal stent placement. In the remaining 10 patients, the guidewire could not be passed through the biliary stricture because it was too tight and the bile ducts were too kinked. No symptoms of biliary stricture were observed in two patients after ERC. Seven patients required percutaneous transhepatic biliary drainage (PTBD), and five patients underwent stenting. Consequently, six patients underwent Roux-en-Y reconstruction to repair the stricture; however, 16 months after transplantation, one patient died of sepsis secondary to chronic cholangitis.

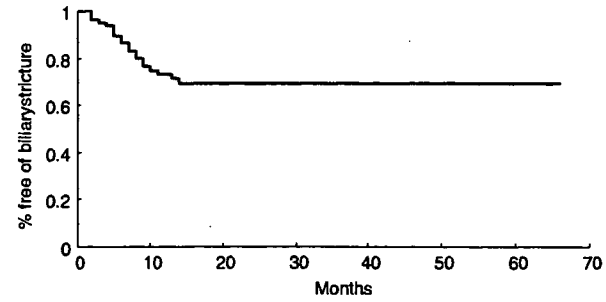

Fig. 2 Biliary stricture-free rate after LDLT

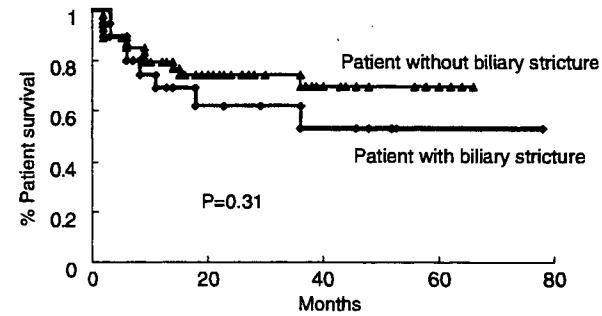

Fig. 3 Impact of biliary strictures on patient survival (Kaplan-Meier) in months

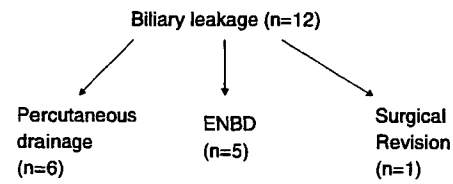

Fig. 4 Summary of the treatment modalities used for biliary leakage: endocsopic retrograde nasobiliary drainage (ENBD), percutaneous drainage, and surgical revision

Discussion

In the present study we observed that post-transplantation anastomotic biliary leakages and strictures occurred, respectively, in 15% and 25% of our patients who

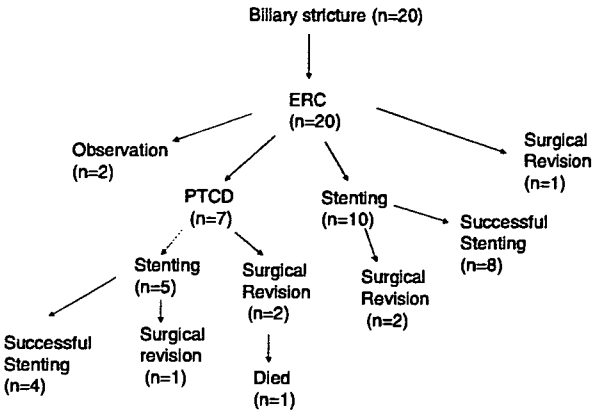

Fig. 5 Summary of the treatment modalities used for biliary stricture: endocsopic retrograde cholangiography (ERC), percutaneous transhepatic cholangiography (PTC), and surgical revision

underwent LDLT with duct-to-duct biliary reconstruction. In previous reports the incidence of biliary stricture in patients who have undergone LDLT with duct-to-duct biliary reconstruction ranges from 20% to 60% [15, 16], whereas the reported incidence of biliary stricture in patients who have undergone cadaveric-donor liver transplantation with duct-to-duct biliary reconstruction is less than 30% [17–19]. Fan et al. reported that while the incidence of biliary leakage was comparable in the two groups, the incidence of biliary stricture was significantly higher in the LDLT group than in cadaveric whole-graft liver transplantation [20].

The present study sought to identify the risk factors for biliary leakage and stricture unique to duct-to-duct biliary reconstruction. We found that patients who developed biliary leakage were vulnerable to biliary stricture. Bile leakage after liver transplantation has previously been suggested as a risk factor for the development of anastomotic strictures [4, 21, 22]. It is likely that the leakage of bile causes local inflammation, thus increasing the risk of fibrosis. Further, the leakage of bile may merely represent ischemia of the extrahepatic bile duct, and in this manner, is associated with anastomotic stricture. The immunosuppressive regimes that included basiliximab were found to be a protective factor against anastomotic leakage. Biliary leakage developed less frequently in patients treated with the basiliximab-based immunosuppressive regimes than in those treated with the non-basiliximab-based ones. The initial 1-week doses of methylpredonisolone were significantly lower in the basiliximab-based immunosuppressive regimes than in those without basiliximab. In general, glucocorticosteroids impair fibroblast proliferation and collagen synthesis, and they delay wound healing [23]. Possibly, the steroid-sparing basiliximab-based immunosuppressive therapy contributed to protection against biliary leakage. Consequently, the development of biliary strictures tended to be less frequent in the basiliximab-based immunosuppressive therapy than in the non-basiliximab-based one. However, there was no significant difference in the incidence of biliary stricture with respect to the immunosuppressive therapies by using stepwise multivariate analysis.

In our series, 40% of the liver grafts had multiple bile duct orifices. The poor outcome might be related to the complicated procedures. The Kyoto Group has reported that they experienced a higher rate of biliary complications with the use of stents for biliary reconstruction; further, no difference was observed with respect to the presence of single or multiple biliary anastomoses [4]. Salvalaggio et al. studied the impact of multiple bile duct anastomoses on the development of biliary complications after liver transplantation in children. They demonstrated that patients with multiple ducts had a higher incidence of leaks as compared to those in the single duct group, but that the incidence of strictures, both early and late, was similar in the two groups [24]. Similarly, in our study, the presence of multiple or single hepatic ducts was not a significant risk factor for biliary stricture. The extensive dissection enabled us to overcome the technical difficulty encountered with multiple and widely separated bile ducts in the liver grafts, as the corresponding orifices in the recipient hilar plate could be freely selected. The use of a bile duct with a wide orifice might result in a good outcome. Hwang et al. reported that duct-to-duct biliary reconstruction involving a small-sized duct (diameter <4 mm) was a risk factor for anastomotic stenosis [25]. However, in our study, the diameter of the graft bile duct was not related to the incidence of biliary strictures.

Ischemic changes around the anastomosis are known to be a major cause of biliary stricture. In donor operations, particularly in cases of right lobe graft, the tissues surrounding the right hepatic duct at the bifurcation must be removed in order to correctly identify the anatomy of the anterior and the posterior segmental branches. The anterior branch of the hepatic duct might be more widely exposed than the posterior branch during the dissection. Dissecting these tissues might cause the biliary stump in the right-lobe graft to become ischemic, which may then facilitate formation of a biliary stricture. The arterial blood supply of the biliary system has been described by several investigators [26–29]. A fine arterial plexus covering the surface of the biliary tract is one of the main sources of the blood supply of the biliary system. Furthermore, the vascular supply for both hepatic ducts depends on an arterial network that is bilaterally fed by the plexus that is formed by the branches of both the right and left hepatic arteries. The blood supply to the bile duct of the graft tends to be tenuous. Shortening of the segment of the donor duct results in an improved circulatory status around the biliary anastomosis, and this technique is therefore suitable for LDLT with a limited length of the graft hepatic duct [9].

Our patients with biliary strictures were treated satisfactory by ERC, PTBD, surgery, or a combination of these. Hisatake et al. reported that 14 (73.4%) of their patients with strictures were treated endoscopically by inserting internal stents ranging from 7 to 12 Fr in size [13]. The complication of biliary stricture occurred only in one patient. Our initial treatment for duct-to-duct biliary reconstruction recipients was endoscopic stenting. Forty percent of our patients with anastomotic strictures failed to respond to endoscopic treatment because it was impossible to pass an endoscopic guidewire through the stricture because of the extreme narrowness of the duct. Those patients who failed to the respond to the endoscopic treatment were treated via a percutaneous transhepatic approach. The total success rate of stenting therapy was 60%. Surgical correction had been effectively performed in patients with recurrent symptoms of cholangitis after stent removal or in those patients for whom stenting was not possible. As demonstrated in several studies, balloon dilatation and stenting via either an endoscopic or percutaneous transhepatic approach were safe and effective. Nonetheless, surgical correction should be reserved for patients who fail to respond to nonsurgical treatment.

Our patients with biliary leakages were treated satisfactory by ENBD, percutaenous drainage, or surgery. We preferred interventional treatment rather than surgery as mentioned by Hwang et al [25]. All patients with biliary leakages were successfully treated by ENBD or percutaneous drainage, except one patient who was treated by surgery.

In summary, our experiences revealed that biliary stricture was associated with biliary leakage and that steroid-sparing basiliximab-based immunosuppressive therapy contributed to the decreased incidence of biliary leakage. The incidence of biliary stricture was not associated with the type or method of biliary reconstruction. The radiological, endoscopic, or surgical treatment for biliary complications was effective and satisfactory. However, the incidence rate of biliary stricture after LDLT is still high. Further improvements in surgical modalities and postoperative management should contribute to the decrease in the incidence of biliary complications.

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RAPID COMMUNICATION

Effects of a 24-week course of interferon-α therapy after curative treatment of hepatitis C virus-associated hepatocellular carcinoma

Soo Cheol Jeong, Hiroshi Aikata, Yoshio Katamura, Takahiro Azakami, Tomokazu Kawaoka, Hiromi Saneto, Kiminori Uka, Nami Mori, Shintaro Takaki, Hideaki Kodama, Koji Waki, Michio Imamura, Hiroo Shirakawa, Yoshiiku Kawakami, Shoichi Takahashi, Kazuaki Chayama

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Correspondence to: Hiroshi Aikata, MD, Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan. aikata@hiroshima-u.ac.jp
Telephone: +81-82-2575192 Fax: +81-82-2575194
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Abstract

AIM: To assess whether a 24-wk course of interferon (IFN) could prevent hepatocellular carcinoma (HCC) recurrence and worsening of liver function in patients with hepatitis C virus (HCV)-infected patients after receiving curative treatment for primary HCC.

METHODS: Outcomes in 42 patients with HCV infection treated with IFN-α, after curative treatment for primary HCC (IFN group), were compared with 42 matched curatively treated historical controls not given IFN (non-IFN group).

RESULTS: Although the rate of initial recurrence did not differ significantly between IFN group and non-IFN group (0%, 44%, 61%, and 67% *vs* 4.8%, 53%, 81%, and 87% at 1, 3, 5, and 7 years, $P = 0.153$, respectively), IFN group showed a lower rate than the non-IFN group for second recurrence (0%, 10.4%, 28%, and 35% *vs* 0%, 30%, 59%, and 66% at 1, 3, 5 and 7 years, $P = 0.022$, respectively). Among the IFN group, patients with sustained virologic response (SVR) were less likely to have a second HCC recurrence than IFN patients without an SVR, or non-IFN patients. Multivariate analysis identified the lack of SVR as the only independent risk factor for a second recurrence, while SVR and Child-Pugh class A independently favored overall survival.

CONCLUSION: Most intrahepatic recurrences of HCV-related HCC occurred during persistent viral infection. Eradication of HCV is essential for the prevention of HCC recurrence and improvement of survival.

Key words: Hepatitis C virus, Hepatocellular carcinoma, Recurrence, Survival, Sustained virological response

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide. Chronic infection with hepatitis C virus (HCV) has been causally associated with HCC[1-3]. Recent advances in imaging and treatment have brought about some improvement in prognosis of patients with HCV-related HCC, but outcomes are still unsatisfactory. The 5-year survival rate is only 50%-70%, even after curative treatment such as hepatic resection or local ablation[4]. Reasons for this unfavorable prognosis are considered to include high intrahepatic tumor recurrence rates and sustained hepatic damage, both resulting from HCV infection[5].

Even after curative hepatic resection for HCV-related HCC, the rate of intrahepatic tumor recurrence within 1 year is 20%-40%, rising to about 80% by 5 years[4,6-8]. Intrahepatic recurrence of HCC may result from intrahepatic metastasis originating from the primary HCC, or from ongoing multicentric carcinogenesis related to chronic HCV infection. Underlying HCV-related hepatic damage may also compromise hepatic functional reserve, and worsen clinical outcome. Thus prevention of HCC recurrence and preservation of liver function are both high priorities for improving prognosis of patients with HCV-

related HCC.

Interferon (IFN) therapy for patients with HCV infection is effective in reducing serum alanine transaminase (ALT) activity and in eradicating HCV[9,10], and thus IFN could have value in minimizing hepatic necrosis, inflammation and fibrosis, as well as reducing the incidence of HCC. Several recent studies have reported that IFN therapy, even after curative treatment for HCV-related HCC, could prevent HCC recurrence and improve survival[11-17]. Unfortunately, since these studies are characterized by differing IFN regimens, definitions of IFN responses, and background characteristics of patients, results have varied and no standard IFN regimen has been established for after curative treatment of HCV-related HCC. As well, the mechanisms by which IFN suppresses HCC recurrence, including possible direct anti-tumor and anti-inflammatory effects, remain uncertain.

In the present study, recurrence and survival outcomes in matched historical controls were compared with those in patients receiving a 24-wk course of IFN-α therapy after receiving curative treatment for HCC.

MATERIALS AND METHODS

Patients

We retrospectively reviewed 495 consecutive patients treated for primary HCC associated with HCV infection at Hiroshima University Hospital from March 1992 to March 2004. Of these, 384 with HCC initially underwent therapeutic intervention with curative intent. Curative treatment was defined as complete tumor eradication, with no residual tumor visible by computed tomography, or resection of all evident tumor tissue. Medical treatment included percutaneous radiofrequency ablation (RFA), ethanol injection, and microwave coagulation therapy (MCT). Surgical treatment included hepatic resection and ablation during laparotomy.

Among these 384 patients, we administered IFN therapy to 42 who met the following eligibility criteria: age under 70 years; up to three tumors with none exceeding 30 mm in diameter, or a solitary tumor less than 50 mm in diameter; tumor-node-metastasis (TNM) stage I, II, or III; detectable serum HCV RNA; seronegativity for hepatitis B surface antigen; chronic hepatitis or compensated cirrhosis with a Child-Pugh class of A or B; platelet count above 70000/μL; absence of local recurrence during the follow-up period; and absence of ectopic intrahepatic recurrence within 24 wk after treatment for primary HCC. We used the TNM classification system of the Liver Cancer Study Group of Japan as the staging system for HCC[18]. Underlying liver conditions such as hepatitis or cirrhosis were confirmed by laboratory, pathologic and radiologic examinations. We classified liver function in chronic hepatitis as Child class A because chronic hepatitis is a known pre-cirrhotic condition. There were only a few chronic hepatitis cases: three in the IFN group and four in the non-IFN group.

As historical control subjects, we selected 42 patients with no IFN therapy after treatment for primary HCC (non-IFN group). These 42 patients, who met the eligibility criteria noted above, were matched by age, gender, tumor size, TNM stage of HCC, serum albumin, platelet counts, and Child-Pugh class with patients who received IFN therapy (IFN group).

Table 1 Patient characteristics

	IFN group (n = 42)	Non-IFN group (n = 42)	P
Median age in years (range)	62[1] (45-69)	63[1] (40-69)	NS
Gender (male/female)	36/6	29/13	NS
Alb (g/dL)	3.9[1]	3.9[1]	NS
PLT (× 10000/μL)	12[1]	11.5[1]	NS
ICG R-15 (%)	17[1]	18[1]	NS
CH or Child A/B	35/7	35/7	NS
Size of main tumor (mm)	20[1] (10-50)	15[1] (10-50)	NS
AFP (ng/mL)	26[1]	31.4[1]	NS
No. of HCC (single/two or three)	30/12	36/6	NS
Stage (I / II or III)	14/28	23/19	NS
Treatment of HCC (medical/surgical)	18/24	20/22	NS

IFN: interferon; Alb: albumin; PLT: platelet; ICG-R15: indocyanine green retention at 15 min; CH: chronic hepatitis; AFP: alpha-fetoprotein; HCC: hepatocellular carcinoma. [1]Median.

Thus, a total of 84 patients (42 in the IFN group and 42 in the non-IFN group) were enrolled. All agreed to participate in the research protocol, which was approved by the hospital research ethics board. Table 1 shows the baseline characteristics of the two groups, indicating no significant differences for age, gender, liver function, tumor characteristics, or therapeutic methods used against HCC.

IFN therapy

In the IFN group, patients received 6 MIU of natural IFN-α (human lymphoblastoid IFN, Sumiferon; Dainippon Sumitomo Pharmaceuticals, Osaka, Japan) intramuscularly every day for 2 wk, followed by three times weekly for 22 wk. IFN therapy began within 24 wk after the initial treatment for HCC. All patients were evaluated every week in an outpatient setting during IFN treatment. Qualitative detection of HCV-RNA was performed by a standardized qualitative reverse transcription-polymerase chain reaction (RT-PCR) assay at every 4 wk during and after IFN treatment.

Among the patients who received IFN therapy, 28 were of HCV genotype 1 and 14 were of HCV genotype 2. These 42 patients had various pretreatment viral loads. Twenty patients (genotype 1, n = 11; genotype 2, n = 9) had high viral loads (≥ 100 kIU/mL by PCR), and 22 (genotype 1, n = 17; genotype 2, n = 5) had low viral loads (≤ 100 kIU/mL by PCR). The 42 patients were divided into two subgroups according to virologic response, i.e. patients with *vs* without a sustained virologic response (SVR). SVR was defined as the sustained absence of serum HCV RNA for more than 24 wk after completion of IFN treatment. Absence of SVR included both persistent viremia (no response) and transient viral disappearance (transient response) during or after IFN therapy. Biochemical response was defined as ALT activity declining to a value within the normal reference range in the presence of viremia.

Follow-up

After curative treatment for primary HCC, all patients studied underwent liver function tests, serum tumor marker assays, such as those for α-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist II (PIVKA-II) every month, abdominal ultrasonography every 3 mo, and dynamic computed tomography (CT) every 6 mo. If recurrence of HCC was suspected, additional examinations including CT during arteriography or tumor biopsy were performed. Recurrence of HCC was defined as any new nodules indicated by CT as hyperattenuation during hepatic arteriography or by hypoattenuation in CT performed during arterioportography. Hypovascular HCC was confirmed histopathologically after fine-needle aspiration biopsy. Patients with recurrent HCC were treated medically or surgically, with curative intent if possible.

In IFN patients, including those with or without SVR, and in the non-IFN group, we compared both the rate of HCC recurrence and the survival rate. We also sought to identify significant prognostic indicators for survival and recurrence after curative treatment of primary HCC.

Statistical analysis

Chi-squared and Fisher exact tests were used for categorical variables, while Student's *t* test and the Mann-Whitney *U* test were used for continuous and ordinal variables, as appropriate. The Kaplan-Meier method was used to assess cumulative survival and recurrence rates, calculated from the date of diagnosis to the date of disease recurrence or death. Surviving patients and those who died of causes unrelated to the liver were defined as censored cases, while patients who died of causes related to the liver were defined as non-censored cases. The log-rank test was used to compare survival and recurrence curves. Univariate and multivariate predictors of survival or recurrence time were determined using the Cox proportional hazard model. Hazard ratios and their 95% confidence intervals (95% CI) were computed. $P < 0.05$ was considered to indicate statistical significance. The JMP version 5.1 statistical software package (SAS Institute, Cary, NC, USA) was used for analysis of data.

RESULTS

Virologic and biochemical responses to IFN therapy and side effects

The 42 patients receiving IFN therapy included 29 in the SVR group and 13 in the group without SVR (10 transient virological responders, 3 with no virological response). In the group without SVR, 7 biochemical responders who had a normalized ALT included 5 with transient virological responses and 2 with no virological response. Although there was no significant difference in the population of patients with HCV genotype 1 between the SVR and non-SVR group, patients in the former had significantly lower pre-IFN viral loads than patients in the latter group. In the SVR group, 24 patients received full-dose IFN therapy without dose reduction, while five patients received a reduced dose of IFN until completion of treatment. In the group without SVR, one patient with no response discontinued IFN treatment at 16 wk because of a recurrence of HCC, while three patients with a transient response discontinued treatment because of generalized fatigue. The remainder of the group without SVR received the full course of IFN therapy. Thus, most patients were able to complete the 24-wk course.

Recurrence of HCC

In the IFN group, first recurrences of HCC developed in 20 patients after the initial treatment for HCC during a median follow-up period of 32 mo. Of these recurrences, 10 were in patients with SVR (10/29) and 10 in patients without SVR (10/13), including 7 transient virological responders and 3 with no virological response. For the 7 biochemical responders without SVR, HCC recurred in 6 patients, including 5 transient virological responders and 1 with no virological response. Of these 20 patients with recurrence, 18 were treated with local ablation therapy or surgical resection without leaving any residual tumor. The remaining 2 patients developed uncontrolled multiple HCC and were excluded from the subsequent study concerning the next recurrence. One died of HCC, while the other was treated repeatedly with hepatic arterial infusion, and has survived. Three patients in the SVR group and 7 in the group without SVR (5 transient virological responders and 2 with no virological response) had a second recurrence of HCC. Of these 10 patients with a second recurrence, 3 (2 transient virological responders and one with no virological response) developed uncontrolled HCC, while others were treated curatively with hepatic resection or local ablation therapy. In the non-IFN group, a first recurrence of HCC occurred in 30 patients during a median follow-up period of 31 mo. HCC recurred in 11 of the 17 who had a normal ALT level. Among the 30 patients with recurrent HCC, 25 were treated with local ablation therapy or surgical treatment, with no residual tumor. The remaining 5 patients who did not undergo curative therapy were treated repeatedly with transarterial chemoembolization. A second recurrence developed in 15 of the 25 patients who had curative treatment for a first recurrence. Among these 15 patients, 10 were treated curatively (9 with local ablation and 1 with hepatic resection). The remaining 5 patients had uncontrolled multiple HCC as their second recurrence.

Overall cumulative rates for first and second recurrence of HCC were compared between the groups. The 1-, 3-, 5- and 7-year rates for first recurrence in the IFN and non-IFN group were 0% *vs* 4.8%, 44% *vs* 53%, 61% *vs* 81%, and 67% *vs* 87%, respectively (Figure 1A, $P = 0.153$; no significant difference between groups). However, the 1-, 3-, 5-, and 7-year rates for second recurrence in the IFN and non-IFN group were 0% *vs* 0%, 10.4% *vs* 30%, 28% *vs* 59%, and 35% *vs* 66%, respectively (Figure 1B, $P = 0.022$). Thus, the second-recurrence rate was significantly lower in the IFN group than in the non-IFN group.

Next, the recurrence rates of HCC were compared between the SVR group, the non-SVR group and the non-IFN group. The rate of first recurrence was significantly lower in the SVR group than in the non-SVR and non-IFN group (Figure 2A). The rate of second recurrence in the

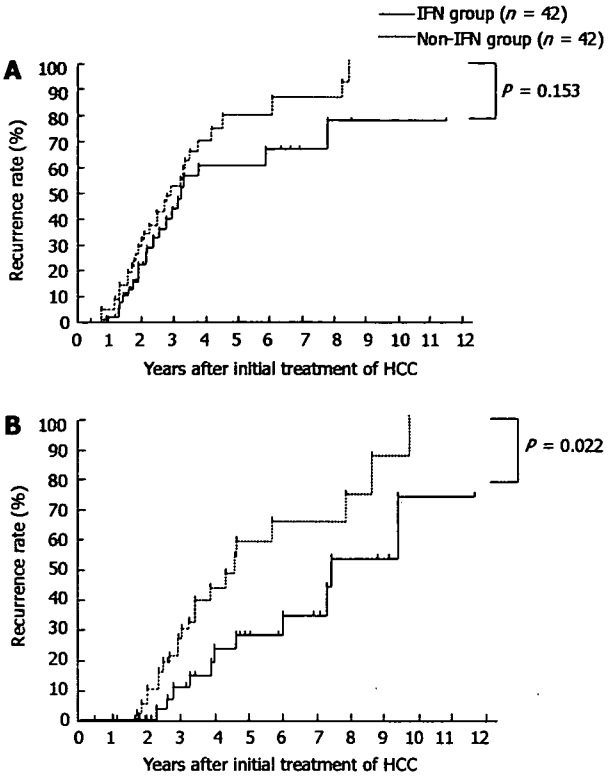

Figure 1 Cumulative recurrence rates after curative treatment of HCC. **A**: Rates of first recurrence compared between IFN and non-IFN groups, showed no significant difference ($P = 0.153$); **B**: Rates of second recurrence compared between IFN and non-IFN groups. The second recurrence rate for the IFN group was lower than that for the non-IFN group ($P = 0.022$).

SVR group was also lower than that in the non-SVR and non-IFN groups; this decrease was significantly greater than that for the rate of first recurrence (Figure 2B). No significant difference was seen in cumulative rates for first or second recurrence between the non-SVR and non-IFN groups. We also confirmed that biochemical responders in the non-SVR and non-IFN groups showed similar Kaplan-Meier curves for cumulative recurrence (data not shown). Recurrence curves were similar between the non-SVR group, including biochemical responders, and the non-IFN group, therefore, we defined these two groups as "non-SVR status" for statistical analysis. Factors found to be significantly associated with first recurrence by univariate analysis were tumor size (≥ 20 mm) and non-SVR status ($P = 0.019$, $P = 0.0067$, respectively). Multivariate analysis showed that no independent risk factor was associated with the first recurrence of HCC (data not shown), although non-SVR status tended to be associated with first recurrence ($P = 0.0657$). As shown in Table 2, univariate analysis indicated that non-SVR status, low platelet count (< 100 000) and high indocyanine green retention (≥ 20%) were significantly associated with second recurrence. Multivariate analysis identified only SVR status as a significant independent inhibiting factor for second recurrence of HCC.

Survival of patients

During the observation period, 13 of the total patients studied died of liver disease. Nine died of HCC and 4 of liver failure. When we compared cumulative survival rates between the IFN and the non-IFN groups (Figure 3A), the respective rates were 100% *vs* 95% at 3 years, 100% *vs* 72% at 5 years, and 86% *vs* 63% at 7 years. The cumulative survival rate was significantly higher in the IFN group than in the non-IFN group ($P = 0.039$). Median survival time following the first treatment of HCC was 52.3 mo (range, 12-158) in the IFN group and 51.8 mo (range, 11-126) in the non-IFN group. In the IFN group, 2 patients died of advanced HCC, 1 with an SVR and the other without. No patients in the IFN group died of hepatic failure. In the non-IFN group, 7 patients died of HCC and four of hepatic failure.

Figure 3B shows cumulative survival curves for the SVR, non-SVR and non-IFN groups. The rate of survival in the SVR group was significantly better than that in the non-IFN group ($P = 0.029$), while no significant difference was evident between the non-SVR and non-IFN group ($P = 0.248$).

Pretreatment factors found to be significantly associated with survival by univariate analysis subsequently were evaluated by Cox regression analysis to determine independent factors. Multivariate analysis showed that SVR status and Child-Pugh class A were independent factors favorably associated with long survival (Table 3).

Liver function

Compared with the non-IFN group, patients who received IFN therapy were less likely to have worsening of hepatic dysfunction. For the SVR, non-SVR and non-IFN groups, we compared the average score for Child-Pugh classification at initial treatment of HCC with that at the time of data analysis. Median observation time was 59.8 mo in the SVR group, 45 mo in the non-SVR group, and 51.8 mo in the non-IFN group. There were no significant differences in the Child-Pugh classification score among these three groups at the time of initial treatment of HCC; however, at the time of data analysis, scores in the non-IFN group were significantly worse than in the SVR group ($P = 0.003$). No significant difference was seen between the non-SVR and non-IFN groups (Figure 4).

DISCUSSION

The present study compared historical control subjects with no IFN treatment with other subjects who were treated with IFN. Background characteristics showed no significant difference between the groups. IFN and non-IFN group did not differ significantly in their rate of first recurrence, but did differ significantly in their rate of second recurrence. According to IFN response, the recurrence rate in the SVR group was significantly lower than that in the non-SVR and non-IFN group, while recurrence rates in the non-SVR and non-IFN group did not differ significantly. Thus, SVR (i.e. HCV eradication) was the most important, and only, inhibiting factor for decreasing risk of HCC recurrence, associated with a 24-wk course of IFN-α therapy following HCC treatment.

Although several recent studies have reported the

Table 2 Factors associated with second recurrence

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
SVR	0.454	0.246-0.728	0.0005	0.457	0.243-0.757	0.0015
PLT > 100000/μ	0.553	0.373-0.814	0.003	0.694	0.445-1.069	0.0973
ICG R-15 (< 20%)	0.667	0.450-0.965	0.032	0.685	0.447-1.035	0.0721

Cox's proportional hazards model was used.

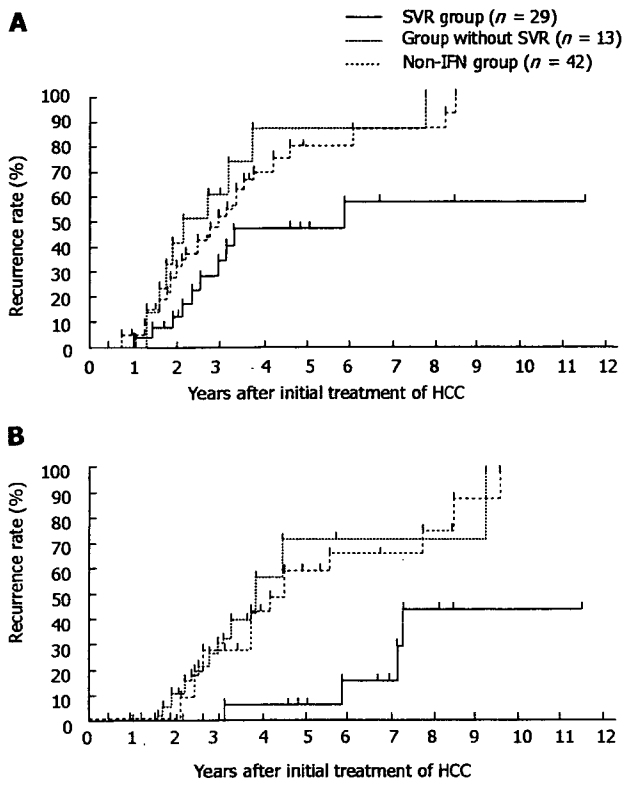

Figure 2 Cumulative recurrence rates according to SVR to IFN therapy after curative treatment of HCC. **A**: Rates of first recurrence compared among SVR, non-SVR and non-IFN groups. The rate of first recurrence of HCC in the SVR group was significantly lower than in the non-SVR and non-IFN groups ($P = 0.002$, $P = 0.016$, respectively). No significant difference in first recurrence rate was seen between the non-SVR and non-IFN groups ($P = 0.381$); **B**: Rates of second recurrence compared among the three groups. Second recurrence of HCC was suppressed in the SVR group compared with the non-SVR and non-IFN groups ($P = 0.0037$, $P = 0.0019$, respectively), and to a more pronounced degree than for the first recurrence rate. No significant difference in second recurrence rate was seen between the non-SVR and non-IFN groups ($P = 0.90$).

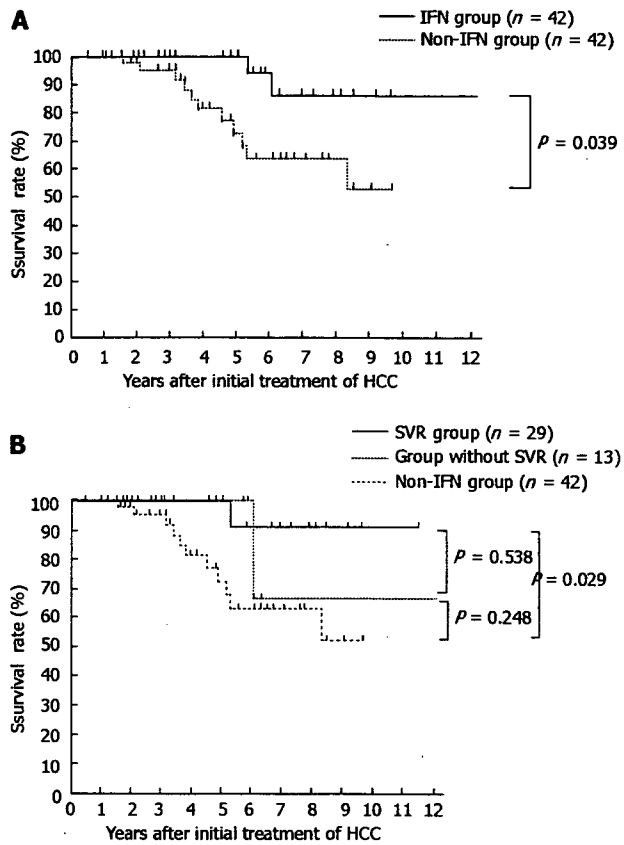

Figure 3 Cumulative survival rates after curative treatment of HCC. **A**: Comparison of cumulative survival rates in the IFN and non-IFN groups. The cumulative survival rate was significantly higher in the IFN group than in the non-IFN group ($P = 0.039$); **B**: Comparison of cumulative survival rates in the SVR, non-SVR and non-IFN groups. Although no significant overall difference was found between the SVR and non-SVR groups ($P = 0.538$), the SVR group had a particularly high survival rate compared with the non-IFN group ($P = 0.029$).

efficacy of chemoprevention with IFN after treatment of HCV-related HCC, the basis of this benefit has not been determined, since IFN has a variety of biologic effects, including antiviral, antiproliferative, immunomodulatory[19-22] and anti-fibrogenic[23,24] activities; growth inhibition through changes in signal transduction[19,25,26]; and activation of natural killer cells[27] and T cells[28,29]. Through these various effects, IFN therapy is thought to suppress tumor recurrence directly and/or indirectly.

Sakaguchi *et al*[15] have reported that low-dose, long-term, intermittent IFN-α therapy can, by a direct anti-cancer effect, inhibit intrahepatic metastasis but not multicentric occurrences. Lai *et al*[29] have reported that IFN-α therapy is effective in advanced HCC. Several experimental studies have shown that IFN inhibits the growth of a human hepatoma cell line[11,15]. In partial disagreement, however, Nishiguchi *et al*[12,14], Suou *et al*[16] and Shiratori *et al*[17] have reported that the rate of HCC recurrence was not different between IFN and non-IFN group during the first few years, but later became significantly lower in the IFN group. They suggested that IFN reduced HCC recurrence in the later period of observation by suppressing multicentric occurrence, as an indirect anti-tumor effect that was related to sustained hepatic inflammation. Although the present study did not have a randomized controlled design, and details of the

Table 3 Factors associated with survival

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
SVR	0.409	0.096-0.922	0.028	0.329	0.076-0.761	0.006
Child-Pugh class A	0.521	0.299-0.922	0.027	0.463	0.238-0.875	0.019
ICG R-15 (< 20%)	0.551	0.286-0.968	0.038	0.724	0.351-1.429	0.350

Cox's proportional hazards model was used.

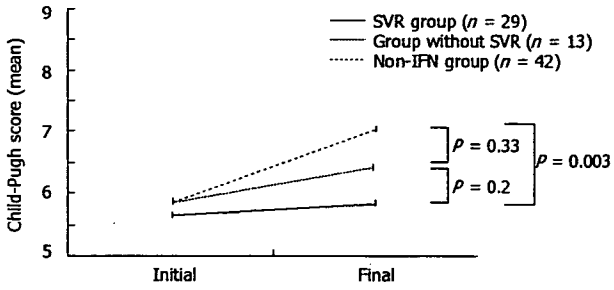

Figure 4 Influence of IFN therapy after curative treatment of HCC on Child-Pugh scores. IFN-treated patients were less likely to show deterioration of hepatic function. In particular, liver function scores in the SVR group were significantly better preserved than in the non-IFN group ($P = 0.003$). Median observation time was 59.8 mo in the SVR group, 45 mo in the non-SVR group, and 51.8 mo in the non-IFN group.

IFN protocol differed from those of others, the long-term results appear to be similar among studies. Recurrence during the first few years might involve undetectable intrahepatic metastasis, or a potential malignant tumor already existing at the time of treatment of the primary HCC; afterward, HCC might recur as multicentric new liver tumor, accompanied by sustained hepatic necrosis and inflammation. Although a direct anti-cancer effect of IFN might to some extent have directly inhibited HCC recurrence, our IFN doses were insufficient to suppress intrahepatic metastatic tumors because there was only a 24-wk treatment. Therefore, in our study, we believe that IFN therapy suppressed HCC recurrence less by a direct anti-tumor effect than by an indirect effect through inhibition of the chronic inflammation associated with HCV infection in the later period of observation.

Several studies have reported that recurrence was suppressed not only in virologic responders to IFN, but also in biochemical responders, even though HCV was not eradicated[12-14]. However, the recurrence rates in our study did not differ significantly between biochemical responders and the non-IFN group. HCV eradication appeared to stand alone as an IFN effect capable of inhibiting recurrence, with eradication having a stronger influence against second recurrence than the first. The differences between the results of the various studies might be due to several reasons. In most previous studies, IFN therapy was given for more than 48 wk, compared with our 24 wk. Differences may also have been present in underlying hepatic inflammatory conditions such as chronic hepatitis and cirrhosis. Although such differences introduce some uncertainty to the conclusions, several recent studies suggest that HCV core protein might directly participate in hepatocarcinogenesis[28,29], which supports the importance of virus eradication.

Although some other recent studies have reported that IFN therapy following HCC treatment also improves liver function and survival of patients with HCV-related HCC, which of the specific IFN actions is important for these benefits remains unknown. We found that overall survival rate and preservation of liver function were significantly better in the SVR group than in the other groups, even including biochemical responders, with all subgroups without SVR resembling non-IFN patients. Favorable independent factors associated with survival by multivariate analysis were SVR and Child–Pugh class A. Thus, with a 24-wk course of IFN-α therapy, HCV eradication appears necessary for prolonging survival, suppressing HCC recurrence, and preserving liver function.

As stated above, effective management of HCV infection is needed, as well as direct treatment of the primary HCC. Although our study had limitations, such as the use of historical controls and a small number of patients, we could demonstrate a clear requirement for HCV eradication to improve survival after a short-course IFN-α therapy. Ribavirin combination or pegylated IFN therapy are considered more effective in HCV eradication than conventional IFN monotherapy[32-34]. Several studies have indicated that pegylated IFN therapy is superior to conventional IFN when administered for 48 wk[34-41]. Pegylated IFN therapy, with or without ribavirin, may improve prognosis in selected patients with no sustained initial response to conventional IFN. For patients who cannot undergo standard-dose IFN therapy because of limited hepatic reserve or thrombocytopenia, low-dose IFN therapy for a longer course might be effective. Nonetheless, further studies with larger controlled groups and long-term follow-up need to be performed to establish what constitutes optimal management of HCV infection after HCC treatment.

COMMENTS

Background

Risk of multicentric recurrence of hepatocellular carcinoma (HCC) and liver function deterioration remains high in hepatitis C virus (HCV)-infected patients even after receiving curative treatment for primary HCC. Most intrahepatic recurrences occurred during persistent viral infection. Although several recent studies have reported the efficacy of chemoprevention with interferon (IFN) therapy after treatment of HCV-related HCC, there was no standard IFN regimen. We investigated whether 24-week course of IFN-α therapy following curative treatment for primary HCC associated with HCV infection could suppress HCC recurrence and improve prognosis.

Research frontiers

To obtain sustained virological response (SVR) was important for suppression of HCC recurrence and for long-term survival in a 24-week course of IFN-α therapy.

Innovations and breakthroughs

Our study demonstrated that only SVR status by a 24-wk IFN-α therapy was the most important factor for decreasing risk of HCC recurrence in the later period of observation including second recurrence.

Applications

This study demonstrated that compared with non-IFN and non-SVR group, SVR group decreased the rate of recurrence, preserved liver function, and prolonged survival time in a 24-wk course of IFN-α therapy.

Peer review

This is a matched historical case controlled study concerning about the effect of 24-week short course IFN-α therapy after receiving curative treatment for primary HCC. The paper is well written and the results show that the most important factor associated with the improvement of prognosis is the SVR status.

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Low-dose intermittent interferon-alpha therapy for HCV-related liver cirrhosis after curative treatment of hepatocellular carcinoma

Soocheol Jeong, Hiroshi Aikata, Yoshio Katamura, Takahiro Azakami, Tomokazu Kawaoka, Hiromi Saneto, Kiminori Uka, Nami Mori, Shintaro Takaki, Hideaki Kodama, Koji Waki, Michio Imamura, Hiroo Shirakawa, Yoshiiku Kawakami, Shoichi Takahashi, Kazuaki Chayama

Soocheol Jeong, Hiroshi Aikata, Yoshio Katamura, Takahiro Azakami, Tomokazu Kawaoka, Hiromi Saneto, Kiminori Uka, Nami Mori, Shintaro Takaki, Hideaki Kodama, Koji Waki, Michio Imamura, Hiroo Shirakawa, Yoshiiku Kawakami, Shoichi Takahashi, Kazuaki Chayama, Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, 734-8551, Japan
Correspondence to: Hiroshi Aikata, MD, Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan. aikata@hiroshima-u.ac.jp
Telephone: +81-82-2575192 Fax: +81-82-2575194
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Abstract

AIM: To assess the efficacy of low-dose intermittent interferon (IFN) therapy in patients with hepatitis C virus (HCV)-related compensated cirrhosis who had received curative treatment for primary hepatocellular carcinoma (HCC).

METHODS: We performed a prospective case controlled study. Sixteen patients received 3 MIU of natural IFN-alpha intramuscularly 3 times weekly for at least 48 wk (IFN group). They were compared with 16 matched historical controls (non-IFN group).

RESULTS: The cumulative rate of first recurrence of HCC was not significantly different between the IFN group and the non-IFN group (0% *vs* 6.7% and 68.6% *vs* 80% at 1- and 3-year, $P = 0.157$, respectively). The cumulative rate of second recurrence was not also significantly different between the IFN group and the non-IFN group (0% *vs* 6.7% and 35.9% *vs* 67% at 1- and 3-year, $P = 0.056$, respectively). Although the difference in the Child-Pugh classification score between the groups at initial treatment of HCC was not significant, the score was significantly worse at the time of data analysis in the non-IFN group than IFN group (7.19 ± 1.42 *vs* 5.81 ± 0.75, $P = 0.0008$). The cumulative rate of deviation from objects of any treatment for recurrent HCC was also higher in the non-IFN group than IFN group (6.7% and 27% *vs* 0 and 0% at 1- and 3-year, $P = 0.048$, respectively).

CONCLUSION: Low-dose intermittent IFN-alpha therapy for patients with HCV-related compensated cirrhosis after curative HCC treatment was effective by making patients tolerant to medical or surgical treatment for recurrent HCC in the later period of observation.

Key words: Hepatitis C virus; Hepatocellular carcinoma; Interferon therapy; Liver cirrhosis; Liver function; Recurrence; Survival

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide. Approximately 80% of Japanese patients with HCC have a history of hepatitis C virus (HCV) infection, and most such patients have liver cirrhosis[1-3]. Although recent advances in imaging techniques and treatment of HCC have improved prognosis of patients with HCV-related HCC, the outcome is still unsatisfactory; the 5-year survival rate is only 50% to 70% even after curative treatment such as hepatic resection and local ablation[4]. The reasons for this unfavorable prognosis is considered to include high intrahepatic tumor recurrence rates and biochemical deterioration by sustained hepatic damage, both resulting from persistent HCV infection[5]. Even after curative hepatic resection for HCV-related HCC, the rate of intrahepatic tumor recurrence within 1 year is 20% to 40%, rising to about