27 Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, Nishioji K, Murakami Y, Kashima K: Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: A retrospective study in 1,148 patients. J Hepatol 1999;30:653–659.
28 Moreno MG, Muriel P: Remission of liver fibrosis by interferon-alpha 2b. Biochem Pharmacol 1995;50:515–520.
29 Tarao K, Takemiya S, Tamai S, Sugimasa Y, Ohkawa S, Akaike M, Tanabe H, Shimizu A, Yoshida M, Kakita A: Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine aminotransferase levels in hepatectomized patients with hepatitis C virus-associated cirrhosis and HCC. Cancer 1997;79:688–694.
30 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.
31 Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M: Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000;132:517–524.
32 Di Bisceglie AM, Carithers RL Jr, Gores GJ: Hepatocellular carcinoma. Hepatology 1998;28:1161–1165.
33 Camma C, Giunta M, Andreone P, Craxi A: Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: An evidence-based approach. J Hepatol 2001;34:593–602.

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Quarterly Review

Locoregional therapy for hepatocellular carcinoma

KENJI IKEDA, HIROMITSU KUMADA

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, with an annual incidence reaching one million new cases.[1,2] In about 90% of patients, HCC is a late complication of cirrhosis.[3] The 5-year incidence of HCC in cirrhosis is 15%-20%.[4,5] The risk of developing HCC has been reported to be 0.5% per year for hepatitis B and 5% per year for hepatitis C.[5-7] Accordingly, the incidence of HCC directly correlates with the epidemiology of the causes of cirrhosis, which is essentially from alcoholic and viral origins.[3] Chronic hepatitis B and C infection appears to be the most important risk factor for HCC. There is, however, a heterogeneous geographical distribution because of its association with chronic viral hepatitis.

In high-risk areas such as Southeast Asia, China and sub-Saharan Africa the prevalence is > 100/100,000 population. Although less common is Western countries, the annual incidence increased from 1.4/100,000 in the period 1976-80 to 2.4/100,000 between 1991 and 1995.[8] In the particular case of the hepatitis C virus (HCV), it takes up to 20 years to develop HCC from chronic viral infection, so that the massive dissemination of this virus in the 1970s and 1980s is now beginning to induce a marked rise in of the incidence of HCC in Western countries. HCC is now emerging as a major health concern for the next decades.[8-11]

The hepatitis B virus has a specific oncogenic action by integration in the host DNA causing chromosomal rearrangements. Another mechanism is inflammation and repeated cycles of necrosis and regeneration associated with chronic inflammation and cirrhosis. Chronic hepatitis C infection, although less prevalent than hepatitis B infection, is another major causative factor for HCC. Alcoholic cirrhosis, hemochromatosis, primary biliary cirrhosis, and autoimmune cirrhosis also increase the risk of developing HCC, with alcohol-induced cirrhosis playing a particularly important role in Western countries.[12,13]

Most patients develop few symptoms while the tumour is small and often present late with multifocal disease. The natural course of HCC is progressive tumor growth compromising hepatic function, intrahepatic metastases and spread to distant sites. In general HCC has a poor prognosis, with a median survival of 3-6 months after the onset of symptoms.[14]

Department of Gastroenterology, Toranomon Hospital, Tokyo, JAPAN
KENJI IKEDA, HIROMITSU KUMADA
Correspondence to: KENJI IKEDA,Department of Gastroenterology, Toranomon Hospital, Tokyo, JAPAN

Nowadays, an increasing number of HCCs are discovered at an early stage because of increasing awareness and screening of asymptomatic patients with cirrhosis.[15] Percutaneous locoregional therapy became a therapeutic option for a small HCC associated with cirrhosis during the last decade, because of poor liver function reserve and a high recurrence rate after surgical resection.

GENERAL CONSIDERATION FOR LOCAL ABLATION THERAPY

A careful clinical, laboratory, and imaging assessment has to be performed on each individual patient by a multidisciplinary term to evaluate eligibility for percutaneous ablation. Cirrhotic patients with a small HCC nodule are candidates for surgery and percutaneous ablation.[16] Multiple percutaneous image-guided therapies currently are available for thermal ablation of localized solid tumors. Thermal sources for these treatment modalities include high-intensity ultrasound, laser, microwave, and radiofrequency.[17,18]

Radiofrequency ablation (RFA) is a safe, predictable, and inexpensive option and has emerged as the thermal modality that most easily can create large volumes of tissue necrosis. The predictability of RFA is adequate to limit collateral damage and complications, however, is limited by biologic and anatomic variability of tissue. The tumor to treat by RFA must be focal, nodular-type lesion. The presence of a clear and easy-to-detect target for needle placement is crucial for the outcome of treatment. Tumor size should be preferentially smaller than 3 to 5 cm in greatest dimension. When using thermal methods of tissue destruction, some additional points are considered. Treatment of lesions adjacent to the gallbladder or to the hepatic hilum risks thermal injury of the biliary tract. Lesions located along the surface of the liver can be considered for thermal ablation, although their treatment requires experienced hands and may be associated with a higher risk of complications. A careful assessment of the coagulation status is mandatory before percutaneous ablation. A prothrombin time ratio (normal time/patient's time) greater than 50% as well as a platelet count higher than 50,000/microliter are required to keep the risk of bleeding at an acceptable low level.

Percutaneous ethanol injection (PEI) is a well-established technique for tumor ablation.[19] PEI induces local tumor necrosis as a result of cellular dehydration, protein denaturation, and chemical occlusion of tumor vessels. It is best administered by using ultrasound guidance because real-time control allows for a faster procedure, precise centering of the needle in the target, and continuous monitoring of the injection. Fine non-cutting needles, with either a single end hole or multiple side holes, are commonly used for PEI. PEI is usually performed under local anesthesia on an out-patient basis. The treatment schedule typically includes a few to several sessions performed once or twice weekly. The number of treatment sessions, as well as the amount of ethanol to inject, may vary greatly according to the size of the lesion, the distribution of the injected ethanol within the tumor, and the compliance of the patient. Several studies have shown that PEI is an effective treatment for small (3 cm or less), nodular-type HCC. HCC nodules have a soft consistency

and are surrounded by a firm cirrhotic liver. Consequently, injected ethanol diffuses within them easily and selectively, leading to complete tumor necrosis in about 70% of the small lesions.[20]

CRYOSURGERY

Cryotherapy was the first technique employed for localized thermal ablation. The use of cryotherapy for treating liver tumors was first described by Cooper in 1963.[21]

Cryotherapy is a method of tumor ablation that uses cooled probes to freeze and destroy areas of tissue measuring up to 8 cm in diameter.[22] The ablative process is carried out by delivering the subfreezing temperature (-20 to -30 degree centigrade) to the target lesion via a penetrating vacuum cryo-probe. The resulting freeze/thaw process causes irreversible cellular damage by different mechanisms. First, the intra- and extracellular ice formation during the freezing process causes direct physical damage by cellular compression, membrane rupture and protein denaturation.[23,24] Second, the freezing process increases the intra- and extra- cellular electrolytes concentration. This, in turn, results in lipoprotein denaturation and, during the thawing process, the highly concentrated cytotoxic free radicals are released. Third, cells are later killed by post-thaw ischemia and infarction as a result of microvascular thrombosis.[25,26]

The procedure is currently predominantly performed intra-operatively, because probes measuring 3 mm in diameter or large are necessary to deliver optimal quantities of liquid nitrogen to the probe tip. Cryo-probes measuring 3-10 mm are usually used. Larger probes tend to produce large areas of tissue destruction (6-8 cm), whereas smaller 3 mm probes produce approximately 3-3.5 cm of tissue destruction. However, as for other methods of minimally-invasive therapy, the proportion of patients who might be usefully treated with this technique is not yet well established. Although cryo-ablation is not seriously considered as a minimally invasive therapeutic option for HCC, several investigators have reported the use of cryoablation for HCC. Zhou et al.[27] reported the use of cryosurgery for the treatment of 60 patients with primary liver cancer, in 1970. Survival at 1, 2, 3, 4, and 5 years was 52%, 34%, 21%, 16% and 11%, respectively. Among the 21 patients with tumor nodules less than or equal to 5 cm in diameter, survival was increased to 76%, 62%, 50.0%, 41% and 38%, respectively. In 1993 Zhou et al.[28] reported a larger series of 113 patients with hepatic cancer, including 107 patients with primary liver cancer and 6 patients with hepatic metastases, who were treated with cryotherapy using similar technique. The 5-and 10-year survival rates were 22% and 8%, respectively, for the 107 with HCC and 49% and 17%, respectively, for the 32 patients with small (< 5 cm) tumors.

LASER THERMAL ABLATION (ILT)

Laser ablation or interstitial laser photocoagulation is another method for inducing thermally mediated coagulation necrosis that has been used for percutaneous tumor ablation. Interstitial laser therapy has been used to treat liver tumors since 1985.[29] Laser is a monochromatic, collimated and coherent radiation with a wavelength of 1024 nm produced by a Nd:YAG generator, which concentrates extremely high energy in small localized areas. It may be transmitted inside the tumor by single or multiple quartz optical fibers inserted through fine needles (21 gauge), thus converting the intense light energy to tissue heating. The laser provides sufficient energy to allow for significant heat deposition surrounding the fiber tip, inducing protein denaturation and cellular death.[30] Thermal profiles have been demonstrated to correlate well with the extent of coagulation necrosis observed histopathologically,[31,32] as well as with ultrasound[32,33] and T1-weighted MR images.[34,35]

Tumors of 1.5-2 cm size can be treated with a single fiber, while larger nodules require the splitting of the laser beam with the insertion of multiple fibers (up to four) by 20 to 21 gauge fine needles, whose precise positioning may be technically difficult. Laser ablation has been mainly used in the treatment of liver metastases,[36-38] while data available on the laser treatment of HCC is limited. Gilliams et al.[39] reported an 86% 1-year survival of 55 patients with colorectal liver metastases and that the mean survival from the detection of metastases was 18 months. Vogl et al.[40] fond that Laser is effective for small liver tumors, with a local control rate of 44% in 1 year. A subsequent study by the same group of authors was performed on 88 patients with colorectal liver metastases.[41] There was a 95% tumor response rate and the mean survival was 35 months. This encouraging result was further supported by a recent study on 676 consecutive patients with malignant liver tumors.[42]

Few studies published to date have long-term follow up of patients. To date, there are few data on the clinical efficacy of laser for HCC.[43-46] Giorgio et al.[43] treated 85 patients with HCC of 1-6.6 cm with one to four laser fibers inserted and single or multiple sessions, obtaining a complete necrosis in 82%. In the study of Pacella et al.[45] 92 patients with HCC of 4 cm were treated with multiple fiber insertion, with an average of 1.3 sessions per tumor. Complete necrosis was obtained in 97%, while a mean follow-up of 25 months showed a local and elsewhere recurrence of HCC in 6% and 49%, respectively. The same investigators treated 30 large HCC (3.5-9.6 cm) by laser thermal ablation followed by segmental transarterial embolization at 30-90 days, obtaining complete tumor necrosis in 90%, with cancer-free survival rate at 1- and 2-years of 74% and 34%, respectively.[46]

Nevertheless, the major limitation of interstitial ablation therapy is the small volume of tumor ablation, although the current new devices may help to overcome this limitation. In practice, laser ablation is not indicated for large liver tumors.

ETHANOL INJECTION

Intra-tumoral injection of ethanol causes dehydration, intracellular coagulation, necrosis, vascular occlusion, and fibrosis of the tumor; it may benefit patients with small tumors and underlying liver disease that limits resectability.

Although there have not been any prospective randomized trials comparing PEI and surgical resection, several series have shown that the long-term outcome of selected PEI-treated patients was similar to that of patients who had undergone resection, with 5-year survival rates of 35-59%.[47-51] A large series from Japan indicated that patients with small (<5 cm) tumors treated with this approach had a 3-year survival rate of 53%. These results are similar to those reported for surgical

resection and are better than those for chemoembolization.[52] Patients with lesions less than 3 cm in diameter had a survival rate of 94% at 1 year, 63% at 3 years, and 29% at 6 years. Survival is better for patients with Child-Pugh stage A or B cirrhosis than for those with stage C. Reported recurrence rates are 29% within 1 year and 63% within 3 year after treatment.[53]

Side effects include mild to moderate pain after needle withdrawal, and fever (>38 degree C°) starting on the day of the procedure and lasting for 2 to 3 days.[53] While PEI is a low-risk procedure, severe complications, including cases of tumoral seeding, have been reported.[54]

The major limitation of PEI, besides the uncertainty of tumor ablation and the long treatment time, is the high local recurrence rate, that may reach 33% in lesions smaller than 3 cm and 43% in lesions exceeding 3cm.[55,56] These high rates of recurrence suggest that this approach treats only the injected lesions and not microscopic or multi-focal liver disease or metastasis.

The injected ethanol does not always accomplish complete tumor necrosis because of its inhomogeneous distribution within the lesion -especially in the presence of intratumoral septa- and the limited effect on extracapsular cancerous spread. Also, PEI is unable to create a safety margin of ablation in the liver parenchyma surrounding the nodule, where satellite nodules are most frequently located.[57]

MICROWAVE COAGULATION (MCT)

Microwave thermal tissue coagulator emits 2,450 MHz electromagnetic radiation. Alternating radiofrequency current agitates ions in the tissue surrounding the needle, creating frictional heat, which denatures and destroys tissue at predictable temperatures, in a relatively predictable volume. Microwave coagulation therapy has been studied mainly in patients with HCC, whereas there are only a few reports of MCT for liver metastasis.[58,59] Percutaneous MCT is applicable for a small HCC (<3 cm).

In an early series, Seki et al.[60] reported complete ablation in 18 patients with small HCC of <2cm, and there was neither any complication nor local recurrence after a short follow-up period of 11-33 months. The response rate of small HCC (<3 cm) to percutaneous MCT is up to 70%, but is only 55% for large HCC (>3 cm).[58] Recently, a larger series has been published, including 50 patients with 107 HCC (mean size 2.7 cm) treated with single or multiple microwave sessions. Complete necrosis was attained in 98% of nodules < 2 cm and in 92% of nodules > 2 cm, with a recurrence rate at 1 year of 45%.[61] Well-differentiated HCC and those superficially located on the liver surface are associated with better prognosis after percutaneous MCT.[58,59,60,62]

Laparoscopic MCT allows effective ablation of large HCC (up to 5 cm) on the liver surface, as it can be safely performed under direct visual guidance as well as with laparoscopic ultrasonography.[63] Using minimally invasive surgery techniques, Seki et al.[64] advocated laparoscopic MCT under local anesthesia and reported complete tumor ablation in 87.5% of patients with small HCC (mean size=2 cm). Another endoscopic technique of MCT using the thoracoscopic trans-diaphragmatic approach has been suggested by Yamashita et al.[65] to treat HCC located just below the diaphragmatic dome, for which percutaneous MCT is impossible and open MCT requires a large incision. Open MCT can be used to ablate large HCC (maximum of 6.5 cm) and tumors whose location is unfavorable for percutaneous or laparoscopic MCT.[66] However, open MCT is contraindicated in tumors at the liver hilum or close to the diaphragm, as injury to bile ducts and/or the diaphragm is possible.

Although the short-term efficacy of MCT appears to be encouraging, there are limited reports on long-term survival. The overall 3-year survival rate reported was 73-86%.[61,67,68] In addition, Itamoto et al.[68] reported a 48.6% overall 5-year survival rate and a 50% overall 4-year survival rate for patients with primary and recurrent HCC, respectively. Disease-free survival depends on local as well as distant tumor recurrence.

The complications of microwave coagulation therapy include abscess formation, biloma, bleeding, hepatic failure and intraperitoneal dissemination of cancer cells.[59,69]

Shimada et al.[59] found that the complication rate in microwave therapy was significantly higher in patients with large (>4 cm) and more advanced tumors and the authors recommended MCT be reserved for liver tumors < 4 cm. With the available technology, microwave ablation seems to be effective in tumors of 2 cm, but requires the insertion of multiple large needles and repeated treatment sessions in case of tumors more than 2.5 cm in diameter.

RADIOFREQUENCY ABLATION

Radiofrequency ablation (RFA) is a relatively new technology that allows for focal coagulation necrosis of hepatic tumors by producing thermal energy with an alternating electric current generator at a radiofrequency of 200 to 1,200 kHz. Percutaneous thermal tissue ablation using radiofrequency current is performed, by keeping the patient in an electrical circuit with adhesive grounding pads on the thighs or back. A needle with a plastic-insulated shaft is usually placed into the tumor percutaneously with imaging guidance, under local anesthesia. It may also be used at laparoscopy or at laparotomy. Complete ablation can usually be achieved in one to two sessions.

Major progress in RF technology was achieved with the introduction of modified electrodes. The devices most frequently used are made by three companies: Radiotherapeutics, Sunnyvale, Calif.; RITA Medical Systems, Mountain View, Calif.; and Radionics, Burlington, Mass.[70,71] Each of the devices uses a different needle design, watt and algorithms. The first two devices use an expandable electrode, which, once positioned in the tumor, opens out into seven to twelve retractable, curved electrodes around the target like an umbrella. The technique determines a reproducible area of necrosis approximately 3-4 cm in diameter. The third device utilizes a cold perfusion electrode with a diameter of 1.2 mm and the tip exposed for 2-3cm.[70,71] By avoiding early increments of impedance linked to carbonization, such electrodes permit application of a greater power with respect to conventional electrodes. To obtain cooling, a physiological solution is circulated within two coaxial lumens situated in the electrode. The technique determines a reproducible area of necrosis of 2 to 4 cm.[72]

A recently constructed electrode with three cooled tips,

permitting a higher current deposition, determines more than 4.5 cm of coagulation necrosis.[73]

To obtain a large and effective ablation area, Kobayashi et al.[74] arranged an algorithm of tumor heating with expandable electrodes. To increase the final necrotic area obtained with the aforementioned techniques, interruption of the tumor arterial supply by means of occlusion of either the hepatic artery with a balloon catheter or the feeding arteries with gelatin sponge particles was recently proposed.[75] These techniques enabled a substantial and reproducible enlargement of the volume of thermal necrosis produced with a single needle insertion, and prompted the start of clinical application of RF ablation.

Several results of early experiences and pilot studies of RFA therapy for HCC were published from 1998 to 2001[76-83] (Table 1). HCCs of 3cm or less were principal candidates for the therapy and almost all authors described a complete response rate of more than 90% after ablation. The majority of these recurrences could be treated by repeated RFA. Livraghi et al.[84] compared the effectiveness of RFA with that of PEI in 112 patients with HCC less than 3 cm. With RFA, complete necrosis was achieved in 90% of tumors (mean, 1.2 sessions), versus 80% complete necrosis (mean 4.8 sessions) for PEI. Microwave has also been compared with radiofrequency ablation in a randomized controlled trial including 94 HCC of 1-3.7 cm in size, reporting equivalent therapeutic effects and complication rates, but fewer treatment sessions for radiofrequency.[85]

The mortality rate following hepatic RFA is less than 1% in the published reports. The morbidity rates associated with hepatic RFA are generally low. Major treatment-related complication arises in only 2% of all patients undergoing RFA.[76-83] Major complications following RFA are frequently associated with thermal injury to bile duct and surrounding structures, including the colon, stomach, and diaphragm, hemorrhage requiring surgical intervention, and portal vein thrombosis. Complication due to bile duct induces varied degrees of biliary tree dilation, bile duct fistula, jaundice, biloma, and liver abscess. Table 2 summarizes the case reports about major and minor complications of RFA therapy for liver malignancy.[86-107] Rhim et al.[95] described an incidence of major complications (2.4%) found in 1139 patients in a multicenter study. The most common complications were hepatic abscess (0.66%), peritoneal hemorrhage (0.46%), biloma (0.20%), ground pad burn (0.20%), pneumothorax (0.20%), and vasovagal reflex (0.13%). Livraghi et al. [108] also reported details of complications encountered in a total of 2320 patients with RFA therapy performed in 41 Italian hospitals: Six deaths (0.3%) were noted, including two caused by multiorgan failure following intestinal perforation; one case each of septic shock following Staphylococcus aureus-peritonitis, massive hemorrhage following tumor rupture, liver failure following stenosis of right bile duct; and one case of sudden death of unknown cause, 3 days after the procedure. Fifty (2.2%) patients had additional major complications. The most frequent of these were peritoneal hemorrhage, neoplastic seeding, intrahepatic abscesses, and intestinal perforation.

COMBINED THERAPY AND LAPAROSCOPIC APPROACH

Although recent clinical studies have shown success in the use of these strategies for single HCC less than 3 cm in diameter, it is clear that further developments are necessary to achieve complete eradication in larger diameter tumors. Similarly, different minimally invasive strategies are better suited for varying disease presentations. While percutaneous therapies are more effective for single tumors, embolization or transarterial chemotherapy is more appropriate for multifocal disease. Therefore, combining several modalities of treatment to achieve complete tumor cell death must not be neglected. A similar, multidisciplinary approach including surgery, radiation, and chemotherapy should also be applied for the treatment of the tumor. Several investigators have explored combination therapies to achieve successful treatment results in primary liver malignancies, including various arrangements of PEI, thermal ablation, transcatheter arterial chemotherapy, vascular occlusion, and chemoembolization.

Table I. Efficacy of radiofrequency ablation therapy for hepatocellular carcinoma

Author (ref)	Year	No. of patient	Liver tumor	Tumor size	Needle device	Observation period	Complete Necrosis (%)	Major Complicatin (%)
Rossi [76]	1998	23	HCC	23-35mm	expandable	15 M	90	0
Livraghi[77]	1999	42	HCC	< 30 mm	cooled tip	10 M	98	2.4
Curley[78]	1999	48	HCC	< 30 mm	expandable	15 M	98	0.8
Francica[79]	1999	15	HCC	10-43mm	cooled tip	15 M	90	6.7
Jiao[80]	1999	8	HCC+Meta*	6pts>3cm	cooled tip	9.4 M	88	0
Allgaier[81]	1999	12	HCC	3.2 ± 1.3cm	cooled tip	4.8 M	100	0
Lencioni[82]	1999	54	HCC	1-3cm	both	23 M	91	0
Llovet[83]	2001	32	HCC	<5cm	cooled tip	10 M	65(76% for< 3cm)	9.4

*Meta – Metastasis
M - Months

Table II. Case reports of complications after radiofrequency ablation therapy for liver tumor.

Complications	(Reference)
Major complications	
Hemobilia, intrahepatic hematoma	(86)
Rapid progression of HCC	(87)
Tumor seeding	(88)
Bleeding requiring transfusion	(89)
Hepatic abscess	(89)
Colonic perforation	[91]
Bacterial peritonitis	[92]
Intrahepatic pseudoaneurysm	[93]
Portal vein thrombosis	[94]
Biloma	[95]
Secondary hemocholecyst	[96]
Diaphragmatic perforation	[97]
Hypertensive crises	[98]
Bilioenteric anastomosis	[99]
Acute renal insufficiency	[100]
Abdominal wall necrosis	[101]
Minor complications	
Arteriovenous shunt	[102]
Perihepatic hemorrhage	[102]
Pneumothorax	[102]
Skin injury	[103]
Hyperkalaemia	[104]
Hemolysis	[105]
Elevation of body temperature	[106]
Sarcomatous change	[107]
Hemoperitoneum	[100]

Combination of Laser Thermotherapy and Arterial Chemoembolization

Laser thermotherapy is a local effective therapy with low morbidity for a few numbers of HCC of 5cm or less in diameter.[109] The radionale for combination of transcather arterial chemoembolization (TACE) and laser ablation is based on the fact that laser therapy can reduce the volume of viable tissue and improve the lesion within the range of TACE effectiveness. Pacella et al.[110] achieved complete response with a single segmental TACE session in 21 (70%) of the 30 patients and reported that the 1 year local recurrence rate was 7% in large HCC. Laser thermotherapy seems to be more beneficial and advisable in combination with TACE for treating patients with relatively larger and multiple HCCs.

Combination of TACE and PEI

Allgaier et al.[111] treated 132 HCC patients with combination TACE/PEI to achieve a median survival of 25 months. Similarly, Bartolozzi et al.[112] treated 86 patients with single HCC tumors (mean diameter, 5.3 cm) with TACE followed by PEI (mean, 6.8 sessions) to achieve complete necrosis in 82%. Overall 1-, 3-, and 5-year survival rates were 92%, 69%, and 47% respectively. A few authors also reported significant increases in survival with combination therapy over TACE alone.[113,114] In 97 patients with recurrent HCC after surgical treatment, Ishii et al.[113] reported a relative risk of cancer death of 0.32 for patients receiving combination therapy. Koda et al.[115] reported reduced local recurrence and lower incidences of new intrahepatic disease in patients receiving combination TACE/PEI. Embolization alone combined with PEI also demonstrated increases in complete necrosis (20-83%) and better 1- and 3-year survival over TAE alone.[116] Combined TACE and PEI is a therapeutic option that has been proposed to overcome the weakness of each of the two procedures in the treatment of large HCC.[115,117,118]

Combination of TACE and MCT

The combined therapy of MCT applied within 1-2 days of TACE can effectively treat HCC>2.0 cm but < 3.0 cm in dimension. Less number of microwave electrode insertions and lower amount of energy for microwave irradiations are needed when both treatment are combined.[119] Ishikawa et al. suggested that MCT destroyed the peripheral part of the tumour that might remain viable after TAE, but combination therapy with TACE is preferable, especially when a viable part existed within tumours. [120] However, larger scale clinical trials are required to define the role of this combined therapy.

Combination of TACE and RFA

RFA achieves complete tumour necrosis for small HCC ($\leq$3.5 cm in diameter) with fewer treatment sessions compared with PEI, and can also create large volumes of tumour necrosis in a shorter period of time than either laser or microwave therapy.

The combination of TACE and RFA induces larger coagulation necrosis areas than RFA alone. Buscarini et al.[121] treated 14 patients with HCC (3.8-6.8 cm; mean diameter, 5.2 cm) with hepatic segmental transcatheter arterial embolization followed by RF ablation. Mean follow-up lasted 13.2 months with 11 patients disease-free at the time of reporting, indicating that larger hepatomas could be treated with this combination of therapies. Bloomston et al.[122] reported that one-year survival was greater in patients undergoing TACE and RFA than TACE alone (100% vs. 67%, P=0.04). Mean survival was longer after TACE with RFA compared with TACE alone (25.3 months $\pm$15.9 vs. 11.4 months $\pm$ 7.3, P<0.05). No patients suffered significant complications in that study. Similarly, Lencioni et al.[123] reported success in 82% of patients with HCCs (diameter, 3.5-8.5 cm) treated with TAE before RF ablation.

For multifocal recurrence, RFA can be useful as a complementary technique for lesions not completely treated by TACE.[124] Goldberg et al.[125] conducted a pilot study in 10 patients with liver tumors, including 4 patients with HCC, and were able to attain 25% to 30% increases in coagulation volume by administering liposomal doxorubicin 24 hours before RF application. More importantly, follow-up imaging studies demonstrated that this particular from of adjuvant therapy resulted in more complete tumor kill as coagulation progressed over time to include residual tumor foci and patent intratumoral blood vessels.

Laparoscopic Approach

The percutaneous approach is least invasive, carries a lower morbidity and complication rate, and is cheapest and most widely used. In the radiology department US, CT, MR guidance or a combination of these approaches can be used. The laparoscopic approach has been used when tumor is adherent

to structures that would be damaged by thermal ablation e.g. tumour adherent to stomach, colon or duodenum. Some centers prefer the laparoscopic approach where there is poor tumor visualization transcutaneously and also for large hepatocellular carcinoma requiring multiple punctures.[126,127] A study that failed selection criteria[128] reported that laparoscopic ultrasonography detected unsuspected extrahepatic disease in 12% and previously unidentified hepatic lesions in 33% of patients. Consistent with this observation, studies of percutaneous radiofrequency ablation without laparoscopic ultrasonography with 10 months' follow-up, reported relapses elsewhere in the liver for 24% to 38% of patients. Data were insufficient to compare outcomes of laparoscopic or open approaches to those of percutaneous ablation. Comparisons between approaches should be made on the basis of "intention to treat".

EVALUATION OF TREATMENT EFFECTS

Contrast-enhanced CT and dynamic MRI are regarded as reliable modalities for evaluation of early responses after radiofrequency ablation and early detection of tumor recurrences.[129-132]

Findings of plain CT show the area after ablation as a low-density area occupying the entire volume of original tumor. In an HCC associated with cirrhosis, radiofrequency heat may be concentrated within a well-encapsulated tumor, and therefore, a successful radiofrequency ablation area tends to be the same size as the original tumor. On contrast-enhanced CT, the ablation area is expected to be nonenhancing. However, a recent ablation area may have an enhancing rim related to hyperemia from thermal injury.[129-131] This is more typically present on the arterial dominant phase but may be present on the portal dominant phase or both phases. Discrete nodular noncircumferential enhancement, especially at the ablation margin, is suspicious for residual or recurrent tumors. Differentiation of reactive hyperemia from residual tumors is often difficult.

The characteristic MRI signals of coagulation necrosis after RFA are intermediate to high signal-to-liver parenchyma on T1-weighted and low signal on T2-weighted images. A T2 hyperintense rim around the ablation area is a possible finding, likely related to edema when thermal ablation is performed. Any discrete areas of T1-hypointense and T2-hyperintense signal should raise the possibility of residual or recurrent tumor. However, a recent ablation area may have heterogeneous signal on both T1- and T2-weighted images because of non-uniform evolution of inflammation and necrosis,[130] resulting in difficulty in the interpretation of unenhanced MRI. Gadolinium-enhanced MRI is therefore routinely used to maximize the accuracy of the study.

Dromain et al.[133] reported a higher sensitivity in early detection of local recurrence on MRI than on CT but without significant differences. A baseline study should be obtained within the first week after the procedure. Subsequent follow-up should be performed every 3 months for 1 year, and every 6 months thereafter. In equivocal cases, follow-up may be more frequently performed.

Lesions adjacent to major vessels have a higher risk of incomplete ablation because of a "heat sink" effect.[134] Because radiofrequency heat cannot easily traverse vessels, the ablation extent is usually limited by major vessels and may not provide the desired ablated margin.

Evaluation of long-term follow-up imaging (>6 months) is generally easier than after ablation, because of the resolution of inflammation. On CT, the RF ablation areas and tracts become sharp and decrease in size, without arterial enhancement. Signs of tumor recurrence include development of noncircumferential nodular enhancement and increase in lesion size. On MRI, the ablation area shows more homogeneous T1 hyperintense and T2 hypointense signal. Signs of recurrence include new enhancement, increase in size of lesion, and development of T1 hypointense and T2 hyperintense signal areas.

FUTURE PERSPECTIVE OF LOCAL ABLATION THERAPY

Recent development of locoregional ablative therapies has expanded the range of tools for treating HCC. The main characteristic of these therapies is the localized tumor destruction in situ, with maximal preservation of non-cancerous part of liver parenchyma, in contrast to the significant liver damage caused by other interventional therapies, such as TACE and intra-arterial chemotherapeutic infusion.

Although a complete tumor ablation rate of over 90% was achieved with RFA, the efficacy of the therapy should be critically assessed with randomized controlled trial to compare it with other local regional therapies or even surgery.

Future studies from the technical viewpoint should be focused on (1) the development of optimal ablation techniques that can increase the volume of tissue destroyed, (2) varied efforts to reduce side effects (most favorable analgesic therapy, avoidance of biliary tree complication), (3) the assessment of efficacy of multimodal and combined treatment, and (4) the development of new and less invasive ablation modality such as extracorporeal high intensity focused ultrasound. Furthermore, chemopreventive therapy should be established to decrease hepatocellular carcinogenesis rate in chronic liver diseases and to reduce recurrence after locoregional therapy.

REFERENCES

1. Di Bisceglie AM, Rustgi VK, Hoofnagle JH, et al. NIH conference. Hepatocellular carcinoma. Ann Intern Med 1998;108:390-401.
2. Watkins KT, Curley SA: Liver and bile ducts. In: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, editors. Clinical oncology, 2nd edn. New York: Churchill Livingstone; 2000. pp1681-1748.
3. Shafer DF, Sorrell MF. Hepatocellular carcinoma. Lancet 1999;353:1253-57.
4. Bruix J. Treatment of hepatocellular carcinoma. Hepatology 1997;25:259-62.
5. Ikeda K, Saitoh S, Koida I, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis —— A prospective observation of 795 cases with viral and alcoholic liver cirrhosis. Hepatology, 1993;18: 47-53.
6. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1998;61:1942-56.
7. Di Bisceglie, Simpson LH, Lotze MT, et al. Development of hepatocellular carcinoma among patients with chronic liver disease due to hepatitis C viral infecton. J Clin Gastroenterol 1994;19: 222-6.
8. El Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745-50.

9. Taylor-Robinson SD, Foster GR, Arora S, et al.: Increase in primary liver cancer in the UK, 1979-1994. Lancet 1997;350:1142-43.
10. Deuffic S, Poynard T, Buffat L, et al.: Trends in primary liver cancer. Lancet 1998;351:214-15.
11. Everson GT. Increasing incidence and pretransplantation screening of hepatocellular carcinoma. Liver Transplant 2000;6:S2-S10.
12. Borzio M, Bruno S, Roncalli M, et al. Liver cell dysplasia is a major risk factor for hepatocellular carcinoma in cirrhosis: a prospective study. Gastroenterology 1995;108:812-7.
13. Colombo M, de Franchis R, Del NE, et at. Hepatocellular carcinoma in Italian patients with cirrhosis. N Engl J Med 1991;325:675-80.
14. Farmer DG, Rosove MH, Shaked A, Busuttil RW. Current treatment modalities for hepatocellular carcinoma. Ann Surg 1994;219: 236-47.
15 Bismuth H, Majno P, Adam R. Hepatocellular carcinoma: from ethanol injection to liver transplantation. Acta Gastroenterol Belg 1999;62:330-41.
16 Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999;19:329-38.
17. DeSanctis JT, Goldberg SN, Mueller PR. Percutaneous treatment of hepatic neoplasms: a review of current techniques. Cardiovasc Intervent Radiol 1998; 21:273-96.
18. Scudamore CH, Patterson EJ, Shapiro J, Buczkowski AK. Liver tumor ablation techniques. J Invest Surg 1997;10:157-64.
19. Bartolozzi C, Lencioni R. Ethanol injection for the treatment of hepatic tumours. Eur Radiol 1999;6:682-96.
20. Shina S, Tagawa K, Unuma T, et al. Percutaneous ethanol injection therapy for hepatocellular carcinoma: a histopathologic study. Cancer 1991;68:1524-30.
21. Cooper Is. Cryogenic surgery: a new method of destruction or extirpation of benign or malignant tissue. N Engl J Med 1963;268: 743-9.
22. de Sio I, Castellano L, De Girolamo V, et al. Tumor dissemination after radiofrequency ablation of hepatocellular carcinoma. Hepatology 2001;34: 609-10.
23. Bischor JC, Rubinsky B. Large ice crystals in the nucleus of rapidly frozen liver cells. Cryobiology 1993;30:597-603.
24. Whittaker DK. Mechanisms of tissue destruction following cryosurgery. Ann R Coll Surg Engl 1984;66:313-18.
25. Brown NJ, Bayjoo P, Reed MW. Effect of cryosurgery on liver blood flow. Br J Cancer 1993;68:10-12.
26. Rubinsky B, Lee Cy, Bastacky J, Onik G. The process of freezing and the mechanism of damage during hepatic crysurgery. Cryobiology 1990;27:85-97.
27. Zhou XD, Tang ZY, Yu YQ, Ma ZC.Clinical evaluation of cryosurgery in the treatment of primary liver cancer. Report of 60 cases. Cancer. 1988;61:1889-92.
28. Zhou XD, Tang ZY, Yu YQ, et al. The role of cryosurgery in the treatment of hepatic cancer: a report of 113 cases. J Cancer Res Clin Oncol. 1993;120:100-2.
29. Hashimoto D, Takami M, Ideezuki Y. In depth radiation therapy by Nd: YAG laser for malignant tumors of the liver under ultrasonic imaging. Gastroenterology 1985:88:A1663.
30. Brown S. Laser-tissue interactions. In: Krasner N, ed. Lasers in gastroenterology. London: Chapman & Hall Medical, 1991: 37-50.
31. Dechma AH, McGehee JA, Beam TE, et al. US-guided percutaneous laser ablation of liver tissue in a chronic pig model. Radiology 1990;176:129-33.
32. Jiao LR, Hansen PD, Havlik R, et al. Clinical short-term results of radiofrequency ablation in primary and secondary liver tumors. Am J Surg 1999;177:303-6.
33. Nolsoe CP, Torp-Pedersen S, Burcharth F, et al. Interstitial hyperthermia of colorectal liver metastases with a US-guided Nd-YAG laser with a diffuser tip: a pilot clinical study. Radiology 1993;187:333-7.
34. Diederich CJ, Nau WH, Deardorff DL. Prostate thermal therapy with interstitial and transurethral ultrasound applicators: a feasibility study. In: Thomas P. Ryan, ed. Surgical Applications of Energy, Proceedings of SPIE Vol. 3249. 1998:2-13.
35. Vogl TJ, Muller PK, Hammerstingl R, et al. Malignant liver tumors treated with MR imaging-guided laser-induced thermotherapy: technique and prospective results. Radiology 1995;196:257-65.
36. Amin A, Donald JJ, Masters A, et al. Hepatic metastases: interstitial laser photocoagulation with real-time US monitoring and dynamic CT evaluation of treatment. Radiology 1993;187:339-47.
37. Schroder T, Castren-Persons M, Lehtinen A, Taavitsainen M. Percutaneous interstitial laser hyperthermia in clinical use. Ann Chir Gynaecol 1994:83:286-90.
38. Masters A, Steger AC, Lees WR, et al. Interstital laser hypertheramia: a new approach for treating liver metastases. Br J Cancer 1992;66: 518-22.
39. Gilliams AR, Broker J, Hare C. Follow-up of patients with metastatic liver lesions treated with interstitial laser therapy. Br J Cancer 1997;76:31.
40. Vogl TJ, Muller PK, Hammerstingl R, et al. Malignant liver tumors treated with MR imaging-guided laserinduced thermothrapy: technique and prospective results. Radiology 1995;196:257-65.
41. Vogl TJ, Mack MG, Straub R, et al. Magnetic resonance imaging guided abdominal interventional radiology: laser-induced thermotherapy of liver metastases. Endoscopy 1997;29:577-83.
42. Vogl TJ, Eichler K, Straub R, et al. Laser-induced thermotherapy of malignant liver tumors: general principals, equipment(s), procedure(s), side effects, complication and results. Eur J Ultrasound 2001;13:117-27.
43. Giorgio A, Tarantino L, de Stefano G, et al. Interstital laser photocoagulation under ultrasound guidance of liver tumors: results in 104 treated patients. Eur J Ultrasound 2000;11:181-8.
44. Christophi C, Muralidharan V. Treatment of hepatocellular carcinoma by percutaneous laser hyperthermia. J Gastroenterol Hepatol 2001;16:548-52.
45. Pacella CM, Bizzarri G, Magnolfi F, et al. Laser thermal ablation in the treatment of small hepatocellular carcinoma: results in 74 patients. Radiology 2001;221:712-20.
46. Pacella CM, Bizzarri G, Cecconi P, et al. Hepatocellular carcinoma: long-term results of combined treatment with laser thermal ablation and transcatheter arterial chemoembolization. Radiology 2001;219:669-78.
47. Castells A, Bruix J, Fuster J, et al. Treatment of small hepatocellular carcionoma in cirrhotic patients: a cohort study comparing surgical resection and percutaneous ethanol injection. Hepatology 1993;18:1121-6.
48. Lencioni R, Bartolozzi C, Caramella D, et al. Treatment of small hepatocellular carcinoma with percutaneous ethanol injection. Analysis of prognostic factors in 105 Western patients. Cancer 1995;76:1737-46.
49. Livraghi T, Giorgio A, Marin G, et al. Hepato cellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. Radiology 1995;197:101-8.
50. Yamamoto J, Okada S, Shimada K, et al. Treatment strategy for small hepatocellular carcinoma: comparison of long-term results after percutaneous ethanol injection therapy and surgical resection. Hepatology 2001;34:707-13.
51. Shina S, Teratani T, Obi S, et al. Nonsurgical treatment of hepatocellular carcinoma: from percutaneous ethanol injection therapy and percutaneous microwave coagulation therapy to radiofrequency ablation Oncology 2002;62 Suppl 1:64-8.
52. Arii S, Okamoto E, Imamura M. Registries in Japan: Current status of hepatocellular carcinoma in Japan. Liver Cancer Study Group of Japan. Semin Surg Oncol 1996;12:204-11.
53. Ohto M, Yoshikawa M, Saisho H, et al. Nonsurgical treatment of hepatocellular carcinoma in cirrhotic patients. World J Surg 1995;19:42-6.
54. Ishii H, Okada S, Okusaka T, et al. Needle tract implantation of hepatocellular carcinoma after percutaneous ethanol injection. Cancer 1998;82:1638-42.
55. Khan KN, Yatsuhashi H, Yamasaki K, et al. Prospective analysis of risk factors for early intrahepatic recurrence of hepatocellular carcinoma following ethanol injection. J Hepatol 2000:32: 269-78.
56. Koda M, Murawaki Y, Mitsuda A, et al. Predictive factors for intrahepatic recurrence after percutaneous ethanol injection

therapy for small hepatocellular carcinoma. Cancer 2000;88: 529-37.
57. Okusaka T, Okada S, Ueno H, et al. Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. Cancer 2002;95:1931-7.
58. Matuskawa T, Yamashita Y, Arakawa A, et al. Percutaneous microwave coagulation therapy in liver tumors. A 3-years experience. Acta Radiol 1997; 38:410-15.
59. Shimada S, Hirota M, Beppu T, et al. Complications and management of microwave coagulation therapy for primary and metastatic liver tumors. Surg Today 1998;28:1130-7.
60. Seki T, Wakabayashi M, Nakagawa T, et al. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. Cancer 1994;74:817-25.
61. Lu MD, Chen JW, Xie XY, et al. Hepato cellular carcinoma: US-guided percutaneous microwave coagulation therapy. Radiology 2001;221:167-72.
62. Ohmoto K, Miyake I, Tsuduki M, et al. Percutaneous microwave coagulation therapy for unresectable hepatocellular carcinoma. Hepatogastroenterology 1999;46:2894-900.
63. Abe T, Shinzawa H, Wakabayashi H, et al. Value of laparoscopic microwave coagulation therapy for hepatocellular carcinoma in relation to tumor size and location. Endoscopy 2000;32:598-603.
64. Seki S, Sakaguchi H, Kadoya H, et al. Laparoscopic microwave coagulation therapy for hepatocellular carcinoma. Endoscopy 2000;32:591-7.
65. Yamashita Y, Sakai T, Maekawa T, et al. Thoracoscopic transdiaphragmatic microwave coagulation therapy for a liver tumor. Surg Endosc 1998;12:1254-8.
66. Hamazoe R, Hirooka Y, Ohtani S, et al. Intraoperative microwave tissue coagulation as treatment for patients with nonresectable hepatocellular carcinoma. Cancer 1995;75:794-800.
67. Yamanaka N, Tanaka T, Oriyama T, et al. Microwave coagulonecrotic therapy for hepatocellular carcinoma. World J Surg 1996;20:1076-81.
68. Itamoto T, Katayama K, Fukuda S, et al. Percutaneous microwave coagulation therapy for primary or recurrent hepatocellular carcinoma:long-term results. Hepatogastroenterology 2001;48:1401-5.
69. Sato M, Tokui K, Watanabe Y, et al. Generalized intraperitoneal seeding of hepatocellular carcinoma after microwave coagulation therapy: a case report. Hepatogastroenterology 1999;46:2561-4.
70. Gazelle GS, Goldberg SN, Solbiati L, Livraghi T. Tumor ablation with radio-frequency energy. Radiology 2000; 217:633-46.
71. Mc Gahan PJ, Dodd DG.. Radio-frequency ablation of the liver: current status. Am J Radiol 2000;176:3-16.
72. Goldberg SN, Gazelle GS, Solbiati L, et al. Radio-frequency tissue ablation: increased lesion diameter with a perfusion electrode. Acad Radiol 2000;3:636-44.
73. Goldberg SN, Solbiati L, Hahn PE, et al. Large volume tissue ablation with radio-frequency by using a clustered, internally cooled electrode technique: laboratory and clinical experience in liver metastases. Radiology 1998;209: 371-79.
74. Kobayashi M, Ikeda K, Someya T, et al. Stepwise hook extension technique for radiofrequency ablation therapy of hepatocellular carcinoma. Oncology. 2002; 63:139-44.
75. Rossi S, Garbagnati F, Lencioni R, et al. Percutaneous radio-frequency ablation of nonresectable hepatocellular carcinoma after occlusion of tumor blood supply. Radiology 2000;217: 119-26.
76. Rossi S, Buscarini E, Garbagnati F, et al. Percutaneous treatment of small hepatic tumours by an expandable RF needle electrode. Am J Roentgenol 1998;170:1015-22.
77. Livraghi T, Goldberg SN, Lazzaroni S, et al. Small hepatocellular carcinoma: treatment with radiofrequency ablation versus ethanol injection. Radiology 1999;210:655-61.
78. Curley SA, Izzo F, Delria P, et al. Radiofrequency ablation of unresectable primary and metastatic hepatic malignancies: results in 123 patients. Ann Surg 1999;230:1-8.
79. Francica G, Marone G. Ultrasound-guided percutaneous treatment of hepatocellular carcinoma by radiofrequency hyperthermia with a "cooled-tip needle": a preliminary clinical experience. Eur J Ultrasound 1999;9:145-53.
80. Jiao LR, Hansen PD, Havlik R, et al. Clinical short-term results of radiofrequency ablation in primary and secondary liver tumor. Am J Surg 1999; 177:303-06.
81. Allgaier HP, Deibert P, Zuber I, et al. Percutaneous radiofrequency interstitial thermal ablation of small hepatocellular carcinoma. Lancet 1999;353:1676-77.
82. Lencioni R, Cioni D, Donati F, et al. Percutaneous treatment of small hepatocellular carcinoma in cirrhosis: RF thermal ablation vs. PEI. A prospective randomized trial. Radiology 1999;213:123.
83. Llovet JM, Vilana R, Concepcio B. Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. Hepatology 2001;33:1124-29.
84. Livraghi T, Goldberg SN, Lazzaroni S, et al. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. Radiology 1992;210:655-61.
85. Shibata T, Iimuro Y, Yamamoto Y, et al. Small hepatocellular carcinoma: comparison of radio-frequency ablation and percutaneous microwave coagulation therapy. Radiology 2002;223:331-7.
86. Francica G, Marone G, Solbiati L, et al. Hemobilia, intrahepatic hematoma and acute thrombosis with cavernomatous transformation of the portal vein after percutaneous thermoablation of a liver metastasis. Eur Radiol. 2000;10:926-9.
87. Seki T, Tamai T, Ikeda K, et al. Rapid progression of hepatocellular carcinoma after transcatheter arterial chemoembolization and percutaneous radiofrequency ablation in the primary tumour region. Eur J Gastroenterol Hepatol. 2001;13:291-4.
88. Llovet JM, Vilana R, Bru C, et al.; Barcelona Clinic Liver Cancer (BCLC) Group. Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. Hepatology. 200;33:1124-9.
89. Zagoria RJ, Chen MY, Shen P, Levine EA. Complications from radiofrequency ablation of liver metastases. Am Surg. 2002;68: 204-9.
90. Kim RY, Weintraub JL, Susman J, Haskal ZJ. Radiofrequency ablation for hemobilia secondary to hepatocellular carcinoma. J Vasc Interv Radiol. 2002;13:317-20.
91. Meloni MF, Goldberg SN, Moser V, et al. Colonic perforation and abscess following radiofrequency ablation treatment of hepatoma. Eur J Ultrasound. 2002;15:73-6.
92. Lucey BC, Maher MM, Gervais DA, Mueller PR. Bacterial peritonitis: a postulated cause of death associated with hepatic radiofrequency ablation. Clin Radiol. 2002;57:765-7.
93. Tamai F, Furuse J, Maru Y, Yoshino M. Intrahepatic pseudoaneurysm: a complication following radio-frequency ablation therapy for hepatocellular carcinoma. Eur J Radiol 2002;44: 40-3.
94. Ng KK, Lam CM, Poon RT, Fan ST. Portal vein thrombosis after radiofrequency ablation for recurrent hepatocellular carcinoma. Asian J Surg. 2003;26:50-3; discussion 54.
95. Rhim H, Yoon KH, Lee JM, et al. Major complications after radio-frequency thermal ablation of hepatic tumors: spectrum of imaging findings. Radiographics. 2003;23:123-34; discussion 134-6.
96. Yamamoto T, Kubo S, Hirohashi K, et al. Secondary hemocholecyst after radiofrequency ablation therapy for hepatocellular carcinoma. J Gastroenterol. 2003;38:399-403.
97. Koda M, Ueki M, Maeda N, Murawaki Y. Diaphragmatic perforation and hernia after hepatic radiofrequency ablation. Am J Roentgenol. 2003;180:1561-2.
98. Onik G, Onik C, Medary I, et al. Life-threatening hypertensive crises in two patients undergoing hepatic radiofrequency ablation. Am J Roentgenol 2003; 181:495-7.
99. Bessoud B, Doenz F, Qanadli SD, et al. Enterobiliary fistula after radiofrequency ablation of liver metastases. J Vasc Interv Radiol 2003;14:1581-4.
100. de Baere T, Risse O, Kuoch V, et al. Adverse events during radiofrequencytreatment of 582 hepatic tumors. Am J Roentgenol 2003;181:695-700.

101. Buscarini E, Buscarini L. Radiofrequency thermal ablation with sexpandable needle of focal liver malignancies: complication report. Eur Radiol 2004;14: 31-7.
102. Lim HK, Choi D, Lee WJ, et al. Hepatocellular carcinoma treated with percutaneous radio-frequency ablation: evaluation with follow-up multiphase helical CT. Radiology 2001;221:447-54.
103. Yamagami T, Nakamura T, Kato T, et al. Skin injury after radiofrequency ablation for hepatic cancer. Am J Roentgenol 2002;178:905-7.
104. Verhoeven BH, Haagsma EB, Appeltans BM, et al. Hyperkalaemia after radiofrequency ablation of hepatocellular carcinoma. Eur J Gastroenterol Hepatol 2002;14:1023-4.
105. Tsui SL, Lee AK, Lui SK, et al. Acute intraoperative hemolysis and hemoglobinuria during radiofrequency ablation of hepatocellular carcinoma. Hepatogastroenterology 2003;50:526-9.
106. Goodman EJ, Johnson PA. Radiofrequency ablation of hepatic tumors can cause elevation of the patient's temperature. Anesth Analg 2003;97:1203-4.
107. Koda M, Maeda Y, Matsunaga Y, et al. Hepatocellular carcinoma with sarcomatous change arising after radiofrequency ablation for well-differentiated hepatocellular carcinoma. Hepatol Res 2003;27:163-67.
108. Livraghi T, Solbiati L, Meloni MF, et al. Treatment of Focal Liver Tumors with Percutaneous Radio-frequency Ablation: Complications Encountered in a Multicenter Study. Radiology 2003;226:441-51.
109. Vogl TJ, Straub R, Eichler K, et al. Malignant liver tumors treated with MR imaging-guided laser-induced thermotherapy: experience with complications in 899 patients (2,520 lesions). Radiology 2002; 225:367-77.
110. Pacella CM, Bizzarri G, Cecconi P, et al. Hepatocellular carcinoma: long-term results of combined treatment with laser thermal ablation and transcatheter arterial chemoembolization. Radiology 2001;219:669-78.
111. Allgaier HP, Deibert P, Olschewski M, et al. Survival benefit of patients with inoperable hepatocellular carcinoma treated by a combination of transarterial chemoembolization and percutaneous ethanol injection: a single-center analysis including 132 patients. Int J Cancer 1998;79:601-5.
112. Bartolozzi C, Lencioni R, Armillotta N. Combined treatment of hepatocarcinoma with chemoembolization and alcohol administration. Long-term results. Radio Med (Torino) 1997;94: 19-23.
113. Ishii H, Okada S, Sato T, et al. Effect of percutaneous ethanol injection for postoperative recurrence of hepatocellular carcinoma in combination with transcatheter arterial embolization. Hepatogastroenterology 1996;43:644-50.
114. Dohmen K, Shirahama M, Shigematsu H, et al. Transcatheter arterial chemoembolization therapy combined with percutaneous ethanol injection for nonresectable large hepatocellular carcinoma: an evaluation of the local therapeutic effect and survival rate. Hepatogastroenterology 2001;48:1409-15.
115. Koda M, Murawaki Y, Mitsuda A, et al. Combination therapy with transcatheter arterial chemoembolization and percutaneous ethanol injection compared with percutaneous ethanol injection alone for patients with small hepatocellular carcinoma: a randomized control study. Cancer 2001;92:1516-24.
116. Tanaka K, Nakamura S, Numata K, et al. Hepatocellular carcinoma: treatment with percutaneous ethanol injection and transcatheter arterial embolization. Radiology 1992;185:457-60.
117. Lencioni R, Paolocchi A, Moretti M, et al. Combined transcatheter arterial chemoembolization and percutaneous ethanol injection for the treatment of large hepatocellular carcinoma; local therapeutic effect and long-term survival rate. Eur Radiol 1998;8: 439-44.
118. Kirchhoff T, Chavan A, Galanski M. Transarterial chemoembolization and percutaneous ethanol injection therapy in patients with hepatocellular carcinoma. Eur J Gastroenterol Hepatol 1998;10:907-09.
119. Seki T, Tamai T, Nakagawa T, et al. Combination therapy with transcatheter arterial chemoembolization and percutaneous microwave coagulation therapy for hepatocellular carcinoma. Cancer 2000;89:1245-51.
120. Ishikawa M, Ikeyama S, Sasaki K, et al. Intraoperative microwave coagulation therapy for large hepatic tumors. J Hepatobiliary Pancreat Surg 2000;7:587-91.
121. Buscarini L, Buscarini E, Di Stasi M, Quaretti P, Zangrandi A. Percutaneous radiofrequency thermal ablation combined with transcatheter arterial embolization in treatment of large hepatocellular carcinoma. Ultraschall Med 1999;20:47-53.
122. Bloomston M, Binitie O, Fraiji E, et al. Transcatheter arterial chemoembolization with or without radiofrequency ablation in the management of patients with advanced hepatic malignancy. Am Surg 2002;68: 827-31.
123. Lencioni R, Cioni D, Donati F, et al. Combination of interventional therapies in hepatocellular carcinoma. Hepatogastroenterology 2001;48:8-14.
124. Nicoli N, Casaril A, Marchiori L, et al. Treatment of recurrent hepatocellular carcinoma by radiofrequency thermal ablation. J Hepatobiliary Pancreat Surg 2001;8:417-21.
125. Goldberg SN, Kamel IR, Kruskal JB, et al. Radiofrequency ablation of hepatic tumors: increased tumor destruction with adjuvant liposomal doxorubicin therapy. Am J Roentgenol 2002;179: 93-101.
126. Montorsi M, Santambrogio R, Bianchi P, et al. Radiofrequency interstitial thermal ablation of hepatocellular carcinoma in liver cirrhosis: role of laparoscopic approach. Surg Endosc 2001;15: 141-5.
127. Podnos YD, Henry G, Ortiz JA, et al. Laparoscopic ultrasound with radiofrequency ablation in cirrhotic patients with hepatocellular carcinoma: technique and technical considerations. Am Surg 2001;67:1181-4.
128. Bilchik AJ, Wood TF, Allegra D, et al. Cryosurgical ablation and radiofrequency ablation for unresectable hepatic malignant neoplasms: a proposed algorithm. Arch Surg 2000;135:657-64.
129. Lencioni R, Cioni D, Bartolozzi C. Percutaneous radiofrequency thermal ablation of liver malignancies: techniques, indications, imaging findings, and clinical results. Abdom Imaging 2001;26: 345-60.
130. Goldberg SN, Gazelle GS, Mueller PR. Thermal ablation therapy for focal malignancy: a unified approach to underlying principles, techniques, and diagnostic imaging guidance. Am J Roentgenol 2000;174:323-31.
131. Lencioni R, Cioni D, Goletti O, et al. Radiofrequency thermal ablation of liver tumors: state-of-the-art. Cancer J 2000;6(suppl 4):S304-15.
132. Sironi S, Livraghi T, Meloni F, et al. Small hepatocellular carcinoma treated with percutaneous RF ablation: MR imaging follow-up. AJR 1999;173:1225-9.
133. Dromain C, de Baere T, Elias D, et al. Hepatic tumors treated with percutaneous radio-frequency ablation: CT and MR imaging follow-up. Radiology 2002;223:255-62.
134. Goldberg SN, Hahn PF, Halpern EF, et al. Radio-frequency tissue ablation: effect of pharmacologic modulation of blood flow on coagulation diameter. Radiology 1998;209:761-7.

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HEPATOLOGY

Significance of hepatitis B virus DNA clearance and early prediction of hepatocellular carcinogenesis in patients with cirrhosis undergoing interferon therapy: Long-term follow up of a pilot study

KENJI IKEDA, MASAHIRO KOBAYASHI, SATOSHI SAITOH, TAKASHI SOMEYA, TETSUYA HOSAKA, NORIO AKUTA, YOSHIYUKI SUZUKI, FUMITAKA SUZUKI, AKIHITO TSUBOTA, YASUJI ARASE AND HIROMITSU KUMADA

Department of Gastroenterology, Toranomon Hospital and Okinaka Memorial Institute for Medical Research, Tokyo, Japan

Abstract

Background and Aim: Because the anti-carcinogenic effect and mechanism of interferon (IFN) in patients with hepatitis B virus (HBV)-related cirrhosis have not been elucidated, quantitative analysis of HBV-DNA concentration was carried out sequentially.

Method: Of 60 consecutive patients with cirrhosis who began IFN therapy between 1986 and 1990, 57 patients were completely observed for the appearance of hepatocellular carcinoma (HCC). All patients underwent intermittent administration of IFN for a median period of 18 months. HBV-DNA was quantified using transcription mediated amplification and hybridization protection assay. A HBV-DNA count <3.7 log-genome equivalent (LGE)/mL (equivalent to $10^{3.7}$ or 5000 copies/mL) was considered to be a negative value.

Results: Of 25 patients who had HBV-DNA loss after IFN therapy, nine lost HBV-DNA during the therapy and 16 lost HBV-DNA after cessation of the therapy. The other nine patients showed a transient loss of HBV-DNA, and the remaining 23 retained persistently positive HBV-DNA during and after therapy. Although HCC developed in two (8.0%) of the 25 patients with HBV-DNA loss, carcinogenesis was found in 11 (34.4%) of 32 patients without HBV-DNA loss (Fisher's exact test, $P = 0.026$). In the two exceptional patients, HCC was detected at 1.2 and 3.6 years after loss of HBV-DNA, respectively. When the HBV-DNA concentration decreased by 2 LGE/mL (decrease to 1/100) at 6 months after initiation of interferon, HBV-DNA became negative eventually in 15 (60.0%) of 25 patients.

Conclusion: A significant decrease or loss of serum HBV-DNA prevents development of HCC, and sequential analysis of HBV-DNA could be very useful in both the prediction and the early detection of small HCC.

Key words: cancer prevention, carcinogenesis, DNA, hepatitis B virus, hepatocellular carcinoma, interferon, liver cirrhosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of death in many parts of sub-Saharan Africa and Asia.[1,2] It is also one of the most common neoplasms in Japan. Abundant epidemiological and molecular biological evidence shows that the hepatitis B virus (HBV) is an important factor in the development of HCC,[3–6] but the precise role of HBV-DNA viruses in the oncogenesis of HCC is still unknown. Although increasing evidence indicates that the HBV plays an important role in the development of HCC, particularly after the discovery of integrated forms of HBV,[7,8] current serological and virological markers are still insufficient for establishing this relationship. Because a really curative therapy is not available for HCC at present, the accurate prediction

Correspondence: Dr Kenji Ikeda, Department of Gastroenterology, Toranomon Hospital, Toranomon 2-2-2, Minato-ku, Tokyo 105-8470, Japan. Email: ikedakenji@tora.e-mail.ne.jp

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and early detection of HBV-related HCC is essential in the current situation. Needless to say, a cohort of patients with HBV-related cirrhosis has a significantly high risk for the development of HCC,[6,9] but the degree of risk of carcinogenesis in an individual patient cannot be predicted as yet. Hepatocellular carcinogenesis in patients with HBV infection may be associated with persistence of aminotransferase, concentration of HBV-DNA, or merely the severity of the liver disease.

Interferon (IFN) has been reported to be effective in patients with HBV-related chronic hepatitis, which, on early control studies,[10–12] decreases serum HBV-DNA concentration and improves biochemical data and subsequently suppresses disease progression to cirrhosis.[13,14] Although the various effects of IFN in HBV infection have been well investigated from the virological, biochemical, and medico-economical viewpoints,[15–17] the influence of IFN on the long-term outcome for liver cirrhosis and on hepatocellular carcinogenesis is still controversial.[18–23] In order to clarify the mechanism of the anticarcinogenic activity of IFN, if any, we analyzed HBV-DNA concentration serially in a cohort of 60 patients with cirrhosis.

The purposes of this study are: (i) to elucidate the relation of hepatocellular carcinogenesis to longitudinal clinical courses of consecutive cirrhotic patients with IFN therapy; and (ii) to investigate a prediction of cancer preventative activity by early HBV-DNA elimination.

METHODS

Patients

Of 189 patients who were diagnosed as having HBV-related cirrhosis using peritoneoscopy and/or liver biopsy from 1983 to 1990 in our hospital, a total of 60 patients underwent IFN therapy from 1986 to 1990. Because three patients were lost to follow up, the remaining 57 patients (95.0%) were analyzed for virological outcome, carcinogenesis, and eventual prognosis: the reason for the dropout from the observation in the three patients was simply relocating house.

Table 1 shows the demography and laboratory data of the consecutive 57 patients who began IFN therapy from 1986 to 1990. There were 45 men and 12 women, with an age range from 19 to 60 years and a median of 41 years. Median values of bilirubin and albumin were 0.9 mg/dL and 4.1 g/dL, respectively. All the patients had a high HBV-DNA concentration of 3.7 log-genome equivalent (LGE)/mL or more at the time of IFN therapy.

Interferon treatment

IFN-α was administered in 35 patients (61.4%) and IFN-β in the remaining 22 patients (38.6%). The daily quantity of IFN was three million units in 22 (38.6%) and six million units in 35 (61.4%), twice a week administration was carried out in 54 (94.7%) and three times a week administration in three (5.3%). All patients received intermittent IFN therapy for a median of 18 months (range, 2–132 months), but the duration of the IFN therapy was arbitrary in this pilot study. Although the daily dose of IFN and the duration of the therapy varied in this study, 52 (91.2%) of the 57 patients received IFN for 6 months or longer.

Table 1 Demography and laboratory data of 57 patients with hepatitis B virus-related cirrhosis undergoing interferon therapy

Demography	
Men : women	45:12
Age (median, range)	41 (19–60)
Decompensated cirrhosis	3 (5.3%)
Past alcohol consumption of 500 kg or more	3 (5.3%)
Laboratory data (median, range)	
Bilirubin (mg/dL)	0.9 (0.4–2.6)
Albumin (g/dL)	4.1 (3.0–4.9)
Aspartic transaminase (IU/L)	65 (16–404)
Alanine transaminase (IU/L)	74(12–586)
Platelet count ($\times 10^3$/mm^3)	125 (68–332)
Antibodies to hepatitis C virus positive	0
Hepatitis B e antigen positive	41 (71.9%)
Hepatitis B virus DNA (LGE/mL)	7.2 (3.9–> 8.7)
Observation period (year)	13.6 (6.5–16.1)

LGE/mL, log-genome equivalent, expressed as 10^n copy/mL.

Follow up of patients and diagnosis of HCC

Follow up of the patients was made on a monthly basis after diagnosis of liver cirrhosis using monitoring virological, hematological, and biochemical data, including α-fetoprotein. All results for these laboratory tests, including HBV markers, were obtained throughout the observation period in each patient. Patients were classified into four groups according to patterns of serial concentration of HBV-DNA: type A, disappearance of HBV-DNA during and after IFN therapy; type B, loss of HBV-DNA after cessation of IFN administration; type C, transient loss of HBV-DNA only during IFN administration; type D, persistently positive HBV-DNA during and after the therapy. Clinical courses of alanine aminotransferase (ALT) fluctuation were also classified into four groups according to normalization of the ALT value.

Imaging diagnosis was made two or more times per year for each patient using computed tomography (CT), ultrasonography (US) or magnetic resonance imaging (MRI). HCC was diagnosed using typical hypervascular characteristics on angiography in addition to certain features of CT, US and MRI. Pathological confirmation of surgically resected specimens was carried out in six (46.2%) of 13 patients with HCC development.

Assays of HBV markers

Serum hepatitis B surface antigen was measured using radioimmunoassay (Dainabot, Tokyo, Japan) and reversed passive hemagglutination (Institute of Immunology, Tokyo, Japan) using commercial assay kits. hepatitis B e antigen (HBeAg) and antibody to HBeAg were determined using ELISA (Institute of Immunology) with commercial kits. Anti-hepatitis C virus antibody (third-generation anti-HCV) was assessed using ELISA kits (Dainabot).

HBV-DNA was assayed using frozen sera stored at −80°C, and quantified using transcription-mediated amplification and hybridization protection assay (Chugai Diagnostics Science, Tokyo, Japan), as described by Kamisango *et al.*[24] A HBV-DNA value of <3.7 LGE/mL (equivalent to $10^{3.7}$ copies/mL or 5000 copies/mL) was considered to be a low value. For all serial sera from the diagnosis of cirrhosis to the end of the observation period in each patient, the DNA quantification was simultaneously carried out using identical measurement kits.

Statistical analysis

Standard statistical measures and procedures were used. The Mann–Whitney *U*-test and χ^2 tests were employed for the examination of background characteristics between the groups with and without HBV-DNA elimination. Fisher's exact test was also used to analyze the relation of HBV markers to carcinogenesis. Rates of cumulative HBV-DNA disappearance, carcinogenesis and survival were calculated using Kaplan–Meier analysis,[25] and the differences between the analyzed groups were assessed using a log-rank test. A *P*-value of <0.05 using a two-tailed test was considered to be significant. Data analysis was carried out using the computer program SPSS version 11.[26]

RESULTS

HBV-DNA in clinical courses

HBV-DNA was positive in all patients at the initiation of IFN therapy (3.9–8.7 LGE/mL). HBV-DNA became negative (<3.7 LGE/mL) in 25 of 57 patients (43.9%) during the observation period, with a median of 13.6 years. The remaining 32 patients did not show a sustained negative HBV-DNA after the therapy, although nine patients did show transient negative values for a limited period during the therapy.

Clinical courses of HBV-DNA were classified into the four categories mentioned above. Nine patients (15.8%) lost HBV-DNA during and after IFN therapy (type A), 16 patients (28.1%) lost HBV-DNA after cessation of the therapy (type B). The other nine patients (15.8%) showed a transient loss of HBV-DNA (type C), and the remaining 23 (40.4%) retained persistently positive HBV-DNA (type D).

The cumulative rate of HBV-DNA disappearance was calculated using Kaplan–Meier analysis (Fig. 1).

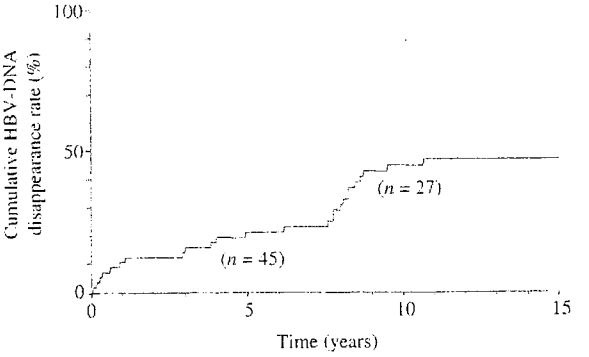

Figure 1 Cumulative hepatitis B virus (HBV)-DNA disappearance rate in the 57 cirrhotic patients with interferon therapy.

DNA became negative in 10.5% at the end of the first year after initiation of IFN therapy, in 12.3% at the third year, 21.0% at the fifth year, 43.7% at the tenth year, and 46.7% at the fifteenth year, respectively.

Hepatocellular carcinogenesis and serial concentration of HBV-DNA

A total of 13 patients developed HCC during the observation period.

The relationship between carcinogenesis and serial concentration of HBV-DNA was analyzed (Fig. 2). None of the nine patients in the type A group developed HCC. Two (12.5%) of 16 patients in the type B group developed HCC: HCC were detected 1.2 years after the disappearance of HBV-DNA in one patient, and 3.6 years after the disappearance of HBV-DNA in the other patient. Three (33.3%) of nine patients in the type C group showed carcinogenesis, and eight (34.8%) of 23 patients in the type D group developed HCC during the observation. Hepatocellular carcinogenesis was significantly associated with persistent positive HBV-DNA after initiation of IFN (2/25 *vs* 11/32; $P = 0.019$ using the $\chi 2$ test, $P = 0.026$ using Fisher's exact test).

Cumulative carcinogenesis rates were analyzed according to the ultimate course of the serial assay of HBV-DNA (Fig. 3). Fifth-year hepatocellular carcinogenesis rates were 0% in patients with HBV-DNA loss, and 9.4% in patients without HBV-DNA elimination; 10-year rates were 8.0% and 22.5%; and 15-year rates were 8% and 44.0%, respectively. The carcinogenesis rate in patients with HBV-DNA elimination was significantly lower than in those without DNA elimination ($P = 0.011$, using a log-rank test).

Hepatocellular carcinogenesis and HBeAg and aminotransferase

The relationship between carcinogenesis and HBeAg positivity during the clinical course was assessed.

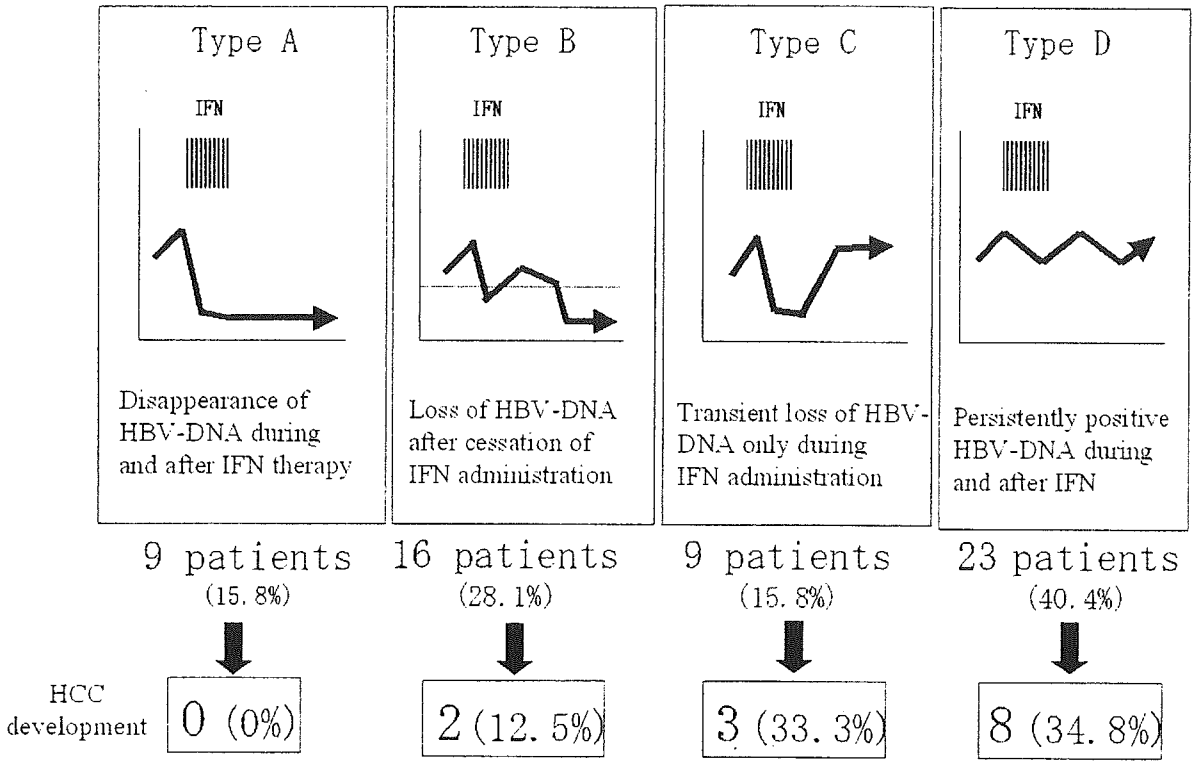

Figure 2 Relation between types of serial hepatitis B virus (HBV)-DNA concentration and carcinogenesis. HCC, hepatocellular carcinoma; IFN, interferon.

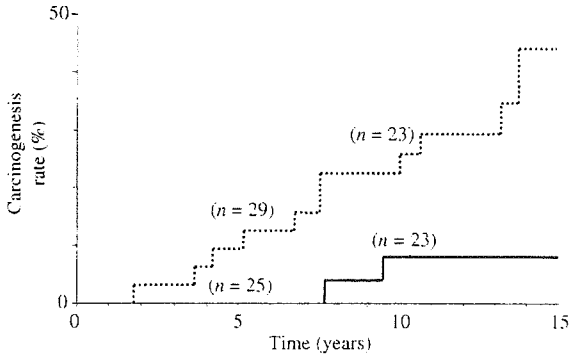

Figure 3 Cumulative hepatocellular carcinogenesis rates in patients (—; $n = 25$) with and (---; $n = 32$) without eventual hepatitis B virus (HBV)-DNA clearance.

HBeAg was positive in 41 patients (71.9%) and negative in 16 (28.1%) at the initiation of IFN therapy. Twenty-eight (68.3%) of the 41 patients showed continuous loss of HBeAg after IFN therapy. HCC developed in four (25.0%) of the 16 patients without HBeAg from the beginning, four (14.3%) of the 28 patients with HBeAg clearance, and five (38.5%) of 13 patients with persistent HBeAg positivity. HBeAg clearance did not significantly decrease the incidence of carcinogenesis risk ($P = 0.12$ using the χ^2 test with Yates' correction).

The relationship between carcinogenesis and a longitudinal course of ALT after IFN therapy was also analyzed. Four (18.2%) of 22 patients with normalization of ALT after IFN therapy developed HCC; nine (25.8%) of 35 patients with persistently abnormal ALT levels developed HCC. The serial values of ALT were not significantly associated with carcinogenesis risk ($P = 0.075$ using the χ^2 test with Yates' correction).

The cumulative HBeAg disappearance rate, HBV-DNA disappearance rate, and ALT normalization rate were calculated in those patients with positive HBeAg at the beginning of IFN treatment (Fig. 4). The HBeAg disappearance rate and DNA disappearance rates were 55.4% and 14.6% at the end of the fifth year, and 55.4% and 40.1% at the tenth year, respectively. The ALT normalization rate at the fifth year was 25.4% and the tenth year rate was 41.2%. Although the incidence of virological and biochemical improvement gradually increased after therapy, the rates evidently differed between virological and biochemical responses.

Influence of the length of interferon therapy on HBV-DNA loss

The influence of the length of the therapy on virological response was assessed.

Although 25 (43.8%) of 57 patients cleared HBV-DNA on overall analysis, 21 (46.6%) of 45 patients who received IFN for more than 6 months and 20 (50%) of 40 patients who received IFN for more than 12 months lost HBV-DNA. Similarly, the HBV-DNA disappearance rate slightly increased correlating with the length of IFN administration: 55.5% in patients who were treated for more than 18 months, 56.0% with more than 24 months' treatment, 64.7% in more than 36 months' treatment, 58.3% in more than 48 months' treatment, and 71.4% in more than 60 months' treatment (Fig. 5). The longer the IFN therapy was carried out, the higher the rate of HBV-DNA disappearance.

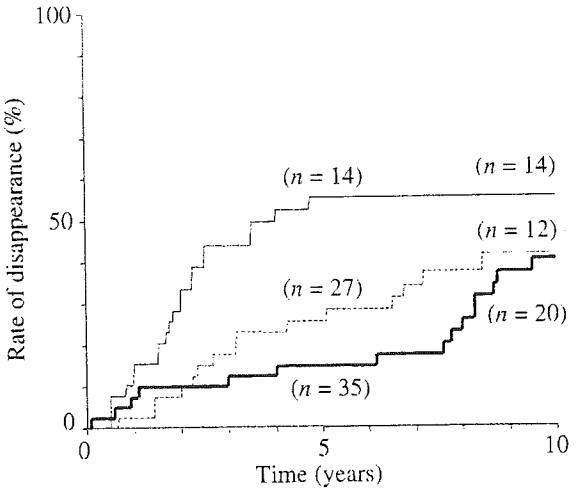

Figure 4 Cumulative (—) hepatitis B e antigen (HBeAg) disappearance rate, (—) hepatitis B virus (HBV)-DNA disappearance rate, and (---) alanine transaminase normalization rate in 41 patients with positive HBeAg at the initiation of interferon therapy.

Prediction of future HBV-DNA elimination

We assessed the relation between an early HBV-DNA response and a future HBV-DNA loss. When the HBV-DNA concentration decreased by ≥2 LGE/mL (decrease to 1/100) during the first 6 months, 15 (60.0%) of 25 patients eventually lost HBV-DNA. In contrast, when the HBV-DNA decrease was <2 LGE/mL during the period, HBV-DNA loss was found in 10 (31.3%) of 32 patients ($P = 0.036$, χ^2 test). Similarly, future HBV-DNA loss was estimated from a decrease in concentration of HBV-DNA at the end of 12 months: HBV-DNA eventually became negative in 15 (62.5%) of 24 patients with a larger DNA decrease of ≥2 LGE/mL at the end of 12 months, eventual DNA loss was found in only 10 (30.3%) of 33 patients with a smaller DNA decrease by <2 LGE/mL. The 12-month decrease of HBV-DNA was significantly associated with future DNA loss ($P = 0.030$, χ^2 test).

The early response of HBV-DNA and the length of IFN therapy were analyzed together for the prediction of eventual HBV-DNA loss. Of 25 patients with a HBV-DNA decrease of ≥2 LGE/mL during the initial 6 months, two (33.3%) of six patients with short IFN therapy of ≤6 months showed a HBV-DNA loss, but 13 (68.4%) of 19 patients with long-term IFN therapy of >6 months lost HBV-DNA. Of 32 patients with a HBV-DNA decrease of <2 LGE/mL in the first 6 months, one (20.0%) of five patients with short IFN therapy showed HBV-DNA loss, but nine (33.3%) of 27 patients with long-term IFN administration lost HBV-DNA. Therefore, according to the early HBV-DNA response and the duration of the therapy, the rate of sustained HBV-DNA decrease to <3.7 LGE/mL varied, with a range of 20.0–68.4%.

Prognosis after IFN therapy

A total of eight patients (14.0%) died in the period of observation: six from development of HCC and the

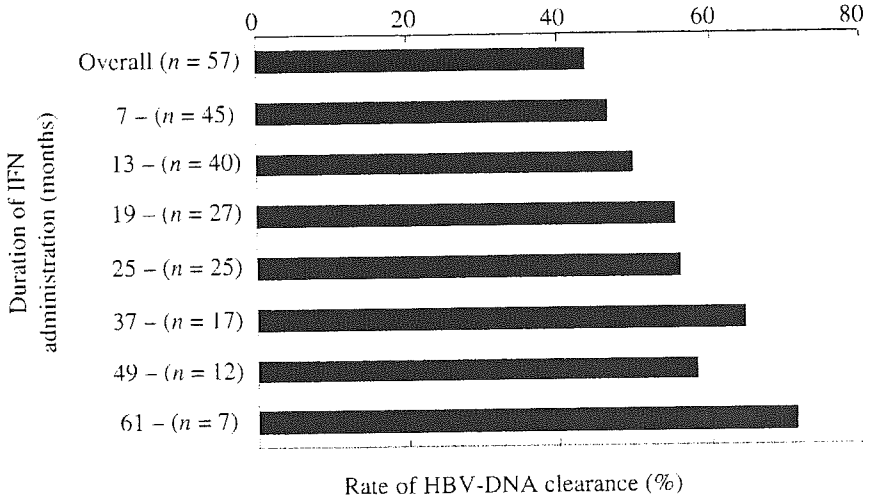

Figure 5 Influence of the length of interferon (IFN) therapy on hepatitis B virus (HBV)-DNA clearance.

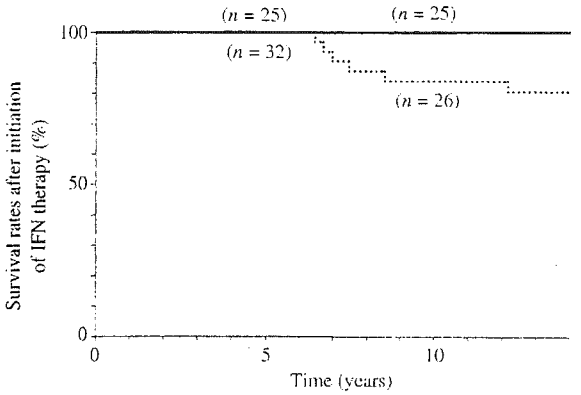

Figure 6 Cumulative survival rates after the initiation of interferon (IFN) therapy in patients (—; $n = 25$) with and (---; $n = 32$) without eventual hepatitis B virus DNA clearance.

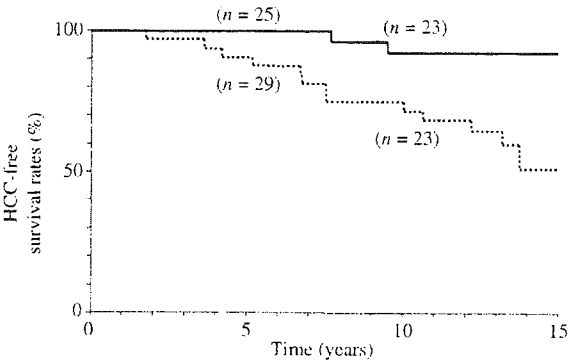

Figure 7 Hepatocellular carcinoma (HCC)-free survival rates in patients (—; $n = 25$) with and (---; $n = 32$) without eventual hepatitis B virus DNA clearance.

other two from liver failure due to aggravation of cirrhosis.

Of 13 patients with HCC development, two patients with HBV-DNA loss have not shown any tumor recurrence after surgical resection, and both patients are alive at the end of the observation. In contrast, nine (81.8%) of 11 patients with persistently high HBV-DNA developed HCC recurrence after therapy, and six (54.5%) of the patients died during the observation period. All six patients died from the development of HCC and none from aggravation of cirrhosis or extrahepatic disease.

Of 44 patients without HCC development until the end of the observation period, none of 23 patients with HBV-DNA loss died, but two (9.5%) of 21 patients with persistently positive HBV-DNA have died from liver failure.

Survival rates were compared between those patients with and without HBV-DNA loss (Fig. 6). Fifth-year survival rates in patients with and without HBV-DNA loss were 100% and 100%, seventh year rates were 100% and 90.5%, tenth year rates were 100% and 84.1%, and twelfth year rates were 100% and 80.6%, respectively. The cumulative survival rate in patients with HBV-DNA loss was significantly higher than that in patients without HBV-DNA clearance ($P = 0.0030$, log-rank test).

The HCC-free survival rates were also assessed in the two patient groups (Fig. 7). Fifth-year HCC-free survival rates in patients with and without HBV-DNA loss were 100% and 90.6%, seventh year rates were 100% and 81.3%, tenth year rates were 92% and 74.8%, and fifteenth year rates were 92% and 51.2%, respectively. The HCC-free survival rate in patients with HBV-DNA loss was significantly higher than that in patients without HBV-DNA clearance ($P = 0.0036$, log-rank test).

DISCUSSION

Until recently, several authors mentioned the anti-carcinogenic activity of IFN in patients with HBV-related cirrhosis. Oon[18] and Ikeda *et al.*[21] have shown that IFN significantly decreased carcinogenesis in patients undergoing IFN therapy with a relative risk of 0.03 and 0.39, respectively. Lin *et al.* also demonstrated an anti-tumor activity of IFN, with a relative risk of 0.11 in a randomized controlled trial for patients with chronic hepatitis and cirrhosis.[23] Mazzella *et al.*,[19] Fattovich *et al.*[20] and the International Interferon-alpha Hepatocellular Carcinoma Study Group in Europe[22] demonstrated a low relative risk for carcinogenesis in patients with IFN therapy, but none could show a statistically significant difference. Aside from the slightly inconsistent results after IFN therapy for cirrhosis, we tried to elucidate the relationship between virological response and HCC development, using a cohort of consecutive patients with cirrhosis who underwent IFN therapy more than 10 years ago. Considering that the disease activity and carcinogenic potency can change significantly in the course of HBV-related liver disease, a longitudinal analysis was carried out for the study of the clinical process and the mechanism of anti-tumor activity of IFN in HBV-positive cirrhosis patients.

In this clinical study, sequential trends of HBV concentration were significantly associated with hepatocellular carcinogenesis, as was found in natural clinical courses of patients without IFN.[27] Although only two of 25 patients who developed HCC showed a disappearance of HBV-DNA during or after IFN therapy, 11 of 32 patients who showed carcinogenesis could not eliminate HBV-DNA using treatment with IFN ($P = 0.019$). A point in common found in the two exceptional patients with HCC development after elimination of HBV-DNA was that the HCC were detected immediately after a significant decrease in the HBV-DNA level after using IFN in the clinical courses: 1.2 years and 3.6 years after in each patient. We can reasonably consider that the discovered HCC in the patients already existed at an indiscernible size at the time of HBV-DNA elimination, and that the minimal HCC automatically grew gradually for the following few years after the decrease in HBV-DNA levels occurred. Even including these two patients with HCC development, the risk of hepatocellular carcinogenesis was significantly associ-

ated with the persistence of a high HBV-DNA concentration. Hepatocellular carcinogenesis was assessed using serial HBV-DNA assay with a cut-off value of 3.7 LGE/mL (or $10^{3.7}$ copy/mL) in this study. Although a detailed analysis of HBV-DNA concentration with a more sensitive measurement may demonstrate a better correlation with the carcinogenesis rate than the present study, setting the HBV-DNA concentration at this cut-off value was significantly valuable in the prediction for HCC appearance.

The mechanism of anticarcinogenic activity of IFN was regarded as an anti-necroinflammatory process through suppression of HBV-DNA concentration from these results. This study dealt with the relationship between carcinogenesis and HBV-DNA principally, but clinical courses of aminotransferase were also significantly related to the HCC development. Aminotransferase values were less valuable than HBV-DNA levels in the prediction of HCC development in the natural clinical course of HBV-cirrhosis,[27,28] and aminotransferase values were also less associated with the future rate of carcinogenesis in patients undergoing IFN therapy.

Although the mere use of IFN does not guarantee a decrease in the rate of carcinogenesis in patients with HBV-related cirrhosis, a serial course of HBV-DNA concentration was significantly correlated with future HCC development during and after treatment. The value of cancer prediction was much higher from the assay of HBV-DNA than that of HBe antigen. Indeed the cut-off values of HBV-DNA concentration seemed to be discretionary; the advantage in clinical practice was marked and conspicuous. When more sensitive ways of measuring HBV-DNA concentration were applied to the analysis, hepatocellular carcinogenesis could be more successfully predicted.

In conclusion, persistence of a high concentration of HBV-DNA was significantly associated with hepatocellular carcinogenesis in cirrhotic patients with IFN therapy, and its sequential analysis would be useful in the early detection of HCC. IFN therapy is recommended to be continued as long as possible until HBV-DNA loss occurs in HBV-cirrhosis patients, from the viewpoint of cancer prevention. Further studies with a greater number of patients are required to confirm the relationship, and future studies should be aimed at defining the role and basic mechanisms by which the carcinogenesis rate was suppressed by IFN in the cohort.

REFERENCES

1 Parkin DM, Stjernsward J, Muir CS. Estimates of worldwide frequency of twelve major cancers. *Bull. World Health Organ.* 1984; **62**: 163–82.

2 Linsell A. Primary liver cancer: global epidemiology and main aetiological factors. *Ann. Acad. Med. Singapore* 1984; **13**: 277–87.

3 Prince AM, Szmuness W, Michon J *et al.* A case-control study of the association between primary liver cancer and hepatitis B infection in Senegal. *Int. J. Cancer* 1975; **16**: 376–83.

4 Ohnishi K, Iida S, Iwama S *et al.* The effect of chronic habitual alcohol intake on the development of liver cirrhosis and hepatocellular carcinoma. Relation to hepatitis B surface antigen carriage. *Cancer* 1982; **49**: 672–7.

5 Lam KC, Yu MC, Leung JWC, Henderson BE. Hepatitis B virus and cigarette smoking: risk factors for hepatocellular carcinoma in Hong Kong. *Cancer Res.* 1982; **42**: 5246–8.

6 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis–a prospective observation of 795 cases with viral and alcoholic cirrhosis. *Hepatology* 1993; **18**: 47–53.

7 Shafritz D, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. *N. Engl. J. Med.* 1981; **305**: 1067–73.

8 Brechot C, Degos F, Lugassy C *et al.* Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. *N. Engl. J. Med.* 1981; **312**: 270–76.

9 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N. Engl. J. Med.* 1993; **328**: 1797–801.

10 Weimar W, Heijtink RA, Kate FJ *et al.* Double blind study of leukocyte interferon administration in chronic HBsAg positive hepatitis. *Lancet* 1980; **1**: 336–8.

11 Alexander GJ, FagenEA, Guarner P *et al.* A controlled trial of 6 months thrice weekly lymphoblastoid interferon versus no therapy in chronic hepatitis B virus infection. *J. Hepatol.* 1986; **3** (Suppl. 2): S183–8.

12 Dusheiko GM, Paterson AC, Pitcher L *et al.* Recombinant leukocyte interferon treatment of chronic hepatitis B. *J. Hepatol.* 1986; **3** (Suppl. 2): S199–207.

13 Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann. Intern. Med.* 1981; **94**: 744–8.

14 Lok AS, Weller IV, Karrayanis P *et al.* Thrice weekly lymphoblastoid interferon is effective in inhibiting hepatitis B virus replication. *Liver* 1984; **4**: 45–9.

15 Koreman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. *Ann. Intern. Med.* 1991; **114**: 629–34.

16 Wong JB, Koff RS, Tine F, Pauker SG. Cost-effectiveness of interferon-alpha 2b treatment for hepatitis B e antigen-positive chronic hepatitis B. *Ann. Intern. Med.* 1995; **122**: 664–75.

17 Dusheiko GM, Roberts JA. Treatment of chronic type B and C hepatitis with interferon alfa: an economic appraisal. *Hepatology* 1995; **22**: 1863–73.

18 Oon CL. Long-term survival following treatment of hepatocellular carcinoma in Singapore: evaluation of Wellferon in the prophylaxis of high-risk pre-cancerous conditions. *Cancer Chemother. Pharmacol.* 1992; **31** (Suppl.): S137–42.

19 Mazzella G, Accogli E, Sottili S *et al.* Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. *J. Hepatol.* 1996; **24**: 141–7.

20 Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with inter-

feron alfa. European concerted action on viral hepatitis. *Hepatology* 1997; **26**: 1338–42.
21 Ikeda K, Saitoh S, Suzuki Y *et al.* Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by the hepatitis B virus. *Cancer* 1998; **82**: 827–35.
22 International Interferon-alpha Hepatocellular Carcinoma Study Group. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. *Lancet* 1998; **351**: 1535–9.
23 Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999; **29**: 971–5.
24 Kamisango K, Kamogawa C, Sumi M *et al.* Quantitative detection of hepatitis B virus by transcription-mediated amplification and hybridization protection assay. *J. Clin. Microbiol.* 1999; **2**: 310–14.
25 Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. *J. Am. Stat. Assoc.* 1958; **53**: 457–81.
26 SPSS Inc. *SPSS for Windows Version 11.0 Manual.* Chicago, IL, USA: SPSS Inc., 2001.
27 Ikeda K, Arase Y, Kobayashi M *et al.* Consistently low hepatitis B virus-DNA saves patients from hepatocellular carcinogenesis in HBV-related cirrhosis–a nested case-control study using 96 untreated patients. *Intervirology* 2003 **46**: 96–104.
28 Ikeda K, Saitoh S, Suzuki Y *et al.* Relationship of hepatocellular carcinogenesis with precore mutant virus and serum hepatitis B virus DNA concentration A longitudinal analysis of patients with cirrhosis. *Hepatol. Res.* 1998; **10**: 142–55.

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Hepatitis B Virus DNA Integration in Hepatocellular Carcinoma After Interferon-Induced Disappearance of Hepatitis C Virus

Akihiro Tamori, M.D.,[1] Shuhei Nishiguchi, M.D.,[1] Susumu Shiomi, M.D.,[2] Takehiro Hayashi, M.D.,[1] Sawako Kobayashi, M.D.,[1] Daiki Habu, M.D.,[1] Tadashi Takeda, M.D.,[1] Shuichi Seki, M.D.,[1] Kazuhiro Hirohashi, M.D.,[3] Hiromu Tanaka, M.D.,[4] and Shoji Kubo, M.D.[4]

Departments of [1]Hepatology; [2]Nuclear Medicine; [3]General Medicine; and [4]Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan

OBJECTIVES: Hepatocellular carcinoma (HCC) has been reported in patients in whom hepatitis C virus (HCV) was eliminated by interferon (IFN) therapy. We examined the pathogenesis of HCC in patients with sustained viral response.

METHODS: Operable HCC developed in 7 of 342 patients cured of HCV infection by IFN monotherapy. No patient abused alcohol or had diabetes mellitus or obesity. Resected specimens of HCC were histologically evaluated. DNA extracted from HCC was examined by polymerase chain reaction (PCR) to locate hepatitis B virus (HBV) DNA. HBV integration sites in human genome were identified by cassette-ligation-mediated PCR.

RESULTS: HBV DNA was not amplified in serum samples from any of the seven patients with HCC and was found in liver in four patients. In the latter four patients, HBV DNA was integrated into the human genome of HCC. In two of these patients, covalently closed circular HBV (cccHBV) was also detected. The patients with HBV DNA integration were free of HCV for more than 3 yr. In two of the three patients without HBV DNA integration, the surrounding liver showed cirrhosis. The liver of HCC with HBV DNA integration had not progressed to cirrhosis. Three of the four tumors with HBV integration had one integration site each, located at chromosomes 11q12, 11q22-23, and 22q11, respectively. The other tumor had two integration sites, situated at chromosomes 11q13 and 14q32. At chromosome 11q12, HBV DNA was integrated into protein-coding genome, the function of which remains unclear.

CONCLUSION: Integrated HBV DNA may play a role in hepatocarcinogenesis after the clearance of HCV by IFN treatment.

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INTRODUCTION

Interferon (IFN) has potent antiviral activity against hepatitis C virus (HCV). Previous studies have shown that IFN can reduce the incidence of hepatocellular carcinoma (HCC) in patients with HCV infection (1–3). After complete eradication of HCV by IFN therapy, HCC was thought to rarely occur (4). Recent studies have shown that HCC develops in 2.5–4.2% of such patients (5–7). These patients may have had advanced liver fibrosis at the time of HCV eradication, and subclinical tumors might have already existed in the liver at the end of IFN therapy (8). In some patients, however, HCC might develop from liver without fibrosis several years after the eradication of HCV by IFN. The etiology of such cases of HCC remains obscure. New regimens combining IFN with antiviral drugs can improve the rate of HCV clearance (9, 10). The risk of HCC might increase in patients with chronic hepatitis who have complete responses to IFN therapy. It is important to delineate the features of HCC occurring after elimination of HCV. Occult hepatitis B virus (HBV) infection is defined as the detection of HBV DNA in the serum or liver of patients without hepatitis B surface antigen (HBsAg) (11). In patients with chronic hepatitis C, occult HBV coinfection may exacerbate liver disease (12). Occult HBV infection is present in a substantial proportion of patients with HCV infection and has a pro-oncogenic effect (13). In the present study, we examined resected liver specimens to evaluate the role of occult HBV infection in the development of cancer after the clearance of HCV by IFN treatment. We also describe the clinical course of such patients with HCC.

METHODS

Patients

At our department, 1,286 patients with chronic hepatitis C without cirrhosis and without HBsAg received IFN

Table 1. Clinical Characteristics of Seven Patients

Case	Gender	Age at Operation	Anti-HBS/ Anti-HBC	Anti-HCV/ HCV-RNA	HCV Genotype Before IFN	Alcohol Intake	Body Mass Index (kg/m^2)	Period from End of IFN to Diagnosis for HCC	HBV DNA Integration	Stage of Fibrosis
1	M	65	−/−	+/−	2a	none	23.1	13 mon	−	2
2	M	59	+/+	+/−	1b	rare	23.7	45 mon	+	2
3	M	65	+/+	+/−	2a	rare	23.7	19 mon	−	4
4	M	61	+/+	+/−	2a	none	23.6	20 mon	−	4
5	M	59	−/−	+/−	2b	rare	23.4	41 mon	+	1
6	M	66	+/+	+/−	2a	none	18.1	80 mon	+	2
7	M	60	−/−	+/−	2a	none	21.5	103 mon	+	1

monotherapy for 24 wk from 1992 through 2002. In 342 patients, serum HCV RNA disappeared, and alanine aminotransferase activity (ALT) was within the normal range for 6 months after the end of IFN therapy. We are now monitoring 144 of these patients every half year. HCC was diagnosed in seven patients, four of whom were regularly monitored (cases 1, 4, 6, and 7). Seven patients underwent hepatectomy at our hospital. Their clinical characteristics are described in Table 1. No patient had alcohol abuse, drug usage, or diabetes mellitus. All patients had a body mass index of less than 25 kg/m^2. The surrounding liver tissue was pathologically classified according to the criteria proposed by Desmet *et al.* (14).

Detection of HBV DNA in Serum and Liver

DNA was extracted from 100 μL of serum or 10 μg of liver tissue by means of proteinase K digestion followed by phenol/chloroform extraction, as described previously (15). HBV DNA in serum or in liver was amplified with specific primers for HBX, HBS, and HBC (sequences of the primers shown in Table 2). Amplification was done in a thermal cycler for 35 cycles: 95°C for 30 s, 55°C for 60 s, and 72°C for 60 s in 40 μL of a reaction buffer containing 30 pmol of the two appropriate primers, four deoxynucleotides each at a concentration of 100 mM, polymerase chain reaction (PCR) buffer, and 2.5 units of Gold Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). With 2 μL of the first PCR product, a second PCR was done. To examine covalently closed circular HBV (cccHBV) in liver, extracted DNA was amplified with primers P23, 24, 25, and p26 (Table 2). The amplification procedure and primers have been described previously (16).

Detection of Integrated HBV DNA in Human Genome

We used cassette-ligation-mediated PCR to detect HBV DNA integrated into the human genome as described previously (15). Briefly, 10 μg of DNA was digested with *Eco*RI, *Hin*dIII, or *Pst*I and ligated to double-stranded DNA cassettes with compatible ends. The cassette-ligated DNA fragments were used as a template for nested PCR with the cassette- and HBV-specific primers. One microliter of the DNA solution was amplified in 40 μL of a reaction buffer containing 10 pmol of the two appropriate primers, four deoxynucleotides each at a concentration of 100 mM, PCR buffer, and 2.5 units of LATaq polymerase. The amplifications were carried out in a thermal cycler for 33 cycles (45 s at 94°C, 2 min at 55°C, 2 min at 72°C), followed by final extension for 10 min at 72°C. With 1 μL of the first PCR product, a second PCR was done. Table 2 shows the sequences of the primers used. The amplified cassette-ligated DNA fragments were subcloned and sequenced with a DNA sequencing system (377A, Applied Biosystems, Tokyo). To identify the integrated site of the host genome, we used the GenomeNet (http://www.genome.ad.jp) to compare the sequences adjacent to the integrated HBV DNA with the human sequence.

Statistical Analysis

Ages, intervals, and tumor sizes in the two groups were compared by Student's *t*-test.

RESULTS

Pathological Findings of the Resected Liver

The seven liver tumors were diagnosed as four poorly differentiated HCC and three moderately differentiated HCC (Table 3). The surrounding liver tissues were diagnosed as chronic hepatitis. The stage of liver fibrosis was IV in two specimens, II in three specimens, and I in two specimens. The activity grade was II in four specimens and I in three specimens. There was no evidence of fat deposits in any of the specimens.

HBV DNA in Serum

HBV DNA was not detected in serum of any of the seven patients with HCC.

HBV DNA in Liver

We detected HBV DNA in five of the seven HCC and three of five noncancerous liver samples (Fig. 1). In detail, HBX was detected in three of the seven tumors and one specimen of noncancerous liver tissue. HBC was detected in four tumors and three liver tissues. HBS was detected in two tumors and three liver tissues. Covalently cccHBV was detected in case two (both HCC and noncancerous liver) and in case four (only liver tissue).