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The long-term outcome of patients with bleeding gastric varices after balloon-occluded retrograde transvenous obliteration

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Background. The purpose of our study was to evaluate the long-term outcome and complications of balloon-occluded retrograde transvenous obliteration (B-RTO) in patients with hemorrhage from gastric fundal varices. ***Methods.*** Thirty-four consecutive patients with bleeding from gastric varices who were treated with B-RTO were enrolled in this study between December 1994 and September 2005 (urgent cases, n = 12; elective cases, n = 22). The long-term outcome, complications, and various liver functions were evaluated. ***Results.*** Complete obliteration was achieved in 31 of 34 (91%) patients with an acute bleeding episode. In one of the remaining patients, there was a technical failure, and the other two had only partial obliteration. The two patients with partial obliteration did not obtain hemostasis. Thus, the rate of hemostasis was 94% (31/33). Gastric varices disappeared in all patients with complete obliteration during the treatment. The rate of gastric variceal eradication was 91%. Variceal rebleeding from esophageal varices occurred in three patients. The rate of rebleeding was 10% (3/31). Rebleeding from gastric varices was not observed after complete obliteration. None of the patients showed worsening of their Child-Pugh score. Although the 5-year cumulative worsening rate of esophageal varices was 52%, neither portal hypertensive gastropathy nor ectopic varices were observed. The patients with worsening esophageal varices were successfully treated with an endoscopic procedure. The 5-year survival rate was 68%. ***Conclusions.*** B-RTO is useful for treatment of bleeding gastric varices, achieving high eradication of gastric varices, a low rebleeding rate, and a fairly good prognosis with improved hepatic function.

Key words: balloon-occluded retrograde transvenous obliteration (B-RTO), gastric fundal varices, bleeding, ethanolamine oleate

Introduction

Gastric fundal varices with hemorrhage are associated with a higher mortality rate than esophageal variceal bleeding,[1–3] and optimal management of gastric varices therefore requires a multidisciplinary approach. Generally, various treatment modalities such as pharmacotherapy, balloon tamponade, endoscopic procedures, interventional radiologic treatment, and surgery have been widely performed. In uncontrolled hemorrhage or rebleeding from gastric varices, a transjugular intrahepatic portosystemic shunt (TIPS) is an important tool.[4–13] However, even patients with gastric varices with portal pressure gradients of <12 mmHg can hemorrhage, and TIPS is not always effective in such patients with low initial portal pressure gradients.[6,8] Balloon-occluded retrograde transvenous obliteration (B-RTO) is an interventional radiologic technique that was developed in Japan.[14–16] This procedure involves occlusion of blood flow by inflation of a balloon catheter into an outflow shunt, such as a gastrorenal shunt, and injection of 5% ethanolamine oleate into gastric varices in a retrograde manner. B-RTO has been safely performed for gastric varices with almost complete eradication.

In patients with gastric varices, low portal pressure gradients are associated with the presence and size of a spontaneous gastrorenal shunt, which is present in up to 85% of such patients.[3,17,18] If a large spontaneous shunt is present and the portal pressure gradient (as measured by hepatic vein wedge pressure gradient) is <12 mmHg, B-RTO should be considered.[19] However, the majority of reports in the literature pertain to prophylactic treatment, and the long-term outcome after

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B-RTO has not yet been fully demonstrated.[14,16,20–24] In the present study, we describe the long-term outcome of patients with bleeding from gastric varices after B-RTO. In addition, we review the literature on the usefulness of B-RTO in the treatment of gastric varices with hemorrhage.[15,25–36]

Patients and methods

Patients

Between December 1994 and September 2005, 133 patients diagnosed with cirrhosis of the liver underwent an urgent endoscopy for a history of hematemesis or melena. In 87 patients, the primary indication was esophageal variceal hemorrhage, in 38 patients, gastric variceal hemorrhage, and in eight patients, acute non-variceal upper gastrointestinal bleeding, such as erosion or ulcer. Among 38 patients with gastric variceal bleeding, 18 had bleeding signs, such as spurting bleeding and adhesion clots. We treated four patients endoscopically for gastroesophageal varices. Among the remaining 14 patients with isolated gastric variceal bleeding, we treated eight by endoscopic hemostasis, such as endoscopic variceal ligation or clipping, or endoscopic procedures such as injection sclerotherapy using 5% ethanolamine oleate or tissue adhesives, and six by balloon tamponade. The other 20 patients had already stopped bleeding at endoscopy. Thus, 14 bleeding patients were treated with B-RTO after the above-mentioned hemostasis, and 20 patients with isolated gastric varices were treated with B-RTO. In this study, these 34 consecutive patients with bleeding from gastric fundal varices were enrolled at Hiroshima University Hospital between December 1994 and September 2005 (Fig. 1). Patient characteristics are shown in Table 1.

An urgent case of bleeding gastric varices is defined as bleeding within 24 h of the initial hemostasis, and an elective case of bleeding gastric varices is defined as bleeding after 24 h of initial hemostasis.[37] Twelve patients had urgent cases, and 22 had elective cases. The patients comprised 26 men and eight women with a mean age of 60 years. Among the 33 patients with liver cirrhosis, the causes were viral liver cirrhosis (n = 17: hepatitis B surface antigen-positive, n = 2; anti-hepatitis C virus antibody-positive, n = 15), alcoholic liver cirrhosis (n = 10), primary biliary cirrhosis (n = 1), autoimmune hepatitis (n = 1), and unknown (n = 4: negative for viral markers). Child-Pugh classifications were grade A (n = 12), grade B (n = 18), and grade C (n = 3). The one remaining patient was diagnosed with an extrahepatic presinusoidal obstruction (EHO). Endoscopic findings for gastric varices were evaluated according to the general rules for recording endoscopic findings of esophagogastric varices.[37,38] Briefly, gastric varices were classified according to their relationship to the cardiac orifice: Lg-c if they were adjacent to the cardiac orifice, Lg-f if they were distant from the cardiac orifice, and Lg-cf if they extended from the cardiac orifice to the fornix. Among 27 patients, the location of the gastric varices was Lg-f, and it was Lg-cf in seven patients. The gastric varices were classified by morphology into F1, tortuous; F2, nodular; or F3, tumorous. The morphol-

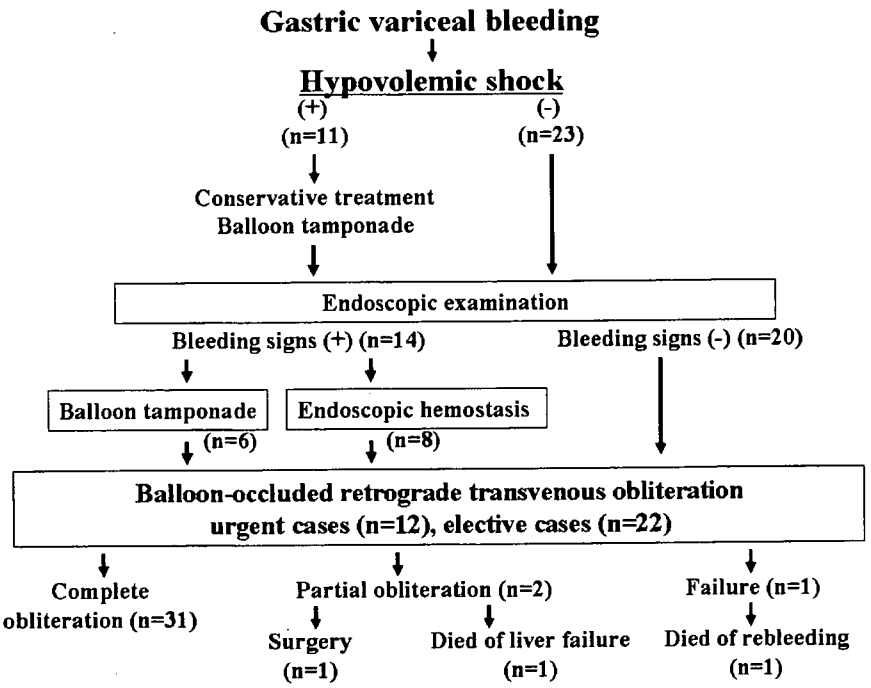

Fig. 1. Schematic flow diagram showing clinical courses and results of patients with bleeding from gastric fundal varices

Table 1. Characteristics of patients

	Urgent cases	Elective cases	*P* value
Number	12	22	
Age (years)[a]	56 (26–80)	60 (24–82)	0.239
Sex (male/female)	9/3	17/5	0.637
Etiology (viral/alcohol/other)	7/3/2	10/7/5	0.781
Child's grade (A/B/C)	4[b]/5/3	9/13/0	0.071
Location of GV[c] (Lg-f/Lg-c/Lg-cf)	10/0/2	17/0/5	0.521
Form of GV[c] (F1/F2/F3)	0/8/4	0/10/12	0.205
Hirota[21] grade[d] (1/2/3/4/5)	3/3/2/4/0	1/4/8/8/1	0.480

B-RTO, balloon-occluded retrograde transvenous obliteration; GV, gastric varices; Lg-f, gastric varices separated from the cardiac orifice; Lg-c, gastric varices adjacent to the cardiac orifice; Lg-cf, gastric varices continuing from the cardiac orifice to the gastric fundus; F1, straight small-caliber varices; F2, moderately enlarged, beady varices; F3, markedly enlarged, nodular, or tumor-shaped varices

[a] Data are mean values (range)

[b] The patient with extrahepatic presinusoidal obstruction was included as Child's grade A

[c] Endoscopic findings for gastric varices were evaluated according to the general rules for recording endoscopic findings of esophagogastric varices[37,38]

[d] Criteria for difficulty of retrograde transvenous obliteration according to retrograde venography under balloon occlusion[21]

ogy was F1 in no patients, F2 in 18 patients, and F3 in 16 patients. When patients showed signs of bleeding on endoscopic examination (Fig. 2a), temporary hemostasis was achieved with balloon tamponade or endoscopic procedures such as injection sclerotherapy using 5% ethanolamine oleate or tissue adhesives, or endoscopic variceal ligation or clipping (Fig. 2b). Portosystemic collaterals such as a gastrorenal shunt were evaluated by contrast-enhanced computed tomography (CT) (Fig. 2c). The study was approved by the institutional review boards of the participating clinical sites before study initiation, and the study was conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients at the time of enrollment.

Balloon-occluded retrograde transvenous obliteration

After we confirmed that gastric variceal bleeding was controlled, we performed angiography and B-RTO. Selective angiography of the celiac and superior mesenteric arteries was performed before B-RTO to evaluate portosystemic collaterals. All patients were in stable condition at the time of treatment. In patients with a gastrorenal shunt, a 6-French balloon catheter (Selecon MP Catheter; Clinical Supply, Gifu, Japan) was inserted into the inferior vena cava through the right femoral vein. In those without a gastrorenal shunt, other catheterizable main draining veins such as a gastrocaval shunt were examined using a 5.5-French balloon catheter (Artec Balloon Catheter, B-RTO type II SML; Create Medic, Tokyo, Japan). The catheter was advanced into any outflow vessels such as a gastrorenal shunt or gastrocaval shunt. If necessary, both shunts were occluded using two balloon catheters. During balloon occlusion of outflow vessels, retrograde venography was performed to determine the hemodynamics of the gastric varices and collateral veins. On the basis of the adrenal venogram obtained during balloon occlusion, the degree of progression of the gastric varices and collateral veins was graded in accordance with Hirota et al.[21] Then, B-RTO was performed by injecting 5% ethanolamine oleate (Oldamin; Grelan Pharmaceutical, Tokyo, Japan) through the outflow vessels during balloon occlusion. Especially in the case of Hirota's grade 3 or 4 varices,[21] additional specialized techniques to treat minor collaterals were utilized.[15,18,21,23,28,30,39,40] If occlusion of minor collateral vessels was necessary, a 50% glucose solution, ethanol, and embolic coils were used. With or without these interventions, a 2.8-French microcatheter (Rapid transit; Johnson and Johnson, New Brunswick, NJ, USA) was introduced through a balloon catheter to the gastric varices, and 2.5–5 ml of 5% ethanolamine oleate was injected intermittently into the gastric varices under fluoroscopy (Fig. 2d). When varices and inflow vessels such as a short gastric vein or a posterior gastric vein could be visualized in their entirety, injection was suspended. Human haptoglobin was administered to prevent renal dysfunction related to hemolysis occurring as a systemic effect of 5% ethanolamine oleate before B-RTO. To avoid incomplete therapeutic efficacy and pulmonary infarction due to an unstable thrombus, we left the catheter in the vein with the balloon inflated for about 20 h and removed it after retrograde venography. If obliteration of gastric varices was insufficient on retrograde venography, additional B-RTO was subsequently performed until opacification of inflow vessels. All patients underwent gastrointesti-

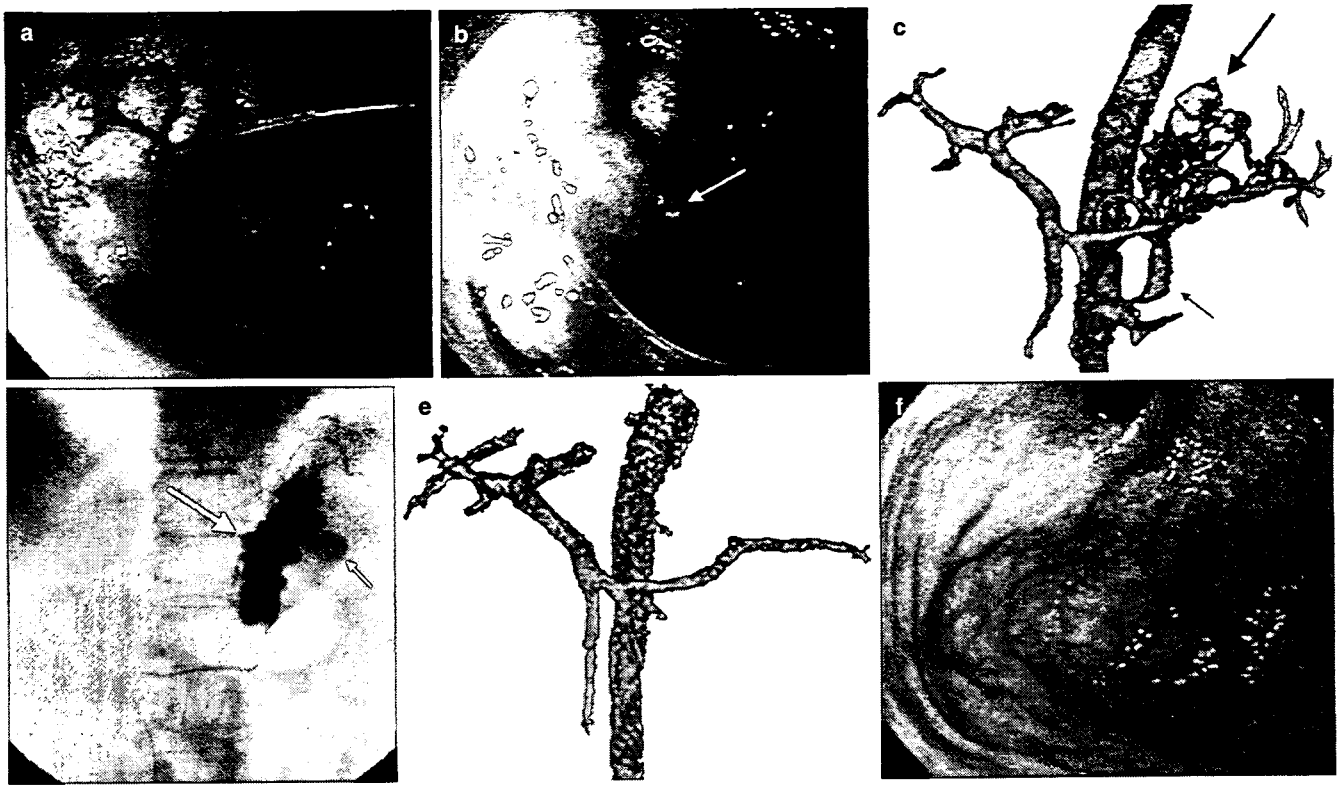

Fig. 2a–f. A 78-year-old man with alcoholic liver cirrhosis. **a** Endoscopic examination reveals huge gastric fundal varices with spurting bleeding. **b** After cyanoacrylate glue was injected intravariceally, gastric variceal bleeding stopped and the glue spilled from the rupture site (*arrow*). **c** Three-dimensional computed tomography (CT) portogram a few hours after endoscopic treatment reveals gastric varices (*large arrow*) and a gastrorenal shunt (*small arrow*). **d** Venogram shows retrograde obliteration. Gastric fundic varices (*large arrow*) were completely obliterated by 5% ethanolamine oleate injected in retrograde manner during balloon occlusion. Subsequently, the posterior gastric vein (*small arrow*) was opacified in retrograde manner. **e** Three-dimensional CT portogram obtained 1 week after balloon-occluded retrograde transvenous obliteration (B-RTO) reveals disappearance of the gastric varices and gastrorenal shunt. **f** Endoscopic examination obtained 1 year after B-RTO reveals complete eradication of the gastric varices

nal endoscopy and contrast-enhanced CT approximately 1 week after B-RTO (Fig. 2e). When the contrast-enhanced CT scan showed gastric varices with low attenuation, including of the afferent veins or the draining veins of the gastric varices, we considered obliteration to be complete. On the other hand, when contrast-enhanced CT showed gastric varices with partial enhancement, we considered the obliteration to be partial. Two radiologists interpreted the angiograms and retrograde venograms during balloon occlusion.

Follow-up and statistical analysis

The hepatic functional reserve was estimated based on the Child-Pugh score. Follow-up diagnostic imaging, such as gastrointestinal endoscopy or contrast-enhanced CT, and examination of hepatic function were performed consecutively at 1, 3, 6, and 12 months, and then every 6 months or 1 year after B-RTO. Any patients who died of causes unrelated to the liver, such as from gastric cancer or leukemia, were withdrawn from the study on the day of death. The cumulative survival rate and cumulative worsened rate of esophageal varices were analyzed using the Kaplan-Meier method and compared with a log rank test. Changes in serum laboratory values were assessed by repeated measures analysis of variance. A value of $P < 0.05$ was considered significant.

Results

Efficacy and complications

A representative clinical course of B-RTO is shown in Fig. 2. Overall complete obliteration was achieved in 31 of 34 (91%) patients with an acute bleeding episode. In urgent cases, complete obliteration was achieved in 10 of 12 (83%) patients, and in elective cases, complete

obliteration was achieved in 21 of 22 (95%) patients. In one of the remaining patients, there was a technical failure because of difficulty with catheter insertion into a gastrocaval shunt, and the other two had only partial obliteration. The patient with failure of B-RTO obtained spontaneous hemostasis but died of gastric variceal rebleeding 1 year later. The two patients with partial obliteration of B-RTO did not obtain hemostasis. One underwent Hassab's devascularization 9 days after B-RTO and survived, and the other died of liver failure (Fig. 1). Thus, the rate of hemostasis was 94% (31/33). Among 31 patients with complete obliteration, the follow-up gastrointestinal endoscopy showed disappearance of gastric varices in 30 patients (Fig. 2f); the other patient died of pneumonia 1 month after B-RTO.

In the patients with complete obliteration, the average dose of 5% ethanolamine oleate was 23.3 ml. Regarding complications, epigastralgia or low back pain was observed in 26 of 34 patients. Pyrexia (>38°C) was observed in nine of 34 patients. However, no patient experienced rebleeding from gastric varices or was treated for shock during angiography or B-RTO. None of the patients developed acute renal failure, hepatic encephalopathy, or hepatic failure.

Cumulative survival

Table 2 lists the outcomes of 34 patients treated with B-RTO. The median follow-up period for all 34 patients was 33 months (range, 1–107 months). Ten patients (29%) died, and the median period to death was 30 months (range, 1–87 months). The causes of death were hepatocellular carcinoma (n = 3), liver failure (n = 6), and bleeding from gastric varices (n = 1). Among 31 patients with complete obliteration of B-RTO, no patients died of hemorrhage from gastric varices or esophageal varices. The overall cumulative survival rate was 90%, 75%, 68%, and 55% at 1, 3, 5, and 7 years after B-RTO, respectively. At 5 years after B-RTO, the cumulative survival rate of urgent cases and elective cases was 74% and 65%, respectively (Fig. 3).

Aggravation of portal hypertension and hepatic function

We examined the aggravation of portal hypertension and hepatic function among patients with complete obliteration of B-RTO. Newly appeared gastric varices were not observed, while ten patients showed worsening of esophageal varices: appearance of red spots on the esophageal mucosa and F2 or F3 morphology of

Table 2. Outcome after balloon-occluded retrograde transvenous obliteration

	Urgent cases	Elective cases	*P* value
Number	12	22	
Complete success	10	21	0.279
Worsening of EV	6	6	0.185
Result (living/died)	9/3	15/7	0.510
Cause of death (HCC/hepatic failure/variceal bleeding)	1/2/0	2/4/1	0.708
Follow-up period (months)[a]	38 (4–97)	32 (1–107)	0.631

EV, esophageal varices; HCC, hepatocellular carcinoma
[a] Data are mean values (range)

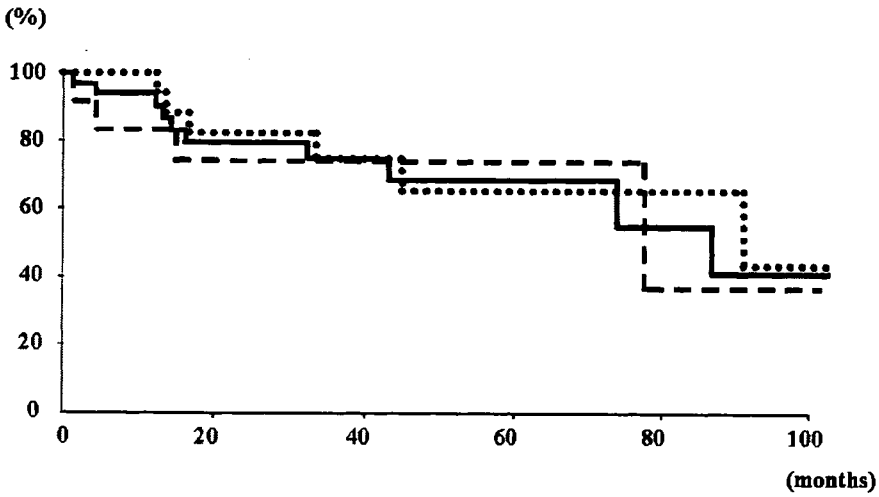

Fig. 3. Cumulative survival after B-RTO. The *solid line* shows overall cases, the *broken line* shows urgent cases, and the *dotted line* shows elective cases

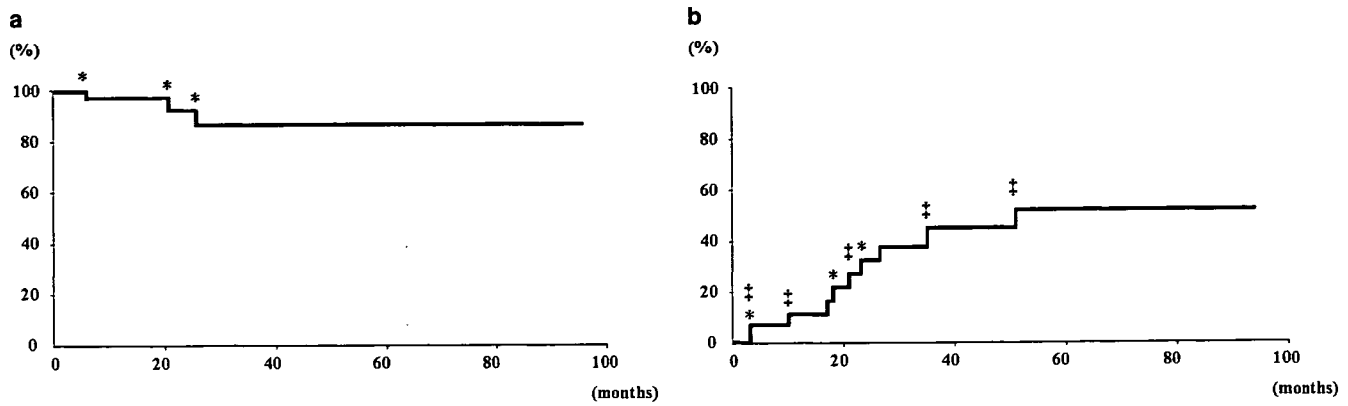

Fig. 4. **a** Kaplan-Meier analyses of cumulative variceal rebleeding. The *asterisk* shows bleeding from esophageal varices ($n = 3$). **b** Overall cumulative worsening rate of esophageal varices after B-RTO: 10 of 31 patients (32%) showed worsening of esophageal varices. The *asterisks* show the times at which patients with bleeding from esophageal varices were treated with endoscopic procedures. The *double daggers* show the times at which patients at risk of hemorrhage from esophageal varices were treated with endoscopic procedures. The remaining patients with worsening esophageal varices without hemorrhage received strict follow-up examinations

esophageal varices. Among these ten patients, three had bleeding esophageal varices and five were in danger of hemorrhage from esophageal varices. They underwent endoscopic procedure (endoscopic injection sclerotherapy, $n = 6$, and endoscopic variceal ligation, $n = 2$). The remaining two patients without hemorrhage were given strict follow-up examinations. No patients died of hemorrhage from esophageal varices. Variceal bleeding occurred in three patients from esophageal varices. The rate of rebleeding was 10% (3/31). Rebleeding from gastric varices was not observed after treatment that achieved complete obliteration. The rate of freedom from rebleeding was 97%, 86%, 86%, and 86% at 1, 3, 5, and 7 years after B-RTO, respectively (Fig. 4a). The overall cumulative worsening rate of esophageal varices was 15%, 39%, 52%, and 52% at 1, 3, 5, and 7 years after B-RTO, respectively (Fig. 4b). Portal hypertensive gastropathy is the term used to describe the endoscopic appearance of gastric mucosa with a characteristic mosaic-like pattern with or without red spots. According to McCormack's classification,[41] almost all patients had mild grade portal hypertensive gastropathy and no patient showed a deterioration of grade or bled from portal hypertensive gastropathy. Ectopic varices were not observed at any time during the study period after B-RTO.

There was no significant difference in Child-Pugh score between before and after B-RTO. Among the various parameters of the Child-Pugh score, the serum albumin level was significantly improved (3.33 ± 0.65 vs. 3.55 ± 0.52 g/dl; $P = 0.04$, before and 4 weeks after B-RTO, respectively). The albumin level improved significantly from 1 to 6 months after B-RTO. However, the albumin level did not continue to improve more than 1 year after B-RTO.

Discussion

At first variceal bleeding, pharmacotherapy should ideally be used to achieve hemostasis and protect against rebleeding.[42] In good responders, drug therapy improves the results in patients with esophageal varices bleeding, although only 30%–40% of patients reduce their portal pressure by >20% from baseline or to levels ≤12 mmHg. However, especially with respect to hemorrhage from gastric fundal varices, the outcome after pharmacotherapy is often unsatisfactory at present. In cases of upper gastrointestinal bleeding, an endoscopic examination is necessary to find the bleeding point and to classify the varices. If active bleeding is observed, then hemostatic procedures are required. Some endoscopic treatments may achieve hemostasis in over 90% of cases; however eradication of gastric varices and rebleeding rates are unsatisfactory.[43–46] It is difficult to treat huge gastric fundal varices by endoscopic injection sclerotherapy without balloon occlusion of gastrorenal shunts.[47,48] B-RTO can obliterate gastric varices from draining veins under balloon occlusion, and also obliterate afferent veins in a retrograde manner, even in patients with huge gastric varices. In Japan, B-RTO has been performed in about 3000 patients.[49] In most cases, the available literature reports prophylactic eradication of high-risk gastric varices. For acute bleeding, B-RTO can be performed after any hemostatic procedure, which is the main limitation of B-RTO. Results of B-RTO in patients with hemorrhage are summarized in Table 3. The success rate was high (95%), which is similar to the prophylactic situation. A low rate of rebleeding was observed after B-RTO. Considering these issues, it is desirable to perform B-RTO after endoscopic hemostatic procedures in patients with bleeding gastric fundal

Table 3. Summary of balloon-occluded retrograde transvenous obliteration results in patients with gastric variceal bleeding

Reference number	Authors	Number of patients	Hemostasis		Eradication of varices
			Endoscopic therapy	Other procedure	
15	Chikamori et al. 1996	6	2	4	6
25	Saeki et al. 1996	2	2	0	2
26	Sonomura et al. 1998	4	0	4	4
27	Kin et al. 1998	6	0	6	6
28	Chikamori et al. 2000	6	5	1	6
29	Fukuda et al. 2001	9	Not described	Not described	9
31	Miyamoto et al. 2003	4	4	0	4
32	Kim et al. 2003	13	Not described	Not described	12
33	Choi et al. 2003	8	Not described	Not described	8
34	Ninoi et al. 2005	35	Not described	Not described	33
35	Sugimori et al. 2005	6	Not described	Not described	6
36	Arai et al. 2005	11	4	0	11
	Our series	34	8	6	31
Total number of patients		144			138

varices. At that time, endoscopic variceal ligation or clipping can be performed. However, patients should be referred to an institution in which B-RTO or injection sclerotherapy using tissue adhesives can be performed immediately after such transient endoscopic hemostatic procedures.

Another problematic long-term sequel of B-RTO is the development or worsening of esophageal varices, which occurs in about 50% of patients.[23,29,31,34] In the present study, a similar rate of worsening esophageal varices was observed. Those patients were all successfully treated endoscopically. Generally, esophageal varices are managed more easily than gastric varices. Esophageal varices that developed after B-RTO were also easier to treat. Elevation of the portal pressure gradient may be expected owing to obliteration of major shunts, such as gastrorenal shunts. Indeed, development or worsening of esophageal varices after B-RTO indicates aggravation of portal hypertension. However, ectopic varices or severe portal hypertensive gastropathy were not observed after B-RTO in the present study.

Although the long-term outcome after B-RTO has not yet been fully demonstrated, some reports have shown improvement of the Child-Pugh score after B-RTO.[24,29] In the present study, liver function, especially the serum albumin value, increased significantly from 1 to 6 months after B-RTO. Moreover, some studies demonstrated that portosystemic encephalopathy improved more after B-RTO than after TIPS.[21,50] An increase in portal blood flow from obliteration of large portosystemic shunts might contribute to improvement in liver function. This was confirmed by Doppler ultrasound before and after B-RTO, and in a hemodynamic study after balloon occlusion of a gastrorenal shunt.[31,51,52] Liver function improved in patients with Child-Pugh class B or C disease as well as in those with class A disease. However, radiological or surgical occlusion of a portosystemic shunt is sometimes accompanied by liver failure when the portal pressure gradient increases 60% or more from baseline after the procedure.[53] Partial splenic arterial embolization can reduce the portal pressure and lead to a good outcome in such cases treated by radiological occlusion of a portal systemic shunt.[54,55] At present, it appears that B-RTO is applicable in Child-Pugh class A or B patients, while the benefit remains unclear in Child-Pugh class C patients. With respect to long-term prognosis, the cumulative survival rate was 68% at 5 years after B-RTO in the present study. Our results are consistent with those of other reports, including with regard to B-RTO for prophylaxis.[23,29,34,56] Considering the factors affecting prognosis, the presence or absence of concomitant hepatocellular carcinoma and the Child-Pugh classification were important factors affecting survival after B-RTO.[29,34] Among the complications of chronic liver disease, B-RTO can reduce deaths due to gastric varices, which are one of the most difficult variceal sites to treat.

The technique of B-RTO is complicated and is not yet standardized in Japan. For successful treatment, additional specialized techniques to treat minor collaterals are required.[15,18,21,23,28,30,39,40] Such techniques result in a decreased use of 5% ethanolamine oleate and may avoid sclerosant-related complications such as hemoglobinemia. In cases with Hirota's grade 3 or 4 in particular,[21] techniques such as stepwise injection of 5% ethanolamine oleate, use of high concentrated glucose or ethanol, coil embolization of minor collaterals, or double-balloon catheterization are needed. By using

these strategies and techniques based on the hemodynamic feature,[15,18,21,23,28,30,39,40] radiologists worldwide could treat patients with gastric varices with gastrorenal shunts by B-RTO. Indeed, usefulness of B-RTO has recently been reported from outside of Japan,[32,33] including in the review, guideline, and educational sections of the journals *Gastroenterology*, *Gut*, and *Radiographics*, respectively.[3,6,18,40]

B-RTO is not feasible in patients without gastrorenal shunts. Ninoi et al.[24] demonstrated that antegrade transhepatic obliteration using metallic coils and 5% ethanolamine oleate eradicated gastric varices in patients difficult to treat with B-RTO. While percutaneous transhepatic obliteration only in afferent vessels resulted in transient hemostasis,[57] antegrade obliteration, including of gastric varices, is reported to be effective as well as B-RTO.[24] Similar efficacy was also demonstrated in patients with ectopic varices.[58] When hemostasis is performed by TIPS in patients with refractory bleeding, embolization together with TIPS should be performed, as recommended by American Association for the Study of Liver Diseases practice guidelines.[12] At the same time, direct obliteration via the TIPS route would be desirable.[10,12,13,58] Especially, the use of long-acting occlusion agents such as liquids in addition to metallic coils could obliterate at the peripheral level of the collateral vessels feeding the varices, and lead to a low incidence of rebleeding.[13,58] These results support the findings that embolization of both esophageal varices and their feeders is essential to lower the recurrence rate after sclerotherapy.[59] It is desirable to obliterate both varices and the peripheral collateral vessels using 5% ethanolamine oleate, the distribution of which can be monitored, although agent-related complications are sometimes reported. No rebleeding from gastric varices after B-RTO might indicate successful embolization of both gastric varices and afferent veins. Thus, it is important in the treatment of gastric varices with hemorrhage to obliterate varices directly in addition to reducing portal pressure. In bleeding gastric varices, direct obliteration using 5% ethanolamine oleate in a retrograde or antegrade manner is desirable with or without TIPS, and whether the portal pressure gradient is more or less than 12 mmHg. When direct obliteration is performed without TIPS, possible ectopic varices or portal hypertensive gastropathy should be considered, although the such aggravation of portal hypertension was not observed in the present study.

In conclusion, B-RTO treatment of bleeding gastric varices achieved high eradication of gastric varices, a low rebleeding rate, and a fairly good prognosis with improved hepatic function.

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RAPID COMMUNICATION

Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma

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Abstract

AIM: To assess the clinical features and prognosis of 151 patients with extrahepatic metastases from primary hepatocellular carcinoma (HCC), and describe the treatment strategy for such patients.

METHODS: After the diagnosis of HCC, all 995 consecutive HCC patients were followed up at regular intervals and 151 (15.2%) patients were found to have extrahepatic metastases at the initial diagnosis of primary HCC or developed such tumors during the follow-up period. We assessed their clinical features, prognosis, and treatment strategies.

RESULTS: The most frequent site of extrahepatic metastases was the lungs (47%), followed by lymph nodes (45%), bones (37%), and adrenal glands (12%). The cumulative survival rates after the initial diagnosis of extrahepatic metastases at 6, 12, 24, and 36 mo were 44.1%, 21.7%, 14.2%, 7.1%, respectively. The median survival time was 4.9 mo (range, 0-37 mo). Fourteen patients (11%) died of extrahepatic HCC, others died of primary HCC or liver failure.

CONCLUSION: The prognosis of HCC patients with extrahepatic metastases is poor. With regard to the cause of death, many patients would die of intrahepatic HCC and few of extrahepatic metastases. Although most of HCC patients with extrahepatic metastases should undergo treatment for the primary HCC mainly, treatment of extrahepatic metastases in selected HCC patients who have good hepatic reserve, intrahepatic tumor stage (T0-T2), and are free of portal venous invasion may improve survival.

Key words: Hepatocellular carcinoma; Extrahepatic metastases; Prognosis; Causes of death

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly malignant tumor with frequent intrahepatic metastasis. The prognosis of HCC patients has improved because of progress in therapeutic procedures, such as surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), and transcatheter arterial chemoembolization (TACE)[1-3]. Moreover, progress in diagnostic modalities, such as ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and digital subtraction angiography (AG) has led to a better detection of patients with early and small HCC or asymptomatic extrahepatic metastases.

The above improvements in survival and diagnostic modalities have resulted in increased detection of extrahepatic metastases from primary HCC and further increases are anticipated in the future. Several groups have investigated extrahepatic metastases from HCC, but many of such cases were in autopsy cases, in a small number of cases or case reports[4-15]. At present, the prognosis of patients with extrahepatic metastases from primary HCC is poor[16,17]. In this regard, there is only little information about the causes of death of such patients[18], and there is no consensus on the treatment strategy for extrahepatic metastases from HCC. For example, what treatment strategy should be used to treat intrahepatic HCC or extrahepatic metastases? Among patients with extrahepatic metastases from primary HCC, which patients should be treated? To our knowledge, there are no reports that

deal directly with these questions. In this relatively large study, we retrospectively assessed the clinical features and prognosis of 151 patients with extrahepatic metastases from primary HCCs, and described the treatment strategy for such patients.

MATERIALS AND METHODS

Patients

From June 1990 to December 2005, 995 consecutive patients with HCC were admitted to our hospital. Among these patients, 880 were initially diagnosed with HCC in our hospital while the others were treated previously for HCC in other hospitals. Extrahepatic metastases from primary HCC were detected in 151 (15.2%) of 995 patients. None of the patients was treated for extrahepatic metastases. All the 151 HCC patients with extrahepatic metastases (117 men and 34 women, median age: 64 years, range: 21-82 years) were enrolled in the present study.

Table 1 summarizes the clinical profile of the 151 patients at the initial diagnosis of extrahepatic metastases. These 151 patients were divided into groups A and B. Group A was consisted of 68 patients presented with extrahepatic metastases together with primary HCC at the initial diagnosis of HCC, group B was composed of 83 patients who received treatment for intrahepatic HCC, and developed extrahepatic metastases during the follow-up period. Among them, 37 (25%) patients were treated previously for primary HCC in other hospitals, 90 patients were of performance status (PS) of 0, 43 patients of 1, 9 patients of 2, 6 patients of 3, and 3 patients of 4[19]. The etiology of the background liver disease was hepatitis B virus (HBV) in 33 patients, hepatitis C virus (HCV) in 89 patients, HBV and HCV in 5 patients, and non-B non-C in 24 patients. The hepatic reserve was Child-Pugh grade A in 88 patients, grade B in 48 patients, and grade C in 15 patients. We evaluated the primary tumor stage according to the Liver Cancer Study Group of Japan criteria[20], based on the following three conditions (T factor): solitary, < 2 cm in diameter, and no vessel invasion. T1 was defined as fulfilling the three conditions, T2 as fulfilling two of the three conditions, T3 as fulfilling one of the three conditions, T4 as fulfilling none of the three conditions. The primary HCC tumor stage at the first diagnosis of extrahepatic metastases was T0 (no intrahepatic HCC) in 11 (7%) patients, T1 in 4 (3%) patients, T2 in 13 (9%) patients, T3 in 43 (28%) patients, and T4 in 80 (53%) patients. Twenty seven of 28 patients with intrahepatic tumor stage T0-T2 were treated previously for intrahepatic HCC. The median size of the main intrahepatic primary tumor was 48 mm (range, 0-160 mm). Intrahepatic tumor morphology was nodular type in 83 (55%) patients, non-nodular type in 57 (38%) patients, and no intrahepatic HCC in 11 (7%) patients. Table 1 lists the sites of extrahepatic metastases at enrollment. Among the 151 patients with extrahepatic metastases, the sites of metastases were the lungs in 63 patients, lymph nodes in 60 patients, bones in 51 patients, adrenal glands in 16 patients and other locations (e.g., peritoneum, pancreas and nasal passages). In some patients, two or more distant metastatic tumors were found in one or more organs.

Table 1 Clinical profile of 151 HCC patients with extrahepatic metastases at the initial diagnosis of extrahepatic metastases

Age (yr)	64 (21-82)
Sex (male/female)	117/34
Etiology (HBV/HCV/HBV + HCV/others)	33/89/5/24
PS (0/1/2/3/4)	90/43/9/6/3
Intrahepatic tumor stage (T0/1/2/3/4)	11/4/13/43/80
Intrahepatic main tumor size (mm)	48 (0-160)
Intrahepatic tumor volume (< 50% / ≥ 50%)	103/48
Intrahepatic tumor morphology (nodular type/non nodular type/no intrahepatic HCC)	83/57/11
Grade of portal vein invasion (Vp 0/1/2/3/4)	74/0/26/28/23
Child-Pugh grade (A/B/C)	88/48/15
AFP (ng/mL)	741.8 (< 5-861600)
DCP (mAU/mL)	1300 (< 10-391400)
Site of extrahepatic metastases, *n* (%)	
Lung	63 (42)
Lymph nodes	60 (40)
Bone	51 (34)
Adrenal	16 (11)
Peritoneum	1 (0.7)
Pancreas	1 (0.7)
Nasal passages	1 (0.7)

Data are expressed as medians and ranges unless indicated otherwise. HBV: hepatitis B virus; HCV: hepatitis C virus; PS: Eastern Cooperative Oncology Group performance status; T0: no intrahepatic HCC; Portal invasion assessed Vp1: tumor thrombus in a third or more of the peripheral branches; Vp2: in the second branch; Vp3: in the first branch; Vp4: in the trunk; AFP: alpha-fetoprotein; DCP: Des-γ-carboxy prothrombin.

Hepatocellular carcinoma

A definitive diagnosis of HCC was based on the finding of typical hypervascular radiological features or histopathological examination of needle biopsy specimen. HCC was also assessed by US, CT, and/or AG. Furthermore, CT was obtained during arterial portography and computerized tomographic hepatic arteriography. Further assessment of HCC was conducted by measuring α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP).

Extrahepatic metastases were diagnosed by CT, MRI, bone scintigraphy, X-ray, and/or positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (FDG), or diagnosed by histopathological examination of surgically resected specimen or biopsy. When we suspected extrahepatic metastases with HCC, we always ruled out other malignancies (such as gastric cancer, colon cancer and lung cancer) by several imaging modalities, serological tumor markers and/or pathological examination.

Follow- up

All the 151 HCC patients with extrahepatic metastases were followed up during the observation period and no one was lost to follow-up. The median follow-up period was 4.9 mo (range, 1-37 mo). After the diagnosis of HCC, all patients were screened at regular intervals for the development of intra/extra hepatic metastases by clinical examination, AFP, DCP, and/or various imaging modalities. Serological tumor markers were measured once every month. US, CT or MRI was performed once every three to six months.

Statistical analysis and ethical considerations

Differences between groups were examined for statistical significance using the Mann-Whitney test (*U*-test) and χ^2 test where appropriate. Cumulative survival rate was assessed by the Kaplan-Meier life-table method and the differences were evaluated by the log rank test. The following 15 potential predictors were assessed in this study: PS (0 *vs* 1-4), age (≤ 65 *vs* > 65 years), sex (M *vs* F), Child-Pugh stage (A *vs* B, C), intrahepatic tumor stage (T0-T2 *vs* T3, T4), main intrahepatic tumor size (≤ 50 *vs* > 50 mm), intrahepatic tumor volume (≤ 50% *vs* > 50%), intrahepatic tumor morphology (nodular type *vs* non nodular type), portal venous invasion (Vp 0-2 *vs* > Vp 3, 4), AFP (≤ 400 ng/mL *vs* > 400 ng/mL), DCP (≤ 1000 mAU/mL *vs* > 1000 mAU/mL), site of extrahepatic metastases (lung *vs* others, bone *vs* others, only lymph node *vs* others), and treatment for extrahepatic metastases (performed *vs* not performed). All factors that were at least marginally associated with the survival after diagnosis of extrahepatic metastases ($P < 0.05$) were entered into a multivariate analysis. The hazard ratio and 95% confidence interval (95% CI) were calculated to assess the relative risk confidence. All analyses described above were performed using the SPSS program (version 11.0, SPSS Inc., Chicago, IL).

The study protocol was approved by the Human Ethics Review Committee of Graduate School of Biomedical Sciences, Hiroshima University and a signed consent form was obtained from each patient.

RESULTS

Site of extrahepatic metastases

Table 2 lists the sites of extrahepatic metastases identified throughout the follow-up period. The most frequent site of metastases that were identified throughout the follow-up period was the lung (n = 71 patients, 47%), followed by lymph nodes (n = 68 patients, 45%), bone (n = 56 patients, 37%), and adrenal glands (n = 18 patients, 12%). Brain metastases were identified in 2 (1%) patients. One (0.7%) patient each had metastases in the peritoneum, pancreas, nasal passages, muscle, skin, diaphragm, and colon. Autopsy was performed in 14 cases with metastases. Despite the detection of extrahepatic metastases in these 14 patients before autopsy, additional extrahepatic metastases were detected on postmortem examination (lymph nodes, diaphragm, and colon). At the first diagnosis of extrahepatic metastases, 109 (72%) patients had single-organ metastases, while the others had multiple organ metastases.

Among the 71 patients with lung metastases, 23 patients had bilateral lung metastases, 14 had additional extrapulmonary site of metastatic disease. The size of pulmonary nodules ranged from 9 to 30 mm at initial diagnosis of extrahepatic HCC. Few patients had symptoms (cough, dyspnea, and pleural effusion) related to lung metastases, and 8 patients who had severe symptoms died subsequently of respiratory failure. The median survival period of these 8 patients was 4.3 mo (range, 2.5-14.4 mo).

Table 2 Sites of extrahepatic HCC detected throughout the entire follow-up period

Site	Patients (n = 151), n (%)
Lung	71 (47)
Lymph nodes	68 (45)
Bone	56 (37)
Adrenal	18 (12)
Brain	2 (1)
Peritoneum	1 (0.7)
Pancreas	1 (0.7)
Nasal	1 (0.7)
Muscle	1 (0.7)
Skin	1 (0.7)
Diaphragm	1 (0.7)
Colon	1 (0.7)

Among the 68 patients with lymph node metastases, metastases were identified in 64 regional lymph nodes. The most common site was in the paraaortic nodes (31/64), followed by portohepatic nodes (21/64), periceliac nodes (6/64) and peripancreatic nodes (6/64). The majority of patients with regional lymph nodes metastases were asymptomatic, but few regional lymph nodes (portohepatic nodes) caused obstructive jaundice. Distant nodal metastases were found at 17 sites. The most common site was the mediastinum nodes (10/17), followed by subclavicular nodes (3/17), iliac nodes (2/17), cardiophrenic node (1/17), and retrocrural node (1/17). All distant lymph node metastases were not associated with clinical symptoms in this study.

Fifteen of 56 patients with bone metastases had multiple bone metastases at the initial diagnosis of bone metastases. The total number of bone metastatic sites was 88. The most frequent site was the vertebra (63/88; cervical vertebrae = 9, thoracic vertebrae = 38, and lumbar vertebrae = 16), followed by the ribs (8/88). Bone metastases were diagnosed by CT, MRI, bone scintigraphy, and/or PET with FDG.

Of the 18 patients with adrenal gland metastases, 13 had right adrenal gland metastases, 4 had left adrenal gland metastases and only one patient had bilateral metastases. These metastases were not associated with symptoms.

Treatments of extrahepatic metastases

All patients with Child-Pugh grade other than C or PS other than 2-4 were treated for intrahepatic HCC, and many of them were continuously treated after the diagnosis of extrahepatic metastases. On the other hand, HCC patients with Child-Pugh grade C or PS of 2-4 received supportive care. Forty-nine (32%) of 151 patients were treated for extrahepatic metastases by surgical resection, TACE, systemic chemotherapy, and/or radiotherapy. The 49 patients had extrahepatic metastases that were considered to worsen prognosis.

Surgical resection was performed in three (2%) patients (with regional lymph node, adrenal gland and lung metastases). The survival periods after surgical resection of extrahepatic metastases were 7 mo (in patients with lymph node metastases), 23 mo (in patients adrenal gland metastases), and 37 mo (in patients with lung metastases).

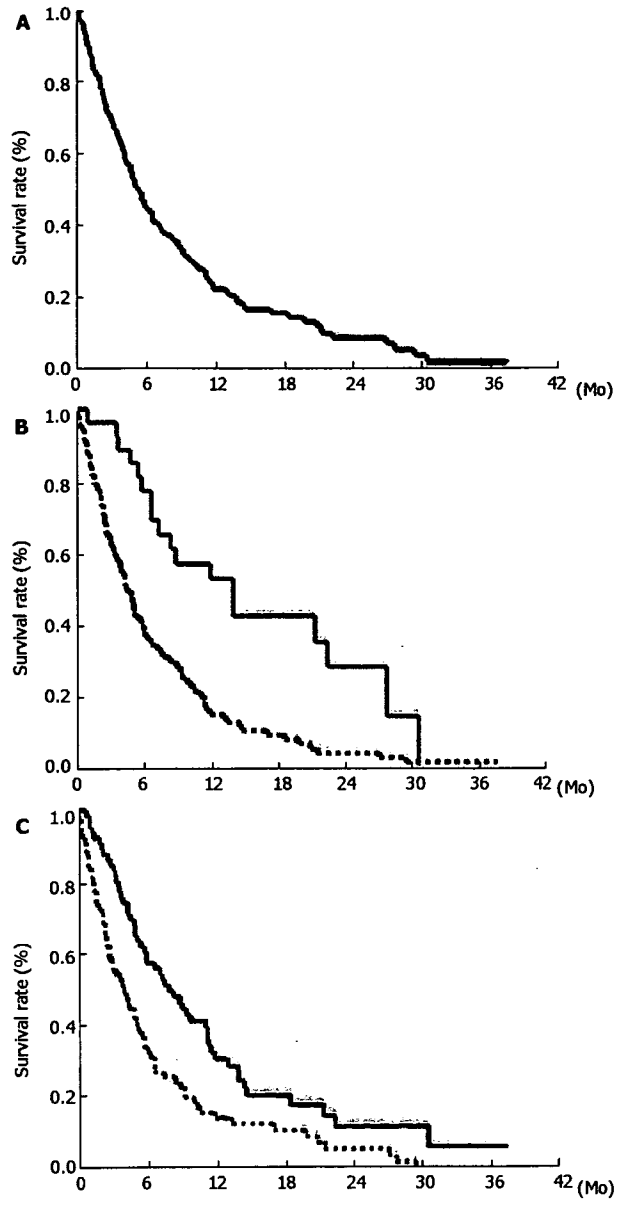

Figure 1 Survival rate of 151 HCC patients with extrahepatic metastases (**A**), intrahepatic tumor stage (**B**) [solid line: T0-T2, dashed line: T3, T4 (log-rank test: $P < 0.001$)], and after treatment of extrahepatic metastases (**C**) [solid line: treatment group, dashed line: no treatment group (log-rank test: $P < 0.001$)].

These three were all alive without recurrence of extrahepatic metastases during the observation period. In each of these 3 patients, hepatic reserve was Child-Pugh stage A, no intrahepatic HCC was not detected, and PS was 0.

TACE was performed in 8 (5%) patients (7 patients with adrenal gland metastases, and one patient with paraaortic lymph node metastases). Systemic chemotherapy was used in 39 (26%) patients. Chemotherapy included 5-fluorouracil, carboplatin, cisplatin. Twenty-five of the 39 patients had lung metastases, 10 had lymph node metastases, 2 had bone metastases, one had lung and lymph node metastases, and one had lung, adrenal gland and lymph node metastases.

Radiotherapy was performed in 36 (24%) patients.

Table 3 Univariate analysis of predictors of survival after initial diagnosis of extrahepatic metastases in 151 patients

Variable	Hazard Ratio	95% CI	*P*
PS (0 *vs* 1-4)	2.181	1.50-3.17	< 0.001
Age (≤ 65 *vs* > 65 yr)	0.988	0.97-1.0	0.18
Sex (M *vs* F)	0.889	0.57-1.38	0.601
Child Pugh stage (A *vs* B, C)	2.323	1.73-3.12	< 0.001
Intrahepatic main tumor size (≤ 50 *vs* > 50 mm)	2.321	1.52-3.54	< 0.001
Intrahepatic tumor volume (≤ 50 *vs* > 50%)	2.523	1.71-3.72	< 0.001
Intrahepatic tumor morphology (nodular *vs* non nodular)	1.506	1.04-2.18	0.03
Vp (0-2 *vs* 3, 4)	2.247	1.53-3.29	< 0.001
AFP (≤ 400 *vs* > 400 ng/mL)	1.158	0.80-1.68	0.439
DCP (≤ 1000 *vs* > 1000 mAU/mL)	1.584	1.08-2.33	0.02
Treatment (performed *vs* not performed)[1]	2.385	1.51-3.77	< 0.001
Site (lung *vs* others)[2]	1.065	0.74-1.52	0.731
Site (bone *vs* others)	1.61	1.11-2.33	0.012
Site (only lymph node *vs* others)	1.133	0.74-1.74	0.567

[1]Treatments: various treatments for extrahepatic metastases (surgical resection, TACE, systemic chemotherapy and/or radiotherapy); [2]Site: site of extrahepatic metastases.

Curative therapy was performed in 10 patients (6 patients with lymph node metastases and 4 patients with adrenal gland metastases). Palliative therapy was performed in the remaining 26 patients who had severe pain due to bone metastases. Furthermore, 9 patients with painful bone metastases were treated with RFA therapy combined with cementoplasty[21]. Nonsteroidal anti-inflammatory drugs or opioids were used in patients with bone metastases due to severe pain.

Survival data

The cumulative survival rates of the 151 HCC patients with extrahepatic metastases after initial diagnosis of extrahepatic metastases at 6, 12, 24, and 36 mo were 44.1%, 21.7%, 14.2%, and 7.1%, respectively (Figure 1A). The median survival period was 4.9 mo (range, 1-37 mo). Survival was compared among patients with intrahepatic tumor stage T0-T2 and T3, T4 (Figure 1B). The rate was significantly higher in the intrahepatic tumor stage T0-T2 groups than in the T3, T4 groups ($P < 0.001$). We investigated the determinants of survival after initial diagnosis of extrahepatic metastases. Univariate analysis identified the following 9 factors significantly influencing survival: PS, 0 ($P < 0.001$); Child-Pugh grade, A ($P < 0.001$); intrahepatic main tumor size, < 50 mm ($P < 0.001$); intrahepatic tumor volume, < 50% ($P < 0.001$); portal venous invasion, Vp 0-2 ($P < 0.001$); use of treatment for extrahepatic metastases ($P < 0.001$, Figure 1C); bone metastasis ($P = 0.012$); DCP < 1000 mAU/mL ($P = 0.02$); and nodular type intrahepatic tumor ($P = 0.03$) (Table 3). Since the variables could be mutually correlated, multivariate analysis was performed. The analysis identified the following four variables as significant and independent determinants of survival after initial diagnosis of extrahepatic metastases: PS ($P < 0.001$), portal venous invasion ($P < 0.001$), treatment of extrahepatic metastases ($P = 0.003$), and Child-Pugh grade ($P = 0.009$) (Table 4).

Table 4 Multivariate analysis of predictors of survival after initial diagnosis of extrahepatic metastases among 151 patients

Variable	Hazard ratio	95% CI	P
PS (0 *vs* 1-4)	5.576	2.431-12.152	< 0.001
Vp (0-2 *vs* 3, 4)	4.792	2.137-10.712	< 0.001
Treatment (performed *vs* not performed)	4.134	1.539-11.011	0.003
Child pugh stage (A *vs* B, C)	2.372	1.247-4.914	0.008

Causes of death

Twenty-five patients were still alive at the end of this study while 126 patients died. Of the latter group, intrahepatic tumor stages at the first diagnosis of extrahepatic metastases were T0-2 in 17 patients and T3-4 in 109 patients. One hundred and twelve (89%) patients died of intrahepatic HCC or liver failure. Fourteen (11%) patients died of extrahepatic HCC (Table 5). Eight patients died of respiratory failure due to lung metastases. Four patients died of bone metastases-related disease. Two patients died of obstructive jaundice due to portohepatic node metastasis.

Of the 4 patients who died of bone metastases-related disease, 3 died of intracranial hypertension due to skull metastasis. Another patient died of vertebra metastasis-related disease. He was 69-year old at first diagnosis of bone metastases. He suffered from complete spinal cord injury due to vertebral metastasis with gradual worsening of PS. Finally, PS changed to 4 and the patient died of aspiration-related pneumonia. The survival period after first diagnosis of extrahepatic metastases was 11.5 mo.

Among the 14 patients who died of extrahepatic HCC, 3 had chronic hepatitis, 7 had cirrhosis of Child-Pugh grade A, 3 had cirrhosis of Child-Pugh grade B, and 1 had cirrhosis of Child-Pugh grade C. All patients who died of extrahepatic HCC with the exception of that with Child-Pugh grade C had some hepatic reserve until death. Intrahepatic tumor stage at first diagnosis of extrahepatic metastases was T0 (3 patients), T1 (4 patients), T2 (1 patient), T3 (5 patients), and T4 (1 patient). All 8 patients with intrahepatic tumor stage T0-T2 were treated previously for intrahepatic HCC. Eight of 17 (47%) patients with intrahepatic tumor stage T0-T2 died of extrahepatic metastases. On the other hand, 6 of 109 (6%) patients with intrahepatic tumor stages T3 and T4 died of extrahepatic metastases. The mortality rate of patients with intrahepatic tumor stage T0-T2 was significantly higher than that of patients with intrahepatic tumor stages T3 and T4 ($P = 0.001$) (Table 6).

Table 5 Clinical profile of 14 patients who died of extrahepatic metastases during the follow-up period

Case	Presentation	Site	Intrahepatic HCC stage	Sex	Age (yr)	Child-Pugh stage	Etiology
1	R	Lung	T3	M	65	A	HCV
2	R	Lung	T4	M	35	CH	HBV
3	R	Lung	T3	M	56	A	HBV
4	R	Lung, vertebra	T0	M	40	CH	HBV
5	R	Lung, vertebra	T1	M	69	A	HBV
6	R	Lung, LN	T0	M	63	B	HBV
7	R	Lung, vertebra, nasal	T0	M	50	A	HBV
8	R	Lung	T3	M	73	A	NBNC
9	I	Skull	T1	M	57	A	HCV
10	I	Skull	T2	F	72	C	HCV
11	I	Skull	T3	M	56	B	HCV
12	A	Vertebra	T3	M	69	A	HCV
13	O	Lung, rib, LN	T1	M	74	A	HCV
14	O	Vertebra, LN	T1	M	70	B	HCV

All patients with intrahepatic tumor stage T0-T2 were treated previously for intrahepatic HCC. R: respiratory failure; CH: chronic hepatitis; LN: lymph node; NBNC: no hepatitis B virus or hepatitis C virus; I: intracranial hypertension symptom; A: aspiration-related pneumonia; O: obstructive jaundice.

Table 6 Causes of death of 126 HCC patients with extrahepatic metastases

Intrahepatic tumor stage	Intrahepatic HCC or liver failure	Extrahepatic HCC
T0-2 ($n = 17$)	53% (9/17)	47% (8/17)
T3-4 ($n = 109$)	94% (103/109)	6% (6/109)

DISCUSSION

The prognosis of HCC patients with extrahepatic metastases is unsatisfactory[16,17] and often not well known[18]. In the present study, we assessed the clinical features and prognosis of 151 consecutive HCC patients with extrahepatic metastases. The incidence of extrahepatic metastases from HCC was 15.2%. The most frequent metastatic sites were the lung, lymph nodes, bone, and adrenal gland. The cumulative survival rates of the 151 patients after the initial diagnosis of extrahepatic metastases at 6, 12, 24, and 36 mo were 44.1%, 21.7%, 14.2%, 7.1%, respectively. The median survival period was 4.9 mo (range, 1-37 mo). The mortality rate due to extrahepatic metastases from HCC was 11% (14/126).

Extrahepatic metastases have been reported to occur in 13.5%-42% of HCC patients[22-24]. In this study, the prevalence of extrahepatic metastases was 15.2%. Though we screened all HCC patients at regular intervals for intra/extra hepatic metastases, not all patients received a full metastatic follow up based on the use of several diagnostic techniques. Since the majority of HCC patients with extrahepatic metastases were asymptomatic, it is possible to miss asymptomatic metastases such as those in the lungs, distant lymph nodes, muscles and rectum.

Based on the initial diagnosis of intrahepatic HCC, Natsuizaka *et al*[16] reported that patients with advanced HCC develop extrahepatic metastases significantly more frequently than those with less advanced HCC. At the initial diagnosis of extrahepatic metastases, many HCC patients with extrahepatic metastases have been reported

to have advanced intrahepatic stage[16,22]. In our study, 123 (81%) patients with extrahepatic metastases had intrahepatic tumor stages T3 (28%) and T4 (53%), at the initial diagnosis of extrahepatic metastases, suggesting that HCC patients with advanced intrahepatic tumor stage (T3, T4) are at risk of developong extrahepatic metastases, and that such patients should be followed up carefully.

On the other hand, our study identified 28 (19%) patients with early intrahepatic tumor stage (T0-T2) at the initial diagnosis of extrahepatic HCC. Eight of the 17 (47%) patients later died of extrahepatic metastases. With regard to previous treatment, 27 of 28 patients with early intrahepatic tumor stage were treated previously for intrahepatic HCC. Considering the possibility of extrahepatic metastases, HCC patients with early intrahepatic tumor stage should be followed up carefully, particurally those who have been treated previously for intrahepatic HCC. This also includes HCC patients who have received complete resection or ablation.

In this study, the most frequent metastatic sites were the lungs, lymph nodes, bones, and adrenal glands. Other studies have reported similar findings[16,22]. HCC is thought to spread mainly *via* the hematogenous route, thus causing intra/extra hepatic metastases. Most of HCCs are hypervascular tumors. Moreover, HCC tends to invade vessels, such as portal and hepatic veins. Therefore, HCC could spread through the lung and systemic circulation via the hepatic or portal vein. This could explain why the lung is the most frequent site of metastases in HCC. Most of HCC patients with lung metastases are asymptomatic. To detect lung metastases from HCC, chest CT should be performed at regular intervals during routine metastasis follow-up.

Though there is no standard treatment for extrahepatic metastases of primary HCC, several authors have reported the use of various treatment modalities for extrahepatic m etastases[7,15,23,25-29]. Some reports have described successful treatment of extrahepatic metastases with no or few intrahepatic HCC[7,25-27]. However, only few HCC patients can undergo surgical resection of extrahepatic metastases because of hepatic reserve or intraheatic tumor stage. In this study, the prognosis of 3 patients after surgical resection of extrahepatic metastases seemed good. These 3 patients had good hepatic reserve, no intrahepatic HCC (PS = 0) and no intra/extra hepatic HCC and are expected to have good prognosis. The clinical features of HCC patients with extrahepatic metastases varied widely. All patients were not symptomatic and thus not necessary to receive treatment of extrahepatic metastases. Thus, treatment of extrahepatic metastases from primary HCC must be performed carefully taking into consideration the clinical features.

Multivariate analysis in our study identified PS, portal venous invasion, treatment for extrahepatic metastases, and Child-Pugh grade as important determinants of survival after the initial diagnosis of extrahepatic metastases. Ishii *et al*[17] reported that brain metastases, number of metastatic tumors and primary tumor status are important factors for survival. In our study, only two patients had brain metastases. With regard to the number of metastatic tumors, we might miss asymptomatic metastases. Thus,

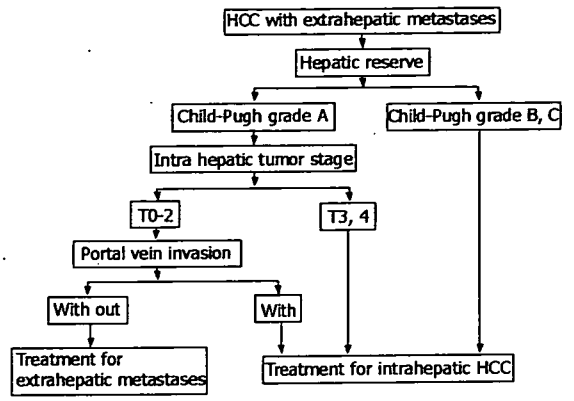

Figure 2 Initial sites to be treated.

we did not include brain metastasis and number of metastatic tumors in this multivariate analysis. Treatment of extrahepatic metastases was an important determinant of survival in our study. There might be selection bias of patients treated for extrahepatic metastases because many of them had good hepatic reserve. HCC patients with poor hepatic reserve did not receive treatment for extrahepatic metastases in this study. Regardless of such bias, treatment of extrahepatic metastases might be important for improvement of prognosis.

With regard to the cause of death, many HCC patients with extrahepatic metastases died of intrahepatic HCC or liver failure and few (11%) died of extrahepatic HCC. Of the 14 patients who died of extrahepatic metastases, 10 had good hepatic reserve and 8 had early intrahepatic tumor stage, at the initial diagnosis of extrahepatic metastases. Usually, HCC patients with good hepatic reserve, no or few intrahepatic HCCs, and those without portal venous invasion show relatively good prognosis. According to the univariate analysis of HCC patients with extrahepatic metastases, patients with early intrahepatic tumor stage have a significantly better prognosis than those with advanced intrahepatic tumor stage. In our study, the mortality rate due to extrahepatic metastases with early intrahepatic tumor stage was significantly higher than that due to those with advanced intrahepatic tumor stage. This might be explained by the differences in survival periods between these intrahepatic tumor stage groups. Extrahepatic metastases with early intrahepatic tumor stage can spread during the relatively long survival period, and few patients die of extrahepatic metastases. Extrahepatic metastasis with early intrahepatic tumor stage is a very important cause of death of HCC patients. Successful treatment of extrahepatic metastases in HCC patients with early intrahepatic tumor stage might improve the prognosis.

In conclusion, the majority of HCC patients with extrahepatic metastases should undergo treatment for intrahepatic HCC. Selected HCC patients with critical extrahepatic metastases could undergo treatment for extrahepatic metastases. However, these selected patients must have good hepatic reserve, intrahepatic tumor stage: T0-T2, and are free of portal venous invasion (Figure 2). The important sites of critical metastases from primary

HCC are the lungs, bones and the portohepatic node. Further studies are needed for the improvement of the prognosis of HCC patients with extrahepatic metastases.

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Successful Treatment of an Entecavir-Resistant Hepatitis B Virus Variant

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Emergence of a lamivudine (LAM)-resistant hepatitis B virus (HBV) with amino acid substitutions in the YMDD motif is a well-documented problem during long-term LAM therapy. Entecavir (ETV) is a new drug approved for treatment of HBV infection with or without LAM-resistant mutants. This report describes an ETV-resistant strain of HBV, which emerged after prolonged ETV therapy in a patient who did not respond to LAM therapy. Direct sequence analysis of the ETV-resistant strain showed appearance of amino acid substitution rtS202G in the reverse transcriptase (RT) domain, together with rtL180M + M204V substitution that had developed at the emergence of LAM-resistant mutant. In vitro analysis demonstrated that the rtL180M + M204V + S202G mutant strain displayed a 200-fold and a 5-fold reduction in susceptibility to ETV compared with the wild-type and the rtL180M + M204V mutant strain, respectively. Adefovir was effective against the ETV-resistant strain both in vitro and during the clinical course. In conclusion, this study showed that virological and biochemical breakthrough due to ETV could occur in patients infected with LAM-resistant HBV and confirmed that the addition of rtS202G substitution to the rtL180M + M204V mutant strain is responsible for ETV resistance and we could treat the resistant mutant successfully. ***J. Med. Virol. 79:1811–1817, 2007.***

KEY WORDS: HBV; rtS202G; lamivudine; adefovir; in vitro

INTRODUCTION

Hepatitis B virus (HBV) is a small enveloped DNA virus known to cause chronic hepatitis and often leads to liver cirrhosis and hepatocellular carcinoma [Bruix and Llovet, 2003; Ganem and Prince, 2004]. To date, interferon and three nucleoside and nucleotide analogs (lamivudine [LAM], adefovir dipivoxil [ADV], and entecavir [ETV]) have been approved for the treatment of chronic HBV infection. Nucleoside and nucleotide analogues suppress HBV replication in most patients and improve transaminase levels and liver histology [Nevens et al., 1997; Lai et al., 1998; Suzuki et al., 1999]. However, prolonged therapy results in the emergence of drug-resistant mutants.

LAM is associated with a higher rate of emergence of drug-resistant mutants than ADV or ETV, which is 24% and 70% after 1 and 4 years of therapy, respectively, followed by increases in viral load and re-elevation of transaminase levels [Lai et al., 2003]. Most LAM-resistant

Abbreviations used: HBeAg,hepatitis B e antigenHBsAg,hepatitis B surface antigenHBV,hepatitis B virusORF,open reading framePCR,polymerase chain reactionRT,reverse transcriptase

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strains show amino acid substitutions in the YMDD (tyrosine–methionine–aspartate–aspartate) motif in the C domain of HBV polymerase. In addition to the emergence of the YMDD mutation, rtL180M and rtV173L mutations in the B domain of HBV polymerase are frequently observed [Allen et al., 1998; Delaney et al., 2003].

Both in vitro and clinical studies have shown recently that ADV and ETV could suppress both wild-type and LAM-resistant strains and were confirmed as salvage therapy for LAM-refractory patients [Levine et al., 2003; Sherman et al., 2006; Rapti et al., 2007]. However, a few studies have already reported the emergence of resistant mutants to these drugs.

ADV-resistant mutations are infrequent and their appearance is delayed in treatment-naïve patients; mutation occurs at 0% after 1 year and 28% after 5 years and the selection of rtA181V/T or rtN236T mutant was associated with resistance to ADV [Maecellin and Asselah, 2005]. On the other hand, the emergence rate of ADV-resistant mutations in LAM-resistant patients was 18% after 48 weeks of ADV monotherapy [Lee et al., 2006]. A recent study reported patients treated with combination therapy of ADV with LAM did not develop resistance to ADV for 3 years [Rapti et al., 2007].

ETV is the most novel nucleotide analogue of the three drugs and displays greater in vitro potency than LAM or ADV against wild-type HBV. ETV-resistance is reported to be rare in treatment-naïve patients [Colonno et al., 2006]. However, ETV-resistant mutants appeared at 6–9% per year in LAM-refractory patients [Tenney et al., 2004, 2007; Sherman et al., 2006].

In the present study, an ETV-resistant strain of HBV was identified after prolonged ETV therapy in a patient who did not respond to LAM therapy. To our knowledge, this is the first report that breakthrough hepatitis was induced by emergence of an ETV-resistant strain and was successfully treated with ADV. This study checked the importance of amino acid substitutions in the HBV polymerase for resistance to ETV in vitro. Furthermore, the susceptibility of the mutant strain to ADV was analyzed.

MATERIALS AND METHODS

Antiviral Compounds

LAM [( )-β-L-2′, 3′-dideoxy-3′-thiacytidine] was provided by GlaxoSmithKline (Stevenage, Herts, UK). Adefovir {9-[2-(phosphonomethoxy)ethyl]-adenine} was provided by Gilead Sciences (Foster City, CA), and ETV {2-amino-1,9-dihydro-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one, monohydrate} was provided by Bristol-Myers Squibb Pharmaceutical Research Institute (Wallingfold, CT).

Analysis of Virological Markers

Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) were determined by enzyme immunoassay kits (Abbot Diagnostics, Chicago, IL). HBV-DNA was measured by real-time PCR using the Light Cycler (Roche, Mannheim, Germany) by the polymerase chain reaction (PCR). The primers used for amplification were 5′-TTTGGGCATGGACATTGAC-3′ and 5′-GGTGAA-CAATGTTCCGGAGAC-3′. The amplification condition included initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 sec, annealing at 58°C for 5 sec and extension at 72°C for 6 sec. The lower detection limit of this assay was 300 copies.

Cloning of HBV-DNA and Plasmid Construction

HBV-DNA was extracted from 100 μl of serum samples by SMITEST (Genome Science Laboratories, Tokyo, Japan) and was dissolved in 20 μl H_2O. The full-length HBV-DNA was amplified using the above HBV-DNA samples by the method of Gunther et al. [1998]. Nucleotide sequence positions were numbered from the unique *Eco*RI site. The 1.4 genome lengths HBV-DNA amplified from the serum of a patient who showed ETV resistance was cloned into a plasmid vector pcDNA3 (Invitrogen, San Diego, CA). In brief, the PCR product amplified using serum from the patient was cleaved with *Bam*HI and *Apa*I (HBV positions 1,400–2,600) and cloned into pcDNA3, which was named pcDNA3-1. Similarly, the PCR product was cleaved with *Apa*I and *Bam*HI (HBV positions 2,600–3,215, 1–1,400) and cloned into pBluerscript SK+ (Stratagene, La Jolla, CA), which was named pB-1. The *Kpn*I-*Bam*HI fragment from pB-1 and *Kpn*I-*Apa*I fragment from pcDNA3-1 were cloned into pcDNA3-1. To introduce the nucleotide substitutions into the rtL180M, M204V, and S202G, site-directed mutagenesis was performed using the QuickChange Site-Directed Mutagenesis kit (Stratagene). Four plasmids with/without amino acid substitutions were created and are listed in Table IV.

Cell Culture, Transfection, and Determination of IC_{50}

HepG2 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum (FBS) at 37°C under 5% CO_2. Cells were seeded to semi-confluence in 6-well tissue culture plates. Transient transfection of the plasmids into HepG2 cell lines was performed using TransIT-LT1 (Mirus, Madison, WI) according to the instructions provided by the supplier. To determine 50% inhibitory concentrations (IC_{50}s) for each anti-viral drug, various concentrations of LAM, ADV, and ETV were added after 24 hr to the culture plate containing the cells, and harvested after 5 days. The medium containing the drugs was changed at days 1, 3, and 4. All experiments were performed in triplicate. GraphPad prism (GraphPad Prism Software, Inc., San Diego, CA) was used to determine the best-fit values for individual dose–response equations.

Analysis of Replicative Intermediate of HBV by Quantitation

The cells were harvested at 5 days after transfection and lysed with 250 μl of lysis buffer (10 mM Tris-HCl [pH

7.4], 140 mM NaCl, and 0.5% (v/v) NP-40) followed by centrifugation for 2 min at 15,000*g*. The core-associated HBV genome was immunoprecipitated by mouse anti-core monoclonal antibody 2A21 (Institute of Immunology, Tokyo) and subjected to Southern blot analysis after SDS/proteinase K digestion followed by phenol extraction and ethanol precipitation. Quantitative analysis was performed by real-time PCR with cyber green using Light Cycler. The HBV-specific primers used for amplification were 5′-TTTGGGCATGGACATTGAC-3′ and 5′-GGTGAACAATGTTCCGGAGAC-3′. The amplification conditions included initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 sec, annealing at 58°C for 5 sec, and extension at 72°C for 6 sec. The lower detection limit of this assay was 300 copies.

Statistical Analysis

Data are expressed as mean SD. Group comparisons were performed using the Student's *t*-test. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Patient's Profile

An ETV-resistant strain of HBV was isolated from a 44-year-old Japanese woman with hepatitis B e antigen-positive chronic HBV infection (Fig. 1A). In this patient, LAM successfully reduced the HBV at the initial stage of treatment. However, viral breakthrough was observed at 11 months after the beginning of LAM therapy and the HBV viral load reached up to 7.5 log copies/ml. After 17 months of LAM, interferon was added to LAM therapy for 6 months. However, after withdrawal of IFN, the viral load and ALT rebounded. Thus, the patient was switched to 0.5 mg of ETV. This resulted in reduction of HBV-DNA and normalization of ALT. After 12 months of ETV therapy, the viral load rebounded, and following 12 more months of ETV, breakthrough hepatitis was observed. After stopping ETV, because of the inadequate effect of IFN monotherapy for one month, the patient was switched to 10 mg of ADV. This treatment reduced both the viral load and ALT level to acceptable levels (Fig. 1).

treatment		month	ALT (IU/L)	HBV-DNA (log copies/ml)
		-3	246	7.2
LAM		0	46	5.2
		5	28	3.7
		11	33	4.1
	IFN	17	72	7.5
		18	1184	5.6
		20	39	3.9
		23	34	3.4
		27	117	7.1
ETV		31	112	7.2
		39	40	2.9
		43	28	4.2
	IFN	56	140	6.8
ADV		57	313	6.8
		60	38	4
	LAM	71	24	3.3
		75	19	3.1

Fig. 1. Clinical course of a patient who developed entecavir resistant mutant.

Isolation of a Multiple Drug-Resistant Hepatitis Strain

Isolates from this patient were analyzed for substitutions in HBV reverse transcriptase (RT). Comparison of the nucleotide sequences by the direct sequence method obtained throughout the clinical course showed three amino acid substitutions in the RT domain of the polymerase (Table I). At the baseline of LAM, all three substitutions were of the wild-type by direct sequence analysis and clonal analysis (Table II). After breakthrough hepatitis induced by LAM, direct sequence analysis showed mixed type (YIDD and YVDD) mutant strain. The rtM204V mutant was detected in 65% of HBV clones and the rest were all the YIDD type. Importantly, at this point, there was no amino acid substitution at rt202. After 12 months of ETV therapy when the viral load was slightly increased, the rtL180M + M204V + S202G mutant was detected in 45% of the HBV clones, followed by decrease of the YIDD and YVDD mutants without substitution at rtS202G. Finally, after 24 months of ETV therapy, when the breakthrough hepatitis occurred, the rtL180M + M204V + S202G mutant was detected in 92% of the HBV clones and the rest were rtL180M + M204V mutants without substitution at rtS202G. Interestingly, the rtM204I + S202G strain never appeared during nucleotide therapy.

Susceptibility of Mutants to Entecavir In Vitro

To analyze the role of the rtL180M, rtG202S, and rtM204V substitutions in ETV resistance, four patient-specific strains were transfected into HepG2 cells (Table III). ETV was added after 24 hr to the culture plate containing the cells, and harvested after 5 days. The core-associated HBV genome was extracted from cells and quantified by real-time PCR. The double amino acid substitutions rtL180M + M204V, which is related to LAM resistance, displayed a 38-fold decrease in susceptibility to ETV compared with the wild-type. Moreover, triple amino acid substitutions rtL180M + M204V + S202G, isolated from the patient